Reviewer's report

Title: LINE-1 Methylation Status and Its Association with Tetralogy of Fallot in Chinese Infants

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Reviewer: Shimul Chowdhury

Reviewer's report:

Overview: The authors present a novel study in which alterations in DNA methylation were found to be associated with Tetralogy of Fallot (TOF). Strengths of the study include the assessment of DNA methylation from DNA isolated from cardiac tissue, and the observations of altered LINE-1, NKX2-5, HAND1, and TBX20 methylation associated with TOF. Weaknesses include a lack of expansion on gene-specific methylation results and analysis as well as omissions of key components of the study.

Major Compulsory Revisions

1. The findings of significant differences in methylation between cases and controls for NKX2-5, HAND1, and TBX20 should be expanded. The paper focuses almost entirely on the LINE-1 methylation analysis. More emphasis should be dedicated to the novel findings of altered methylation in these important cardiac genes. Most significantly, TBX20 was found to have the smallest p-value in the study. Why was the majority of focus on LINE-1 methylation?

2. In the discussion section it is stated that “this finding is of remarkable clinical relevance” and “is a molecular marker capable of accurately indicating the presence of TOF in pediatric patients.” Although the results from the study are promising, it should be noted that obtaining cardiac tissue for methylation analysis is an extremely invasive procedure. Thus, using methylation markers from cardiac tissues its’ potential impact in the clinical realm may be limited. The study provides initial evidence more on the potential pathophysiology of TOF. Overstatement of results from a small population case-control study should be avoided.

3. Why were NKX2-5, HAND1, and TBX20 selected over other key cardiac regulator genes such as GATA4, GATA5, NOTCH1, etc? Please provide rationale for these gene selections.

Minor Essential Revisions

1. In the discussion section is stated “…..NKX2-5, HAND1, and decreased methylation levels in the promoter CpG island of TBX20 in the TOF patients than in controls (Table 4). Table 4 only lists the primers sequences and positions. I believe Table 2 should be the table referenced here.
2. The Introduction section lacks certain references that should be included. Certain sentences include:

“Heredity is likely to play an important role in the development of TOF,”
“…Some studies have proven the existence of a correlation between TOF and gene mutations,”
“In addition to disorders in the DNA sequence, epigenetic regulation has been associated with TOF.”
“….serve as a surrogate marker for global genomic DNA methylation.”

3. The term “kernel” should be changed to “gene.”

4. Please define the difference between CpG sites and CpG units.

5. The number of overall subjects in the higher quartile (6) is a little concerning. I appreciate that the samples obtained for the study are precious. Perhaps stating this as a limitation in the analysis is justified.

6. The explanation of the gene methylation status in the methods sections mentions LINE-1 but makes NO mention of the other genes in the study. I assume the same method was used, but the other genes should be listed.

7. Along the same rationale, what regions were targeted for gene-specific methylation analysis of NKX2-5, HAND1, and TBX20? Promoter regions?

8. The total number of subjects for LINE-1, NKX2-5, HAND1, and TBX20 methylation analysis differs (47, 38, 45, 44, respectively). What accounts for this difference? Sample availability? Failure of usable data?

Discretionary Revisions

The large difference in the age ranges between cases and controls is potentially troubling. The authors have shown that LINE-1 methylation is not associated with age in cases and controls separately. Inclusion of age and gender can easily be included in the logistic model. This analysis should be included for gene-specific methylation as well. How can we be sure that confounding factors do not account for the differences seen between cases and controls for NKX2-5, HAND1, and TBX20?

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
I declare that I have no competing interests