Cardiology in the Young

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Identification of IL-6 as a potential mediator of the myocardial fibrosis that occurs in response to surgery with cardiopulmonary bypass in children with Tetralogy of Fallot

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Abstract

Background: Tetralogy of Fallot is a common CHD. Studies have shown a close link between heart failure and myocardial fibrosis. Interleukin-6 has been suggested to be a post-independent factor of heart failure. This study aimed to explore the relationship between IL-6 and myocardial fibrosis during cardiopulmonary bypass. *Material and Methods:* We downloaded the expression profile dataset GSE132176 from Gene Expression Omnibus. After normalising the raw data, Gene Set Enrichment Analysis and differential gene expression analysis were performed using R. Further, a weighted gene correlation network analysis and a protein–protein interaction network analysis were used to identify HUB genes. Finally, we downloaded single-cell expression data for HUB genes using PanglaoDB. *Results:* There were 119 differentially expressed genes in right atrium tissues comparing the post-CPB group with the pre-CPB group. *IL-6* was found to be significantly up-regulated in the post-CPB group. Six genes (*JUN*, *FOS*, *ATF3*, *EGR1*, *IL-6*, and *PTGS2*) were identified as HUB genes by a weighted gene correlation network analysis and a protein–protein interaction network analysis. Gene Set Enrichment Analysis showed that IL-6 affects the myocardium during CPB mainly through the JAK/STAT signalling pathway. Finally, we used PanglaoDB data to analyse the single-cell expression of the HUB genes. *Conclusion:* Our findings suggest that high expression of IL-6 and the activation of the JAK/STAT signalling pathway during CPB maybe the potential mechanism of myocardial fibrosis. We speculate that the high expression of IL-6 might be an important factor leading to heart failure after ToF surgery. We expect that these findings will provide a basis for the development of targeted drugs.

Background

Tetralogy of Fallot is a rare CHD that affects about 3.5% of infants born with CHD.[1] Currently, the most effective treatment for ToF in children remains is the surgery with cardiopulmonary bypass.[2] Despite surgical repair of defects in patients with ToF, these patients are still at significant long-term risk of heart failure, arrhythmias, exercise intolerance, and sudden death.[3] One study has shown that there is a close link between heart failure and myocardial fibrosis.[4] Inflammation is an important pathological factor leading to myocardial fibrosis. Inflammatory cytokines (TNF-α, AngII, IL-6, *etc.*) can be activated directly or indirectly through a variety of molecular signalling pathways allowing fibroblasts to increase the synthesis of extracellular matrix proteins such that tissue in the damaged area and the surrounding non-damaged area undergo different degrees of fibrosis.[5] Moreover, Takayoshi et al[6] have proposed that interleukin-6 can act as a post-independent factor in heart failure. Despite efforts to protect organs, CPB can still cause a systemic inflammatory response, which contributes to post-operative complications and myocardial failure.[7–9]

In this study, we aimed to explore the potential mechanism of the association between CPB and myocardial fibrosis at the genetic level. We analysed the differential gene expression in right atrium biopsies acquired from children affected by ToF undergoing primary surgical defect repair pre- and post-CPB. Based on a previous study,[6] we focused our attention on expression differences in IL-6. We hope that our findings will contribute to improving management strategies for cardiopulmonary bypass and identify new targets for existing drugs or predict new drugs for known targets. The workflow of our study is shown in Fig 1.

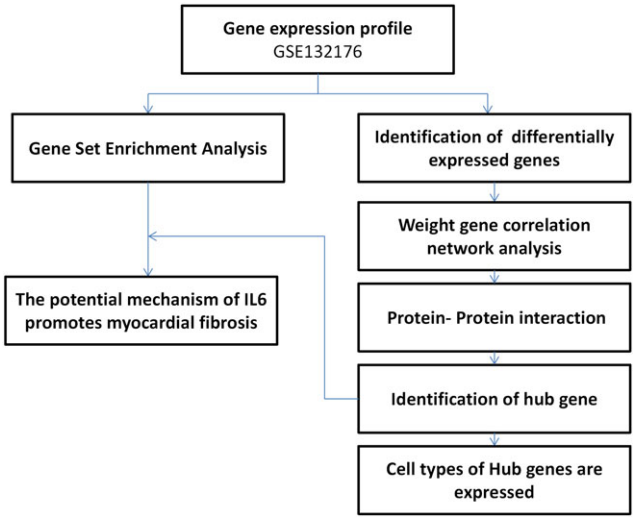

Figure 1. The workflow of the present study.

Material and methods

Data processing

We downloaded the expression profile datasets GSE132176 (Platform: GPL13158) from Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) database.[10] We closely analysed the GSE132176 data to identify any patients with ToF. The data we extracted included atrial tissue samples from 10 children pre- and post-CPB during TOF surgery. R (v3.6) package *limma*[11] was used to normalise the raw data.

Gene Set Enrichment Analysis

We set out to study the effect of CPB on biological function in patients with ToF by comparing gene expression levels pre- and post-CPB. A Gene Set Enrichment Analysis[12,13] was used to analyse the differences of the two groups using KEGG pathways. GSEA was performed using GSEA 3.0 (Java) software (http://www.broadinstitute.org/gsea/). The c2.cp.kegg.v6.2.symbols.gmt datasets from the Molecular Signatures Database[14] were used as reference gene sets. An enrichment analysis was considered statistically significant when meeting a nominal p-value cut-off (NOM p-value) of < 0.05.

Screening for differentially expressed genes

DEGs were identified using the *limma* package in R. A volcano map and a heatmap of DGEs were plotted using the *ggplot2*package and *pheatmap* package, respectively. DEGs with $p < 0.05$ and $|\log_2 FC| > 1$ were considered as being significantly different. Boxplots and paired plots were drawn using the *beeswarm* and *ggpubr* packages to show the different expression levels of IL-6 in the pre- and post-CPB groups.

Weighted gene correlation network analysis

A weighted correlation network analysis can be used to find clusters (modules) of highly correlated genes, which can be used to identify candidate biomarkers or therapeutic targets.[15] We use DEGs to perform WGCNA. The best power was automatically selected by the software, and then the co-expression networks were constructed using the WGCNA R package.

GO enrichment analysis and protein–protein interaction network

A GO enrichment analysis (learn what biological processes genes are involved in and how they function at the molecular level)[16] and the identification of the PPI network (a tool to understand cell functions)[17] were carried out for the module with the strongest correlation in WGCNA. The enrichment analysis was performed using *org. Hs.eg.db* and *enrichplot* packages in R, and the PPI was based on STRING (https://string-db.org/) with the species limited to "Homo sapiens".

HUB gene identification

The data obtained from STRING were then imported into Cytoscape3.70, an open software for visualising and analysing interaction networks.[18] The top 10 genes of Maximal Clique Centrality[19] were selected and visualised using the CytoHubba[19] plugin. The top six genes were identified as HUB genes ranked by MCC.

Cell types associated with the HUB genes

We identified the cell types associated with the HUB genes using PanglaoDB (https://panglaodb.se/index.html), which is a database for the scientific community interested in the exploration of single-cell RNA sequencing experiments from mouse and human.[20]

Results

Identification of DEGs

Through a differential gene expression analysis comparing pre- and post-CPB, we found there were 119 DEGs, of which 112 were up-regulated and 7 were down-regulated (Fig 2a). The top 50 with the most significant differences genes were visualised using a heatmap (Fig 2b). Particularly, we found that IL-6 was significantly up-regulated in the post-CPB group ($p = 4.871e{-}04$) (Fig 2c). A paired analysis showed that IL-6 expression was significantly up-regulated in all the post-CBP samples (Fig 2d).

WGCNA

Through a WGCNA, a total of five modules were identified (Fig 3a). The turquoise module included 116 genes and was positively correlated with the post-CPB group (correlation = 0.84, p = 4e-6; Fig 3b). Furthermore, a biological processes enrichment analysis showed that the turquoise module genes were significantly involved in response to lipopolysaccharide, molecules of bacterial origin, leukocyte cell–cell adhesion, epithelial cell proliferation, and regulation of epithelial cell proliferation (Fig 3c). An enrichment for molecular function showed that turquoise module genes were enriched in DNA-binding transcription activator activity, RNA polymerase II-specific, transcription corepressor activity, and DNA-binding transcription repressor activity, and RNA polymerase II-specific (Fig 3c).

PPI and the identification of HUB genes

We imported the genes in the turquoise module into STRING to build a network with 111 nodes and 556 edges (Fig 4a). The resulting network was then imported into Cytoscape in order to analyse and visualise the top 10 MMC genes (Fig 4b). Next, we selected the top six genes with the most significant MMC as HUB genes (*JUN*, *FOS*, *ATF3*, *EGR1*, *IL-6*, and *PTGS2*).

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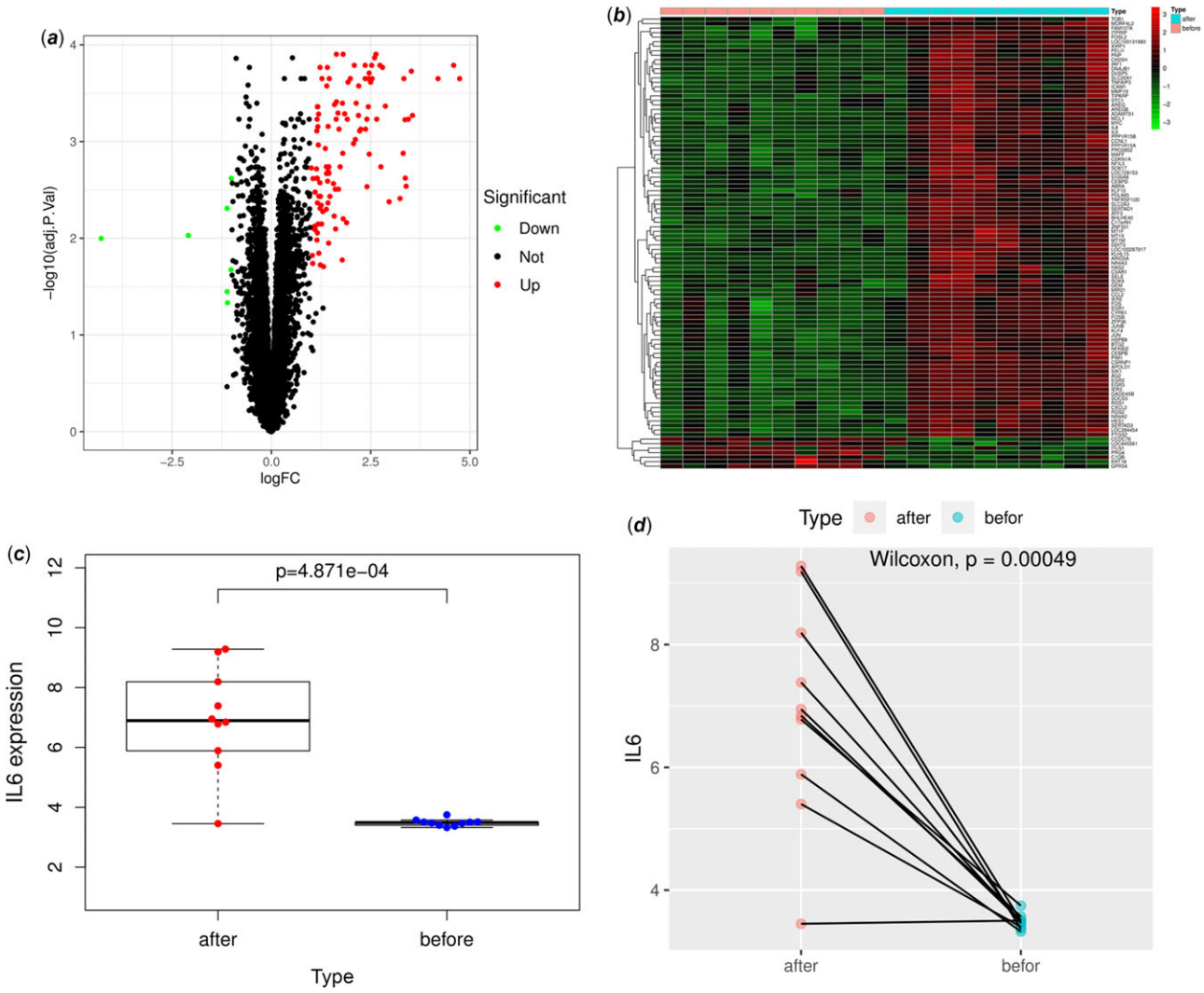

Figure 2. Differential expression gene analysis. (***a***) Volcano plot of the after-before, red dots represents up-regulated genes, green dots represents down-regulated genes, and black dots represents no significantly expressed genes. (***b***) A heatmap of differentially expressed genes. (***c***) IL-6 is up-regulated after CPB. (***d***) Paired analysis of IL-6.

Gene Set Enrichment Analysis

A GSEA showed that compared to the before CPB group, neuroactive ligand–receptor interactions, MAPK signalling pathway, Wnt signalling pathway, Jak-STAT signalling pathway, colorectal cancer, T-cell receptor signalling pathway, ErbB signalling pathway, and bladder cancer were enriched in the post-CPB group (Fig 5a). These pathways were also enriched in the IL-6-high group (Fig 5b). Furthermore, we found that up-regulation of IL-6 affects the myocardium during CPB mainly through activation of the Jak-STAT signalling pathway (Fig 5c and d).

Cell types associated with HUB genes

Data downloaded from PanglaoDB showed that IL-6, ATF3, EGR1, and PTGS2 are closely associated with fibroblasts (Fig 6).

Discussion

ToF is a serious CHD, and early post-operative complications have a long-term impact on the quality of life and survival of children after surgical treatment.[1,3] The CPB-induced inflammatory response is an important factor leading to post-operative complications in ToF.[7] IL-6 is a critical inflammatory cytokine that plays an important role in myocardial fibrosis.[5] At the same time, myocardial fibrosis is an important factor leading to heart failure.[4] Therefore, it is necessary to explore the molecular mechanism of CPB leading to myocardial fibrosis, focusing on IL-6. In our study, we first found that IL-6 was generally up-regulated in the post-CPB group (Fig 2d), suggesting that CPB directly causes high expression levels of IL-6. The WGCNA and PPI network analysis identified six genes that were identified as HUB genes (*JUN*, *FOS*, *ATF3*, *EGR1*, *IL-6*, and *PTGS2*). Amongst these, *FOS, ATGF3*, *EGR1*, and *IL-6* are known to be closely related to myocardial fibrosis.[3,21–23] *JUN* has been reported to play an important role in Inflammation,[24] and as the target of non-steroidal anti-inflammatory drugs, *PTGS2* plays a critical role in regulating inflammation and pain.[25–27] One study showed that inhibition of PTGS2 prolonged allograft survival and reduced inflammation and myocardial damage during acute cardiac allograft rejection in a rat model.[28] Thus, inflammation-related genes appear to be significantly up-regulated post-CPB during ToF surgery. It is known that inflammation is an important inducer of myocardial fibrosis.[29] IL-6 is generally regarded as an inflammatory biomarker, and is commonly used to assess the presence and severity of low-grade inflammation.[30]

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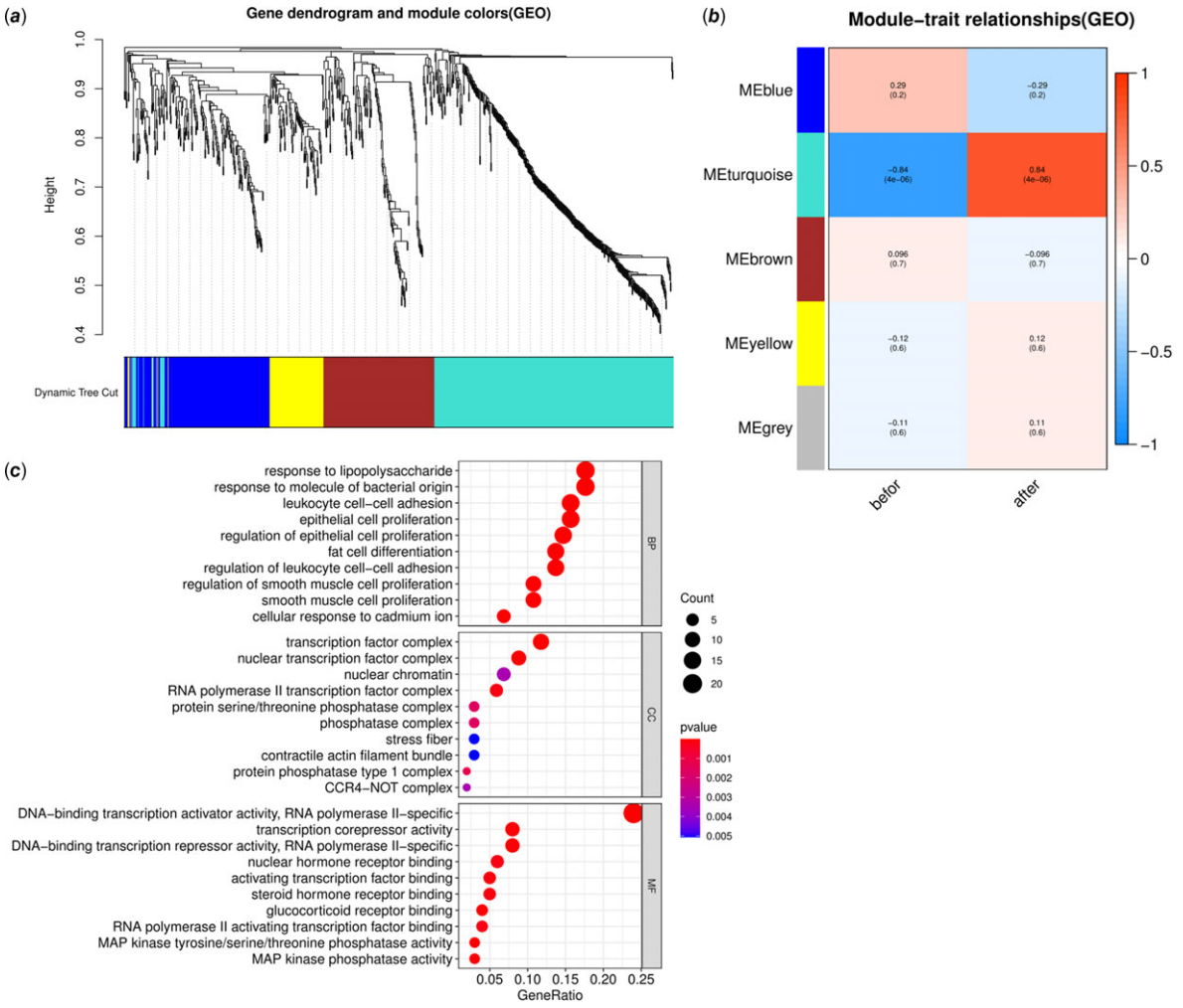

Figure 3. Weighted gene correlation network analysis. (***a***) Recognition module, each module was given an individual colour as identifiers, including five different modules. (***b***) Correlation heatmap of gene modules and phenotypes, the red is positively correlated with the phenotype, blue is negatively correlated with the phenotype. (***c***) GO enrichment analysis of weighted genes.

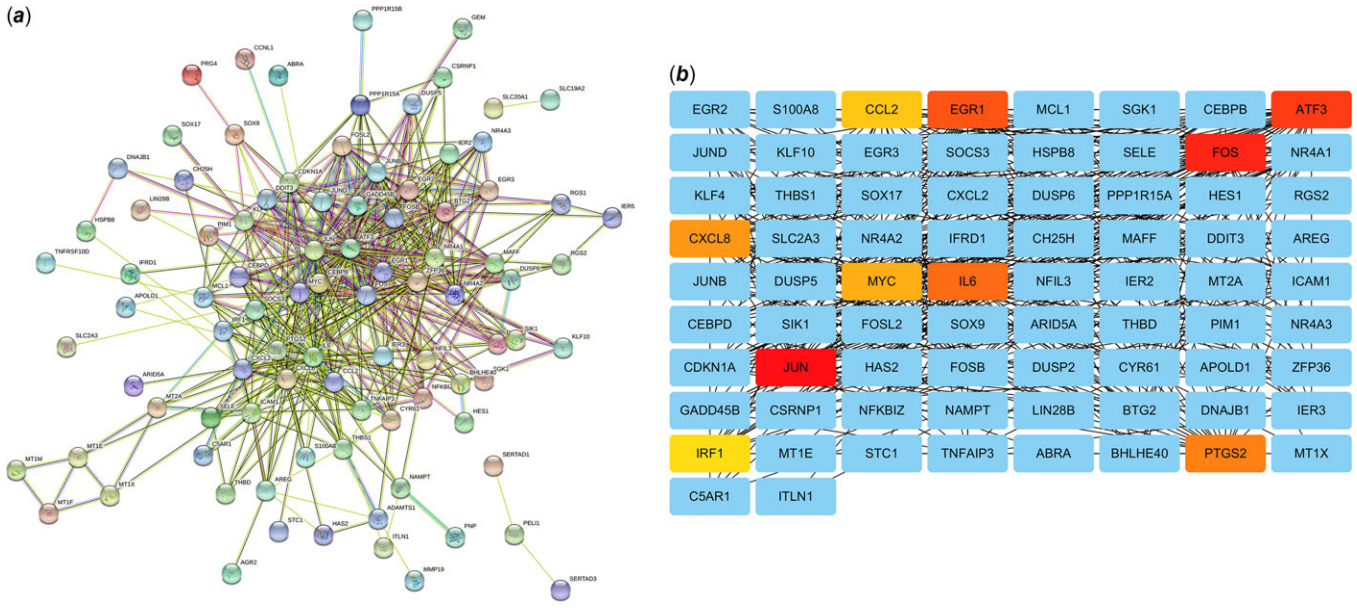

Figure 4. HUB gene identification (***a***) Protein–protein interaction network (***b***) The top 10 genes of Maximal Clique Centrality, the top 6 genes with the most significant MMC as HUB genes (JUN, FOS, ATF3, EGR1, IL-6, and PTGS2).

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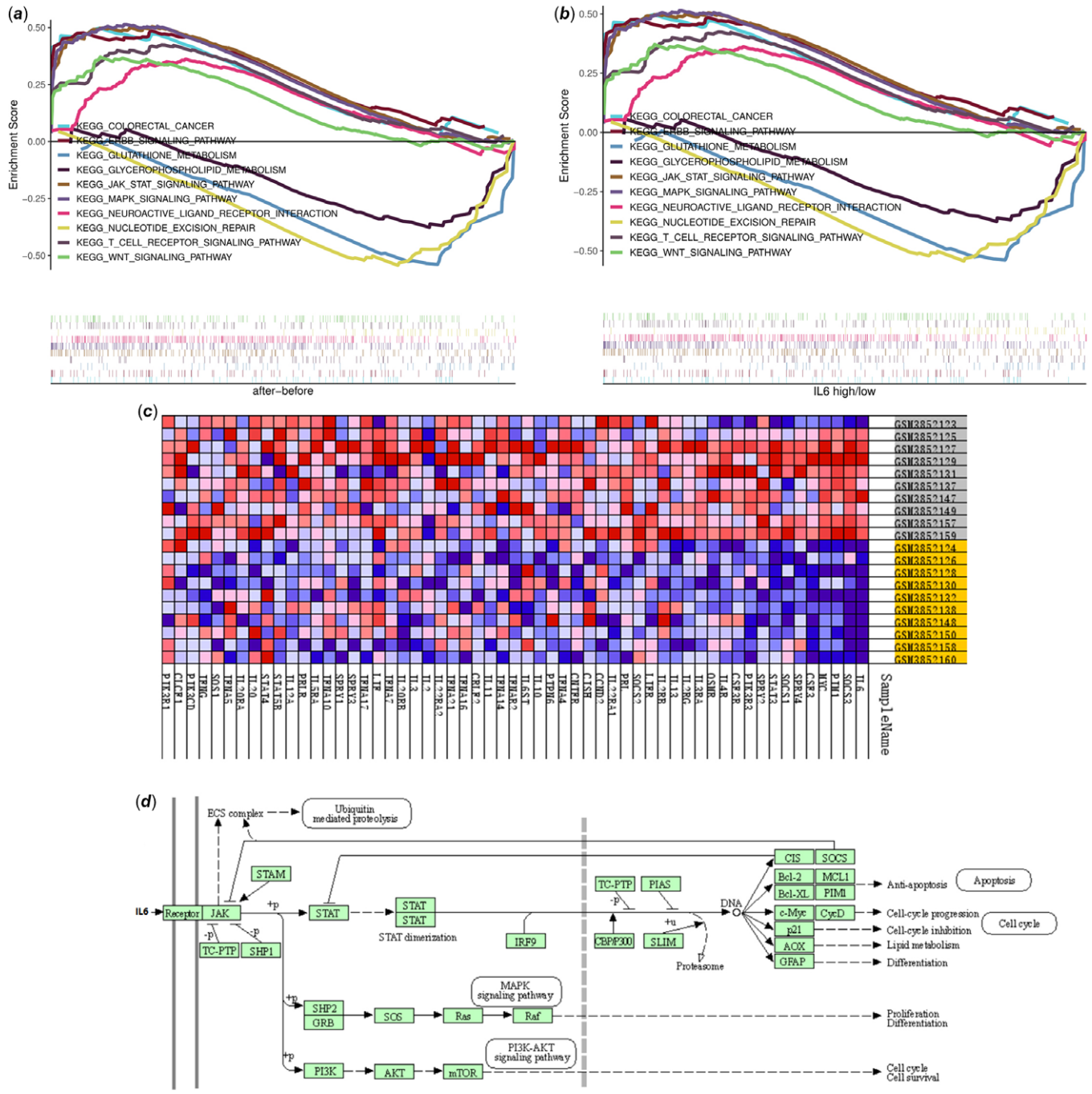

Figure 5. Result of Gene Set Enrichment Analysis. (***a***) KEGG pathways enriched in after CPB group. (***b***) KEGG pathways enriched in IL-6-high group. (***c***) Genes expression in JAK-STAT signalling pathway. (***d***) The potential mechanism of up-regulation of IL-6 affects the myocardium during CPB.

Here, we further explored biological processes associated with CPB. A biological process enrichment analysis shows that genes with a strong correlation with CPB are significantly involved in the response to lipopolysaccharide, molecules of bacterial origin, leukocyte cell–cell adhesion, epithelial cell proliferation, and regulation of epithelial cell proliferation. Furthermore, a molecular function enrichment analysis and GSEA analysis showed neuroactive ligand–receptor interaction and a number of immune-related pathways are enriched in the post-CPB group and the IL-6-high group. One study has shown that neuroactive ligand–receptor interaction is associated with human arrhythmogenic right ventricular cardiomyopathy.[31] Another study has suggested that the myocardial protective effect of sevoflurane is through neuroactive ligand–receptor interactions in patients undergoing coronary artery bypass graft surgery;[32] we, therefore, speculate that neuroactive ligand–receptor interactions are closely related to cardiac function.

It is well known that the MAPK signalling pathway and the T-cell receptor signalling pathway are associated with inflammatory responses.[33,34] In addition, activation of the Wnt signalling pathway leads to adverse remodelling after ischaemic injury, whereas inhibition of Wnt signalling improves cardiac function.[35] The ErbB signalling pathway is closely associated with heart failure, and it is a target

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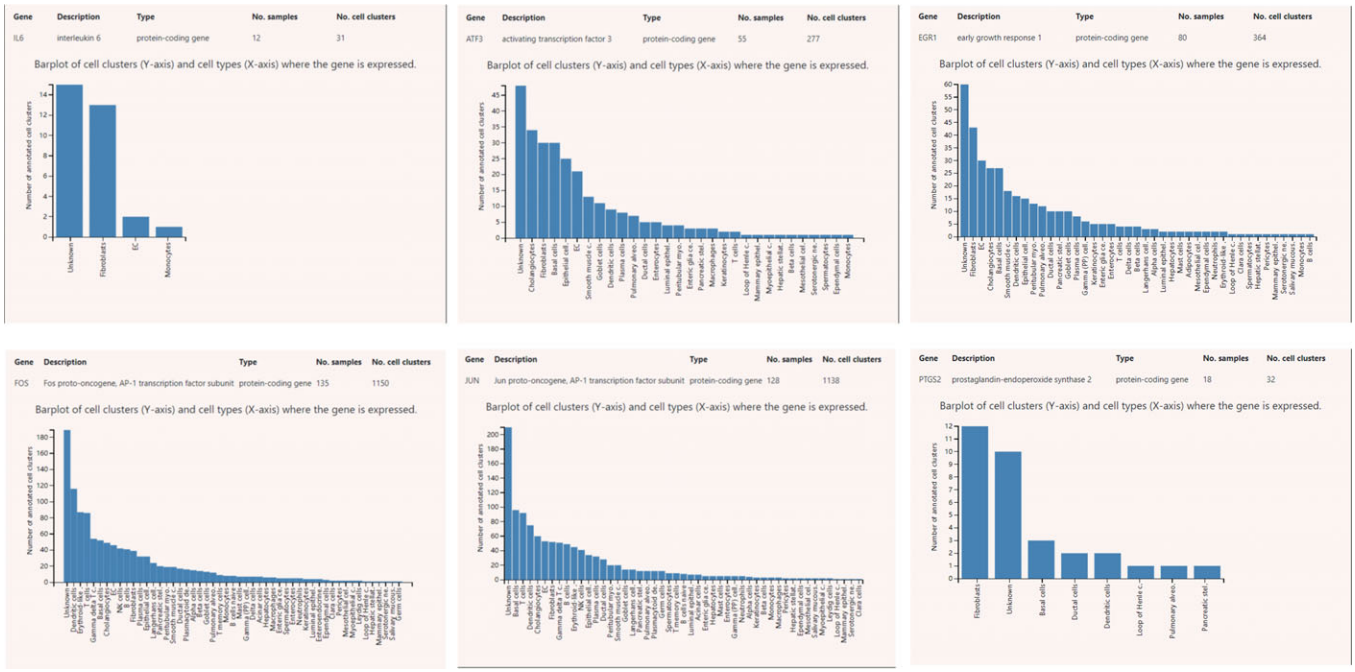

Figure 6. Cell types of HUB genes are expressed.

for drugs to treat heart failure.[36] The JAK/STAT signalling pathway is an important cytokine signal transduction pathway, which regulates diverse physiological and pathological processes including cellular inflammation, differentiation, proliferation, apoptosis, and immunity.[37] Activation of the JAK/STAT signalling pathway as a result of the up-regulation of IL-6 results in pro-inflammatory responses that promote myocardial fibrosis (Fig 5c and d).

From the PanglaoDB analysis, we found that IL-6, ATF3, EGR1, and PTGS2 are closely related to fibroblasts (Fig 6), which further supports our findings. These data suggest that using drugs during CPB procedures that can prevent a pro-inflammatory response may improve prognosis. In this regard, aprotinin and ulinastatin are common anti-inflammatory drugs that are used in CPB, procedures. Propofol, sevoflurane, and dexmedetomidine have also been found to have anti-inflammatory and myocardial protective effects during CPB.[32,38–41]

Although we have uncovered several interesting results in this study, it does have significant limitations. First, the sample size is small and so this may affect the validity of the findings, and second, the proposed molecular mechanisms are theoretical and so require further experimental verification.

Conclusions

In the present study, we identified several HUB genes (*JUN, FOS, ATF3, EGR1, IL-6,* and *PTGS2*) that are associated with myocardial inflammation and fibrosis caused by CPB during ToF surgery in children. High expression of IL-6 would be expected to activate JAK-STATA signalling pathway during CPB, and therefore may be a potential mechanism underlying myocardial fibrosis. It can be speculated that the high levels of IL-6 are an important factor leading to heart failure after ToF surgery. Our study provides a theoretical basis for the formulation of cardiac protective strategies for CPB and to provide a direction for the future development of drugs to address CPB-mediated fibrosis.

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Availability of data and materials. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Conflicts of interest. None.

Ethical standards. The research does not involve ethics, and ethics approval was not required.

Authors' contributions. H.L. analysed the data. Z.W. designed the study and revised the manuscript. Y.Z. prepared figures. L.Z. authored or reviewed drafts of the paper, and approved the final draft. All authors read and approved the final manuscript.

Patient consent for publication. Not applicable.

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S1047951121001803.

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