TP TRANSLATIONAL PEDIATRICS
AN OPEN ACCESS JOURNAL COVERING ALL ASPECTS OF PEDIATRICS RESEARCH
IMPACT FACTOR 2.286

Peer Review File

Article information: https://dx.doi.org/10.21037/tp-21-233

Reviewer A

Authors reported a clinical and genetic analysis of a cohort of newborns suffering of arrhythmias. The manuscript is well-written and results are of high interest.
However, some points should be clarified:

Comment 1 - please specify NICU.
Reply1 : Thanks for your comments. We have added the suggested content "NICU (neonatal intensive care unit)".
Changes in the text of "TP-21-233-manuscript-Revised-3-with line numbers" : page 3, line 70

Comment 2 - please include classification of variants following ACMG recommendations. In addition, please divide cohort in carries of VUS or anyone carrying LP/P variants.
Reply 2: Thanks for your comments. In the revised supplementary table 3, we have supplemented the classification of each variant follow the ACMG guideline.
Changes in the text: revised supplementary table 3.

Comment 3 - please include genes analyzed.
Reply 3: Thanks for your comments. In this study, we performed whole exome sequencing, and we did not target candidate genes during data analysis. In fact, from the results of this study, we identified the pathogenic variants in syndromic and metabolism-related genes, rather than the arrhythmia-related genes only. We have added the description of the genetic testing in the MATERIALS AND METHODS section.
Changes in the text of "TP-21-233-manuscript-Revised-3-with line numbers": page 4，line 89-104

Comment 4 - All rare variants identified using NGS analyzed were confirmed By Sanger?
Reply 4: Thanks for your comments. All identified SNV/indel variants were

confirmed by Sanger sequencing. We have added this description in the revised manuscript.

Changes in the text of "TP-21-233-manuscript-Revised-3-with line numbers": page 4，line 96-104

Comment 5 - all exons of all genes analyzed were covered at least at 20x by NGS? If not, exons were amplified using Sanger?

Reply 5: Thanks for your comments. In this study, the average on-target sequencing depth was 120×, and fraction of official target covered with at least 20X is higher than 96.5%. We have added this description in the revised manuscript.

Changes in the text of "TP-21-233-manuscript-Revised-3-with line numbers": page 4，line 93-94.

Comment 6 - CNVs identified were confirmed by MLPA or HRM?

Reply 6: Thanks for your comments. CNVs were analyzed with the use of a home-modified CANOES17 using a read depth calculated by BEDtools (V.2.17). We have added this description and the reference in the revised manuscript. Since both copy number variants detected were trisomy 21, karyotype analysis was used for verification.

Changes in the text of "TP-21-233-manuscript-Revised-3-with line numbers": page 4, line 97-104.

Comment 7 - Newborns with malignant arrhythmias were treated with ICD, pharmacs or any intervention (such as cardiac ablation)?

Reply 7: Thank you for your comments. In this study, infants with atrial flutter (8 infants) and ventricular fibrillation (3 infants) were treated with electric defibrillation. Infants with malignant arrhythmias were treated with anti-arrhythmic drug and/or heart failure drugs. But there was no intervention treatment including radiofrequency ablation.

Comment 8 - Family segregation and clinical assessment of relatives were performed in all relatives?

Reply 8: Thank you for your comments. Among the 20 probands with single gene defects, 4 newborns were identified variants in genes with autosomal recessive inheritance model. Of these 4 patients, all of them have siblings with unexplained neonatal deaths except patient 7. Among the 20 probands with single gene defects, 16 newborns were identified variants in genes with autosomal dominant inheritance model. Of these 16 patients, only 5 were verified by the parents' sample and

confirmed to be *de novo*, so the further verification of other family members were not performed.

Comment 9 - Family history of sudden death or any arrhythmia?

Reply 9: Thank you for your comments. The family history was provided in supplementary table 1. Three of four infants diagnosed with carnitine palmityl transferase II deficiency had similar sibling histories. One infant had atrioventricular junctional escape beat and grade I atrioventricular block, and his father and grandfather both had bradycardia. Unfortunately, however, these family members have not been tested.

Changes in the text: supplementary table 1.

Comment 10 - Please explain in detail the difference in clinical practice if a VUS or LP/P were identified. Any different adoption o measures in newborns concerning genetic variant identified?

Reply 10: Thank you for your comments. Arrhythmias in newborns can be caused by a variety of factors, including abnormal cardiac conduction, structural abnormalities, and internal environment disturbance. For the newborns with metabolic and syndrome-related gene defects, precise treatment can be targeted at the etiology. For the neonates with ion channel-related gene defects, we are exploring specific treatments that target different disease-causing genes.

Comment 11 - Any variant identified in the cohort was previously reported?

Reply 11: Thank you for your comments. In supplementary table 3, we provided the PMID numbers of previously reported literatures.

Reviewer B

Nice study looking at genetic causes of arrhythmia in the neonatal period. I think this study adds to the literature, I just have a few minor issues.

Comment 1. It would be helpful for the authors to better describe the guidelines they used for variant interpretation. They provide the tools they used in the Methods but not the criteria that they applied. This is important to allow for comparison between studies evaluating genetic findings from this population.

Reply 1: Thanks for your comments. We have supplemented the description of the genetic testing, and cited references of variation annotation in the MATERIALS AND METHODS section. In the revised supplementary table 3, we have supplemented the classification of each variant follow the ACMG guideline.

Comment 2. Results presented in the discussion:
"In our cohort, four infants died within 3 days after birth; ultimately, they were 254 diagnosed with carnitine palmityl transferase II deficiency based on genetic 255 sequencing reports (3 SLC25A20 genes and 1 CPT2 gene), and three of them had similar sibling histories."
Reply 2: Thank you for your suggestion. We have modified our text as advised: "In our cohort，four infants with malignant arrythmia died early after birth, ultimately they were diagnosed with carnitine palmityl transferase II deficiency based on genetic sequencing reports."
Changes in the text of "TP-21-233-manuscript-Revised-3-with line numbers": page 8-9, line 221-223.

Comment 3. Page 3 line 77- I suggest change "next-generation sequencing" to "exome sequencing"
Reply 3: Thank you for your suggestion. We have revised it according to your suggestion and "next-generation sequencing" was changed to "whole exome sequencing" .
Changes in the text of "TP-21-233-manuscript-Revised-3-with line numbers": page 4, line 83.

Comment 4. Page 4 line 132- I suggest changing "refused genetic sequencing" to "declined exome sequencing"
Reply 4: Thank you for your comments. We have modified our text as advised.
Changes in the text of "TP-21-233-manuscript-Revised-3-with line numbers": page 5, line 117.

Comment 5. Page 6 line 175-
"Infants with premature beats had some syndrome-, respiration- and immune-related genes, though these genes had no direct relationship with their arrhythmias (Figure 1).
Change to
"Infants with premature beats had disease-causing variants in syndromic, respiration- and immune-related genes, though these genes had no direct relationship with their arrhythmias (Figure 1)."
Reply 5: Thank you for your comments.. We have modified our text as advised.
Changes in the text of "TP-21-233-manuscript-Revised-3-with line numbers": page 6, line 152-154.

Comment 6. I would also suggest replacing "mutation" with "disease-causing variant" throughout the manuscript.

Reply 6: Thank you for your comments. We have modified the "mutation"to "disease-causing variant" throughout the manuscript.

Changes in the text of "TP-21-233-manuscript-Revised-3-with line numbers": page 3, line 60 , page 6, line 153 and page 8, line 212..