Reviewer’s report

Title: Methylation of the genes ROD1, NLRC5, and HKR1 is associated with aging in Hainan centenarians

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Reviewer: Yong-Han He

Reviewer’s report:

Zeng et al. reported the methylation of several genes associated with longevity in Hainan. This provides some useful information on the epigenetics in the oldest-old population. However, I have some concerns on this manuscript.

1. The authors showed the methylation was associated with longevity, how did they draw this conclusion? They should exclude that the methylation was not age-related, because age-dependent changes in DNA methylation have been well studied. Aging is a major influential factor.

2. The authors described that "Hainan centenarians remain an under-studied population", after searching the PUBMED database, there are dozens of papers related to this population study, the author should reference them where necessary.

3. The significance is p<0.01, how did the author set this significance? Is there any support about this threshold? I think the author should set a corrected p values (e.g. multiple testing correction for the p value).

4. The GO and related analysis should be described in the results instead of discussion section.

5. According to the published papers, there are several studies reporting the DNA methylation in centenarians, but not just "In previous longevity-methylation studies, the age range of the oldest study subjects has typically been from 80 to 99 years of age[23, 24]", even in the Chinese population, the authors should cite the references.

6. The authors identified the longevity-associated methylations, and also revealed the genes that may be regulated. Regarding the transcriptional regulation of methylation, and the enriched TSS location of methylation sites, I suggest that gene expression levels should be tested in this study to support the conclusions, at least those with the strongest significance.

7. Please proofread the language used in the manuscript, avoiding any spelling or grammar mistakes. For example, "ROD1 was found to be a member of the heterogeneous nuclear ribonucleoprotein family, which regulate the alternative splicing of pre-RNA"

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
Yes

**Does the work include the necessary controls?**  
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**  
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**  
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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Please indicate the quality of language in the manuscript:

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