COMMENTS TO THE AUTHOR

Shi and colleagues performed a case-control study of the relationship between the ITLN1 polymorphisms with Ischemic stroke in Chinese population well. This study encompasses some interesting points however several points should be addressed before acceptance for the publication. Comments and suggestions are below.

Generally, the manuscript could be improved with linguistic revision by a native speaker. Some passages should be rephrased.

Abstract:

1) Add the numbers of the study groups and briefly explain the sanger sequencing and add the NM names of reference sequences in the Methods section as in the examples below.

Example 1)"We sequenced deoxyribonucleic acid from 96 patients with myelomeningocele. The 11 exons were amplified by polymerase chain reaction, and the products were sequenced with the Sanger method. Results were compared with reference sequences (NM_000454, NM_000636, and NM_001024466) obtained from University of California Santa Cruz Genome Browser. Observed alleles that differed from the reference sequences were considered novel variants."

Example 2) "Methods: MMP8 rs1940475 and rs3765620, and MMP10 rs17860949 from 700 IS patients and 700 controls were genotyped by the MassARRAY iPLEX platform. The impact of polymorphisms on IS risk was evaluated by logistic regression analysis."

2) The sentence "By comparing with the Sanger sequencing results and the reference sequence of the NCBI website" which is written in the result part, should be explained in methods.

3) "Logistic regression analysis showed that *ITLN1* (rs6427553, rs7411035 and rs4656958) were associated with a decreased risk of IS, especially in people less than 60 years old and males" Who do you mean by men over sixty? Is it IS patient or Control group?

4) The sentence "In short, we observed a correlation between *ITLN1* variants (rs6427553, rs7411035 and rs4656958) and IS risk in the Chinese subjects, laying the foundation for *ITLN1* gene as a biomarker for ischemic stroke." is not appropiate for the conclusion part of the study. Please state comment about this correlation.

Introduction:

1) The incidance of IS has been mentioned too much in the first paragraph. It can be shortened.

2) **"** Intelectin-1**"** should be used instead of "Omentin-1" in all over manuscript.

3) Please state information about *ITLN1* gene, its polymorphisms and the IS relationship.

4) “In the present study, we used Sanger sequencing to complete the PCR amplification of the *ITLN1* gene promoter region, and then compared the obtained sequence with the sequence on NCBI to identify SNPs of the *ITLN1* gene promoter region. In addition, the association of these loci with the risk of IS was analyzed using logistic regression.The results will lay a foundation for finding the diagnostic markers of IS”.

Please modify this paragraph to include the purpose of the study.

Materials & Methods:

1) The subsections “DNA extraction, Primer design and DNA amplification” and “Sanger sequencing of PCR products” should be combined under the subheading “Sanger sequencing of PCR products”.

*2) The sentence “*We used G*power 3.1.9.7 software to estimate the sample size of the case group and the control group through t-test. The parameter settings are: Tail=2, Effect size = 0.20, ³)= 0.05, Power=0.945, Allocation ratio =1.” should be replaced in Statistical analyses.

3) Please specify in which program the primer design was designed.

4) Line 82-83. The sentences “The positive and negative primers were shown in the Online Resource 1” should be deleted.

5) Line 84. please state the full name of the Genewiz company in parentheses.

6) “According to the results of agarose gel electrophoresis, 4% L of the PCR target fragment amplification product with clear and complete bands was sent to Tsingke Biotechnology Co., Ltd. for two-way Sanger sequencing. After obtaining base sequence of the target fragment, it was compared with the reference sequence of *ITLN1* gene promoter region provided by NCBI to determine whether there were single nucleotide polymorphisms in *ITLN1* gene promoter region.”

The paragraph is very detailed and hard to follow. Authors should simplify the methods and specify the program the sanger sequencing was analyzed.

7) Line 96. This sentences “After the sequence alignment was completed, the SNP was obtained.” should be deleted.

8) “According to Fisher’s exact test, significant SNPs were screened in the control group and the case group ($p < 0.05$). Then, chi-square test was used to perform Hardy-Weinberg equilibrium (HWE) test on significant sites to evaluate whether the enrolled samples were representative of the population.”

Instead of this explanation Authors may explain the method by using the sentence as below.

“ Genotype and allele distributions of detected SNPs were compared using Fisher’s exact test. The Hardy–Weinberg equilibrium and genotype distribution in case and control subjects were analyzed using the $\chi 2$ test.

9) Please specify the models used in the Genotype–phenotype associations, such as dominant and recessive model, in the statistical analysis section.

10) Please state the adjusted for confounding variables used in logictic regression analysis.

11) Please specify whether the age and gender classification were done in the whole group or among the case-control groups in table 4.

12) Please provide an explanation of the abbreviations FPRP and MDR.

Results:

1) In line 115, As shown in Table 1 should be written instead of table 1.

2) “As shown in the allele model, people with rs6427553-T, rs7411035-G and rs4656958-A had a lower risk of IS (OR : 0.84, 95%CI: 0.72-0.99 p= 0.032; OR : 0.82, 95%CI: 0.70-0.97, p= 0.017; OR : 0.76, 95%CI: 0.64-0.90 p= 0.002).”

In the sentence please identify the people whether they are IS patients or control group.

Also, in line 119 please state the word respectively in the end of the sentence.

3) In lin 121 please mention the general population whether they are IS patients or control group.

4) The lines of123-127 are also mentioned in the table 2, just $p<0.05$ can be specified.

5) Instead of the lines 128-133 as shown in table 3, the significant result should be mentioned.

6) “We did age and gender stratification analysis to assess the association between polymorphisms and IS risk.”

In which group this stratification was done ? Authors should mention this.

7) In line 136 please specify the people.

6) Please state the number of case and control in figure 1 and tables.

7) Please delete the “A: minor alleles; B: major alleles.” in table 1.

9) Please delete the column of the gene in table 1.

10) Please delete the “logistic regression analysis with adjustments for age and gender.” in table 4.

Discussion:

1) In the discussion section, it is recommended that the article ** mentioned below be discussed and included in the sources.

2) The functional effect of *ITLN1* variants (rs6427553, rs7411035 and rs4656958) should be discussed by performing in silico analysis.

3) The association between SNPs and IS should be discused more detailed.