Author’s response to reviews

Title: Association of limbic system-associated membrane protein (LSAMP) to male completed suicide

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Author’s response to reviews: see over
Dear Editors of the BMC Medical Genetics:

Please find enclosed our revised manuscript entitled as “Association of limbic system-associated membrane protein (LSAMP) to male completed suicide”.

We have addressed the comments by reviewers as well as corrected our manuscript accordingly.

First, about most important changes in updated version of the manuscript. We decided to leave out two loci from our analysis because we felt the results should be replicated with a different method before publishing. Unlike the other loci in our SNP set, they were not performing so well on SNPlex, resulting in poor yield in automatic genotype analysis, so at first we adjusted a number of genotypes manually and included the results into the previous version of manuscript. Having discussed it lately, we have come to a conclusion that this procedure could be a source of type I error and we wish to re-check the results with a different genotyping method. Since applying extra arrays consumes time and resources, we are leaving these two loci (rs9821809 and rs2918219) out from the current publication and going to consider them in future ones.

The second important change is that we corrected our single-marker associations by permutation test, and adjusted the text accordingly.

Please find the responses to the reviewers’ comments below.

Yours sincerely,

Anne Must
Answers to concerns raised by Referee 1

1. Provide the Odds Ratio for any allele and genotype test that are significant.

We included the OR values as well as 95% confidence intervals in the Results section:

“The odds ratios for associated loci were respectively 1.3 (CI: 1.01-1.67), 1.3 (CI: 1.01-1.67), 1.3 (CI: 1.00-1.62) and 1.5 (CI: 1.01-2.29).”

and

“In case of rs4831129, frequency of TT genotype was significantly higher in suicide group, as opposed to GG or GT genotypes (chi square(1)= 6.624, p=0.011; OR=1.5, CI: 1.11-2.11). The same principle applied for rs9874470 where frequency of T-homozygotes in suicide group exceeded that of controls (chi square(1)=5.964, p=0.017; OR=1.5, CI: 1.08-2.07).”

2. Provide the D’ for the block 1, 2, 3 and 5 (3snp block).

We have added Figure 1 which shows the locations of SNPs as well as degree of LD expressed in D’.

3. Provide the OR for the haplotype tests.

After leaving out two SNPs from the study, the haplotype blocks defined by confidence interval algorithm did not yield any significant associations, so we did not provide the OR values.

4. Authors should apply multiple correction such as the permutation test.

We performed the permutation test (10 000 permutations) implemented in Haploview 3.32. All the initial associations disappeared.

5. The results should be discussed after the correction is applied.

We have done it.

6. The authors specified that they used the Tagger but they did not specify the minor allele frequency cutoff.

It was 5% and we have explained it in the “Marker selection and genotyping” subsection:

“Thirty tag SNPs were selected from LSAMP gene and flanking regions (150kb fragment from 116931222-117081732; and 242kb fragment from 117428563-117670401) using the Tagger algorithm ($r^2=0.8$, minor allele frequency >0.05) implemented in Haploview 3.32.”

7. Also, they should specify for each SNPs the location (i.e. intron, exon, 3’UTR).

We have added a column in the Table 1 that specifies the location of each locus.

8. In the methods they said that they selected the SNPs only in the 3’ and 5’ regulatory regions. How did they identify such regions?
We have corrected the term into “flanking regions” and provided the chromosome location of the areas in the “Marker selection and genotyping” subsection.

9. HWE p-values should be provided for all SNPs.

We have added a column in the Table 1 providing these values.

10. Authors can try to analyze the different suicide methods for further genetic associations.

It would be interesting but currently we have too small sample to do that. We have 288 suicide victims, 88% of them have committed suicide by hanging. All other suicide causes are represented in too small numbers to perform a reliable association analysis.

11. The blocks are numbered form the 3’ to the 5’. They should be consistent with the gene physical map.

We have corrected it and numbered the blocks in reverse order.

12. Please provide the link for the electronic citations.

We have done it.
Answers to concerns raised by Referee 2

1. The major weakness of the study is the fact that the authors did not apply a Bonferroni correction for their significant results. After correction for multiple testing their results are no longer statistically significant.

Since we skipped two loci from the second version of manuscript, we were left with no significant differences between haplotype frequencies and the need for Bonferroni correction was called off. However, we corrected the single marker results for multiple comparisons with a permutation test implemented in Haplovew 3.32.

2. The authors investigated male suicide victims, but there is no description in the text whether there are also no females in the control sample.

We have stated in Subjects section that “two groups of male subjects were investigated in this study”.

3. I do not understand the haplotype analysis. The authors did not give any overview about the degree of LD between all investigated SNPs, but determine 5 haplotype blocks according to the LD measurement. This is not understandable for the reader.

We have added Figure 1 in order to provide the reader with a better visual overview of the SNPs locations, as well as the degrees of LD expressed as D’ value. The haplotype blocks were delineated using the confidence interval method by Gabriel implemented in Haplovew 3.32.

4. The abbreviation of the gene name (LSAMP) should be mentioned in the title.

We have done that and corrected the title into “Association of limbic system-associated membrane protein (LSAMP) to male completed suicide”.

5. In table 1 the allele frequencies should be presented for the major or minor allele and not for the associated allele (not all alleles are associated).

We have presented the allele frequencies for major alleles.