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.