Comments to the Authors,

In this manuscript, Dr. VRANEKOVIĆ and colleagues conducted an association study between LINE-1 methylation, MTHFR (rs1801133) polymorphism and occurrence of congenital heart defects (CHDs) in children with Down syndrome (DS) in a Caucasian population (N=90). As the investigation shown the LINE-1 methylation and rs1801133 don’t have significantly difference between mothers of DS-CDH+ and DS-CHD-. However, the authors found the LINE-1 methylation could be significantly predicted by BMI and rs1801133 polymorphism in mothers of DS-CDH- surprisingly. The accident findings is quite interesting. However, I have several concerns:

1, Now that LINE-1 and rs1801133 don’t have significant difference between mothers of DS-CDH+ and DS-CHD-, I don’t understand why the significant prediction can only be found in DS-CDH+ subgroup. I highly suspected the significant prediction is false positive result caused by non-random sampling. Is there any further data could provide more confident support to the conclusion. It is hard to validate the relationship between BMI and LINE-1, however, it is quite easy to validate the relationship between rs1801133 and LINE-1 methylation with cell biology technique. I prefer the authors to provide this evidence to avoid the false positive association.

2. Please check carefully about 677CT and C677T in the manuscript.

3. I prefer the author to replace ‘influenced’ with ‘associated or correlated’ in the the following scenario: We found that the MTHFR C677T genotype/diet combination significantly influenced global DNA methylation.

4. Meanwhile, the authors should consider another question about the technique deviation in the measurement of LINE-1 methylation. In the present study, the authors didn’t provide the technique reproducibility which might be completed in the previous study, However, the author should estimate the influence from the technique variation which I am not sure whether the technique variation is larger than that in samples or not. Meanwhile, I am not sure which region or CpG sites the author measured in this study (primers have not shown). What kind of percentage for this value can be applied to indicate the genome-wide DNA methylation.