Volume overload initiates an immune response in the right ventricle at the neonatal stage

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Background Pulmonary regurgitation caused by the correction or palliation of pediatric tetralogy of Fallot (TOF) leads to chronic right ventricular (RV) volume overload (VO), which induces adolescent RV dysfunction. A better understanding of the molecular mechanism by which VO initiates neonatal RV remodeling may bring new insights into the post-surgical management of pediatric TOF.

Methods and Results We created a fistula between the abdominal aorta and inferior vena cava on postnatal day 1(P1) using a rat model to induce neonatal VO. Echocardiography revealed that the velocity and velocity- time-integral of the pulmonary artery (PA) were significantly elevated, and hematoxylin and eosin (H&E) staining showed that the diameter of the RV significantly increased. RNA-seq analysis of the RV on P7 indicated that the top 10 enriched Gene Ontology (GO) terms and the top 20 enriched terms in the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were associated with immune responses. Flow-cytometric analysis demonstrated that the number of CD4+and CD8+ immune cells were significantly augmented in the VO group compared with the sham group.

Conclusions A neonatal cardiac VO rat model on P1 was successfully created, providing a platform for studying the molecular biology of neonatal RV under the influence of VO. VO - induces an immune response at the neonatal stage (from P1 to P7), suggesting that immune responses may be an initiating factor for neonatal RV remodeling under the influence of VO and that immunosuppressants may be used to prevent pediatric RV remodeling caused by VO.

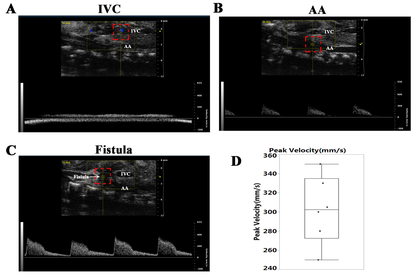


Figure 1. Establishment of the abdominal aorta and inferior vena cava fistula (AVF). (A) The inferior vena cava (IVC) manifested no pulsatile blood flow. (B) There was pulsatile blood flow in the abdominal aorta (AA), with a peak blood flow velocity of 300 mm/s. (C) Representative image of the pulsating blood flow at the fistula, with a peak blood flow velocity of 400 mm/s. (D) Quantification of peak velocity at the fistula.

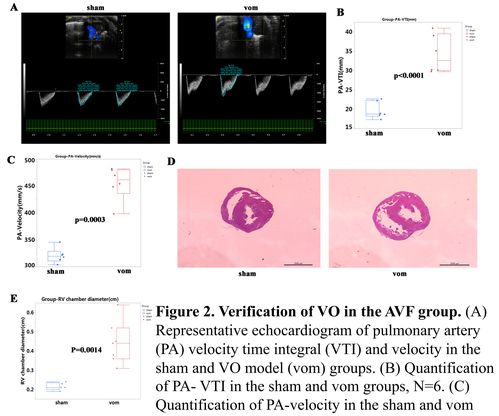
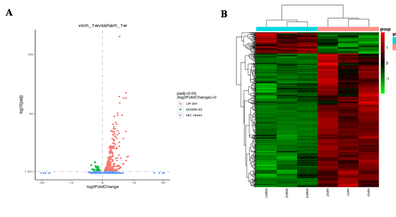
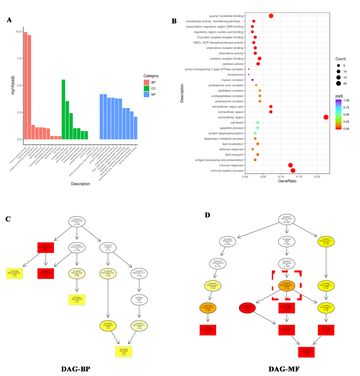


Figure 2. Verification of VO in the AVF group. (A) Representative echocardiogram of pulmonary artery (PA) velocity time integral (VTI) and velocity in the sham and VO model (vom) groups. (B) Quantification of PA- VTI in the sham and vom groups, N=6. (C) Quantification of PA-velocity in the sham and vom groups, N=6. (D) Middle panel, representative H&E staining of hearts from the sham and vom groups. (E) Middle panel, quantification of the diameter of the RV chamber.





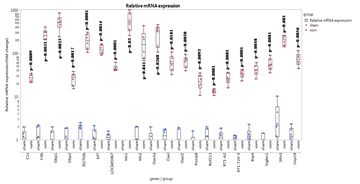


Figure 6. Relative mRNA expression of top 20 differentially expressed genes in the RNA-seq data. The relative fold change was calculated using the ΔΔCT method [25]. One sample in the sham group was set as "1"; the other samples referred to the sample.

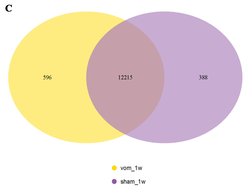


Figure 4. GO enrichment analysis indicates that the differentially expressed genes altered by VO are primarily associated with immune response. (A) Histogram of the GO analysis. From the results of the GO enrichment analysis, we displayed the 10 most significant terms. The abscissa is the GO -term, and the ordinate is the significance level for GO term enrichment; the higher the value, the more significant the result. The different colors represent three different GO subclasses: biological process (BP), cellular component (CC), and molecular function (MF). (B) Scatter plots of GO analysis. From the results of the GO-enrichment analysis, we selected the 30 most significant terms to construct scatter plots for display. The size of the dots represents the number of genes annotated to the GO term, and the colors from red to purple represent the significance level of the GO term enrichment. (C) DAG analysis of biological process (BP). (D) DAG analysis of molecular function (MF). The intensity of the color represents the degree of enrichment; a darker color indicates a higher degree of enrichment. Each node displays the name of the term and the adjusted P-value (padj).

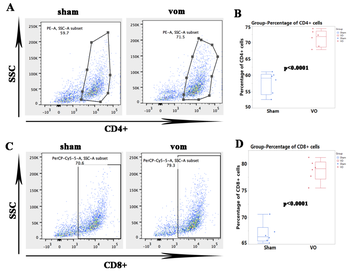


Figure 7. Verification of the RNA-seq data by flow cytometric analysis of immune cells. (A) Representative dots of CD4+ cells. (B) Quantification of the percentage of CD4+ cells. (C) Representative dots of CD8+ immune cells. (D) Quantification of the percentage of CD8+ cells.

Figure 3. The transcriptome of the neonatal RV is altered by VO. (A) Volcano map of the differentially expressed genes between the vom and sham groups. There were 391 upregulated and 63 downregulated genes in the vom group. (B) Cluster analysis of the differentially expressed genes between the vom and sham groups. The clusters of genes in each group were quite different from each other but were similar within the same group. (C) Venn diagram of the differentially expressed genes between the vom and sham groups. These two groups shared 12,215 commonly expressed genes; there were 596 genes expressed only in the vom group and 388 genes expressed only in the sham group.

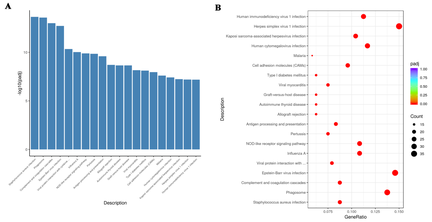


Figure 5. KEGG pathway analysis indicates that the differentially expressed genes altered by VO are principally associated with immune response. (A) Histogram of the 20 most significant KEGG pathways. The 20 most significant KEGG pathways from the KEGG enrichment results are displayed. The abscissa is the KEGG pathway, and the ordinate is the significance level of pathway enrichment; the higher the value, the greater the significance. (B) Scatterplot of the 20 most significant KEGG pathways. The abscissa is the ratio of the number of genes in the KEGG pathway analysis to the total number of differentially expressed genes, the ordinate is the KEGG pathway, the size of the dots represents the number of genes annotated to the KEGG pathway, and the colors from red to purple represent the significance level of KEGG pathway enrichment.