Altered metabolic and inflammatory transcriptomics after cardiac surgery in neonates with congenital heart disease

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The study examines the whole blood transcriptome profile before and after cardiopulmonary bypass (CPB) in neonates with hypoplastic left heart syndrome (HLHS), a severe form of congenital heart disease, that can develop low cardiac output syndrome (LCOS). Whole blood mRNA transcriptome profiles of 13 neonates with HLHS before and after their first palliative surgery were analyzed to determine differentially expressed genes and pathways. The median age and weight at surgery were 4 days and 3.2 kg, respectively. Of the 13 patients, 8 developed LCOS. There was no significant difference between CPB, aortic cross clamp, deep hypothermic cardiac arrest times between patients that develop LCOS and those that do not. Upon comparing differential gene expression profiles between patients that develop LCOS and those that do not in pre-operative samples, 1 gene was up-regulated and 13 were down regulated. In the post-operative samples, 4 genes were up-regulated, and 4 genes were down regulated when patients that develop LCOS were compared to those that do not. When comparing post-operative samples to pre-operative samples in the patients that do not develop LCOS, 1484 genes were up-regulated, and 1388 genes were down regulated; while patients that developed LCOS had 2423 up-regulated genes, and 2414 down regulated genes for the same pre to post-operative comparison. Pathway analysis revealed differential regulation of inflammatory pathways (IL signaling, PDGF, NOTCH1, NGF, GPCR) and metabolic pathways (heme metabolism, oxidative phosphorylation, protein metabolism including amino acid and derivatives, fatty acid metabolism, TCA cycle and respiratory electron transport chain). By identifying altered transcriptome profiles related to inflammation and metabolism in neonates with HLHS who develop LCOS after CPB, this study opens for exploration novel pathways and potential therapeutic targets to improve outcomes in this high-risk population.

Congenital heart defects are the most common birth defects, affecting nearly 40,000 infants each year in the United States of America1–3. The majority of forms of congenital heart disease (CHD) presenting during the neonatal period require surgery on cardiopulmonary bypass (CPB) in early infancy and multiple subsequent surgeries. Of these, hypoplastic left heart syndrome (HLHS) remains the most critical form of congenital heart disease, requiring as a first stage of surgical palliation the Norwood operation or its modifications, typically performed during the early neonatal period. The Norwood operation is one of the highest risk operations for CHD, with mortality rate as high as 19%4.

Cardiopulmonary bypass (CPB) activates an inflammatory response that closely resembles the systemic inflammatory response syndrome (SIRS), which is characterized by alterations in cardiovascular and pulmonary function, fever, fluid retention, coagulopathy, and multisystem organ dysfunction/failure (MODS)5,6. The degree of morbidity attributed to the post-CPB inflammatory response is correlated to its severity7. In particular, low cardiac output syndrome (LCOS) has been associated with this inflammatory response which contributes to post-operative morbidity and mortality8,9.

The cellular and molecular mechanisms regulating the systemic inflammatory response to CPB are not completely understood, but are thought to include complement activation, cytokine release, and endothelial cell

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activation, leading to myocardial and vascular injury. Differences in the host's genotype may determine their individual pro or anti-inflammatory response profile, and thus the magnitude of their systemic inflammatory response to CPB. Whole blood transcriptomic studies have accounted for the heterogeneous inflammatory response seen after CPB in adults. However, there are no studies exploring whole blood transcriptomic profile in neonates with critical CHD, and in particular HLHS. In this study we hypothesized that children with HLHS will have unique transcriptome profiles before and after their Norwood operation, and that they would correlate with the development of a post-operative LCOS.

Methods
Patient enrollment. This was a single-institution prospective observational study performed at the Texas Children's Hospital Heart Center. The study was approved by the Institutional Review Board (IRB) of Baylor College of Medicine. All experiments were performed according to the guidelines approved by IRB. Since the subjects were minor, an informed consent was obtained from legal guardian/parent of each enrolled patients. We enrolled 13 neonates diagnosed with HLHS, between July 2016 and August 2017, who were scheduled for a Norwood procedure. Exclusion criteria involved patients with weight < 3 kg at the time of surgery, a known prenatal chromosomal disorder, or gestational age < 34 weeks.

Anesthetic and surgical technique. Perioperative procedures and techniques (including anesthesia and surgery) were standardized per institution protocol and have been reported previously by Andropoulos et al. Briefly, the patient was anesthetized using isoflurane, fentanyl and midazolam. All patients received one dose of IV methylprednisolone (30 mg/kg/dose) on induction of anesthesia. None of the patients received additional blood transfusion before obtaining second sample after surgery.

Bypass flow rates of 150 cc/kg/min were utilized, and pH-stat blood gas management was used for all cases. For aortic arch reconstruction, regional cerebral perfusion (RCP) was utilized at 18 °C, along with deep hypothermic cardiac arrest for brief periods, and never for more than 20 min. Hematocrit was maintained at 30–35% during cooling and hypothermic periods and increased to 40–45% during rewarming. Regional oxygen saturations of the brain (rSO2) were monitored throughout the perioperative period with a protocol that attempted to maintain rSO2 > 50% before and after bypass and > 90% while on bypass (INVOS 5100B; Somanetics, Inc., Troy, MI, USA).

Blood samples. Initial blood samples were collected from an existing arterial or venous catheter in all patients before induction of anesthesia and then again, 24 h post-operatively.

RNA purification. Blood samples were collected in PAXgene Blood RNA Tubes (Qiagen, Germantown, CA, USA). RNA was extracted using Qiagen PAXgene Blood miRNA kit (Qiagen, Germantown, CA, USA) using automated protocol.

RNA sequencing protocol. Total RNA quality assessment was done using BioRad Experion Automated Electrophoresis Station (Biorad, Hercules, CA, USA) and quantified using Qubit 3.0 fluorometer (Qubit RNA BR Assay Kit) (Life Technologies Corporation, Carlsbad, CA, USA). Sample libraries were prepared using the Illumina TruSeq Stranded mRNA Sample Preparation Kit (Cat # 20020594) and TruSeq RNA Single Index Set A Index (Cat # 20020492) (Illumina Inc., San Diego, CA, USA). Two rounds of purification were performed and during the second elution the RNA was fragmented and primed for cDNA synthesis. DNA was subjected to end repair, A-tailing and adapter ligation. Post ligation cleanup was performed using AMPure XP beads (Agencourt, Beverly, MA, USA). DNA fragments were amplified using PCR and purified using AMPure XP beads (Agencourt, Beverly, MA, USA). The quality of the amplified libraries was checked on an Agilent 4200 Tape Station System (Agilent Technologies, Santa Clara, CA, USA) using the D1000 Screen Tape Assay. The libraries were standardized to 2 nM, equal volumes of these libraries were pooled, and then the pooled library was sequenced using 100 bp paired-end reads on the Illumina HiSeq 2000 platform (Illumina Inc., San Diego, CA, USA). Samples were demultiplexed using Casava (v1.8, Illumina).

RNA-seq alignment and quantification. Fastq data files were trimmed using Trim Galore (version 0.5.0) and the trimmed sequences were aligned against the human genome (GRCH38) using HISAT2 before sorting reads using samtools. Finally, reads were quantified using featureCounts (version 1.6.4) against the GENCODE gene reference.

Differential expression analysis. The counts matrix generated using featureCounts was processed in the R statistical software environment to determine differentially expressed genes for the reported comparisons. The R package RUVseq was used to remove unwanted variation using the RUVr method. DESEQ2 was then used to determine differential gene expression using the likelihood ratio test. Significance was achieved for fdr-adjusted p-value < 0.05 and fold change exceeding 1.5×.

Principal component analysis and data visualization. Data was visualized using GraphPad Prism version 8 unless indicated differently. PCA, heatmaps and volcano plots were generated using the R statistical software environment. Circos plots were generated using the Circos software (version 0.69) and Circos Table Viewer software (version 0.63).
Gene set enrichment analysis (GSEA). All the genes detected for a comparison were ranked according to their log2 fold change in expression regardless of statistical significance. GSEA was performed using the Java-based GSEA software (version 3.0) on this ranked gene lists against the entire MSigDB database (version 6.1), with 1000 permutations; significance was achieved for a q-value less than or equal to 0.25, per the GSEA developers recommended practices.

Quantitative real time PCR analysis (qPCR). Quantitative PCR was performed using the QuantStudio 7 Flex real-time PCR detection system (Thermo Fisher) and SYBR Green (#1725274, Bio-Rad). The thermal cycling conditions used were as follows: one cycle at 95 °C for 1 min, 40 cycles at 95 °C for 15 s, and 60 °C for 15 s. The primers used in real-time PCR test were listed as follows: GRB2 forward primer AAT TAT GTC ACC CCGTGAAG, GRB2 reverse primer TGT TCT GAC TCTCCCTCACAG; LYN forward primer ACC AAG GTG ACT CCC CCT TCC TGA ATT TG, LYN reverse primer CTG GTG ACC ACA GCG TAG AG; PRKACB forward primer CAT GGC CAC TCT CGT CTG AAA AC, PRKACB reverse primer AGA AGT GCT GGA GAG GGA CA, IL2RB forward primer CGG GAG GTG GAA GAA GT; CSF2RA forward primer GGT TTG CCT AGT GCT TTT GC, CSF2RA reverse primer AGC CTC ACT GTT GGG GTA TG; COX8A forward primer AGC TTG GGA TCA TGG AAT TG, COX8A reverse primer CGG GAA CAT GG, ALAS2 reverse primer GAG GCA CAC AAC AAA GCA GA; FOXO3 forward primer GGC AGC ACT CA, FOXO3 reverse primer AAG CCA CCT GAA ATC ACA CC; β-ACTIN forward primer CAT CGA GCA CGG CAT CGT CA, β-ACTIN reverse primer TAG CAC AGC CTG GAT AGC AAC. Relative mRNA levels were calculated using the \(2^{-\Delta\Delta CT}\) method and normalized by β-actin in the same sample.

Whole blood deconvolution. The gene expression read counts profiles were filtered to remove poorly expressed genes, then upper quartile normalized was applied, and finally the counts per million (CPM) were determined for each gene using the R package EdgeR. The relative cell abundance of 22 different immune cells was determined based on the CPM transcriptomic profile for all the samples using the LM22 signature and CIBERSORTx method.

Clinical data analysis. Our primary clinical outcome data was development of low cardiac output syndrome (LCOS) as defined by at least one of the following during the first 48 h period: VIS > 15 at any time, or with pre-operative or post-operative status, or with the LCOS outcome. There is an emergent pattern showing that LCOS status can be attributed to the variation observed in the samples after surgery between patients that develop LCOS and those that do not (post-operative LCOS vs. No-LCOS). There is also a pattern present in

Results

Patient characteristics (Table 1). The median age of patients at surgery was 4 days (IQR 2–6 days) with median gestational age of 39 weeks and median weight at surgery of 3.2 kg. The patients were all Caucasian, predominantly male and of non-Hispanic ethnicity. We stratified the patients by the primary clinical outcome— one group that developed LCOS and the other that did not develop LCOS (No-LCOS). Of the total population, eight patients developed LCOS. Patients that developed LCOS were more likely to have a lower birth weight (\(p<0.05\)) (Table 1). There was no association between the development of LCOS and gestational age, age at time of surgery, CPB time, aortic cross clamp time, deep hypothermic cardiac arrest, or length of stay in the hospital and intensive care unit.

The median mechanical ventilation duration was 3.81 days, median ICU and hospital length of stay was 30 and 55 days respectively. There were 2 hospital deaths. There was no difference between the groups with respect to rate of post-operative complications (Table 2).

Differential gene expression. Principal component analysis was performed to reduce the dimensionality of the data and determine if the predominant gene expression variation in the samples could be associated with pre-operative or post-operative status, or with the LCOS outcome. There is an emergent pattern showing that LCOS status can be attributed to the variation observed in the samples after surgery between patients that develop LCOS and those that do not (post-operative LCOS vs. No-LCOS). There is also a pattern present in
the patients that develop LCOS when samples before and after surgery are compared (LCOS post-operative vs. pre-operative). However, within the first three principal components there is not a clear delineation between pre-operative and post-operative patients that do not develop LCOS (Fig. 1A).

We considered four different comparisons within the patient cohort. We first examined differential gene expression pre-operatively comparing the LCOS (patients that will develop LCOS) and No-LCOS (patients that will not develop LCOS) groups; then we examined differential gene expression post-operatively comparing LCOS and No-LCOS patient groups. Next, we assessed the transcriptomic impact of the surgical procedure. For the LCOS group we compared post-operative gene expression to pre-operative gene expression, and we did a similar before and after comparison for the No-LCOS group.

There were few genes that were statistically significant when pre-operative patients that do not develop LCOS were compared to patients that develop LCOS. A similar trend was observed in post-operative comparison between LCOS and No-LCOS groups (Table 1). In the pre-operative samples (LCOS vs. No-LCOS), 1 gene was up-regulated and 13 were down regulated ($q$-value < 0.05). In the post-operative samples (LCOS vs No-LCOS), 4 genes were up-regulated, and 4 genes were down regulated ($q$-value < 0.05). The surgical intervention had a greater effect on gene expression (Supplemental Table 2). In the No-LCOS group, 1484 genes were up-regulated, and 1388 genes were down regulated ($q$-value < 0.05), while patients that developed LCOS had 2423 up-regulated genes, and 2414 down regulated genes ($q$-value < 0.05) after surgery (Fig. 1B).

Signatures for each comparison were generated by filtering the results for statistical significance ($q$-value < 0.05) and a linear fold change of at least 1.5×. Volcano plots and a Circos plot for these signatures illustrate that patients that develop LCOS have an overall greater fold change in gene expression pre-operative versus post-operative (Figs. 1C and 2); concordantly, the LCOS group shows stronger significance of the gene differences pre-operatively and post-operatively and a larger number of significantly changed genes. In addition, these signatures show no preferential distribution across the human genome (Fig. 2). The deconvolution of the whole blood patient transcriptome and quantification of the relative abundances for 22 different immune cell types was performed to determine if the differences in gene expression could be attributed to differences in the proportion of immune cell types. No significant differences were detected (Supplemental Fig. 1).

### Table 1. Demographic and clinical patient characteristics. LCOS Low cardiac output syndrome, CPB Cardiopulmonary bypass, ACP Antegrade cerebral perfusion, LOS Length of stay.

| Variable                      | No LCOS (n = 5) Median (IQR) | LCOS (n = 8) Median (IQR) | p Value |
|-------------------------------|-----------------------------|---------------------------|---------|
| Male                          | 4                           | 8                         | 0.385   |
| Race–Caucasian                | 5                           | 8                         | 1.00    |
| Gestational age (weeks)       | 34                          | 0                         | 1.00    |
|                              | 35                          | 0                         | 1.00    |
|                              | 36                          | 1                         | 0.804   |
|                              | 39                          | 4                         | 0.667   |
| Birth weight (kg)             | 3.5 (3.4–3.7)               | 3 (2.8–3.2)               | 0.028   |
| CPB (min)                     | 184 (178–210)               | 211 (163–230)             | 0.883   |
| Aortic cross clamp (min)      | 96 (93–124)                 | 99.5 (82.5–117.5)         | 0.341   |
| Circulatory arrest (min)      | 8 (7–11)                    | 8 (7–14.5)                | 0.765   |
| ACP (min)                     | 85 (77–86)                  | 76 (61.5–93.5)            | 0.77    |
| Hospital LOS (days)           | 47 (46–140)                 | 56 (42–132)               | 0.77    |
| CICU LOS (days)               | 29.6 (24.8–38.7)            | 32.8 (19.7–56.1)          | 1.00    |
| Mechanical ventilation duration (days) | 3 (1.8–4.3) | 4 (2.8–10.9) | 0.223   |

### Table 2. Post-operative complications. CPR Cardiopulmonary resuscitation, ECMO Extra corporeal membrane oxygenation, OR Operating room.

| Variable                      | No LCOS (n = 5) | LCOS (n = 8) | p Value |
|-------------------------------|----------------|-------------|---------|
| Arrhythmia requiring therapy (n) | 2             | 2           | 1.00    |
| Post-op ECMO (n)              | 0             | 1           | 1.00    |
| Sepsis (n)                    | 0             | 1           | 1.00    |
| CPR prior to surgery (n)      | 0             | 0           | 1.00    |
| Pre-op infection (n)          | 0             | 0           | 1.00    |
| Left OR with open chest (n)   | 0             | 2           | 0.487   |
Pathway analysis. Gene Set Enrichment Analysis (GSEA) was performed on each of the four different comparisons in order to gain insight into the pathways and mechanisms which may regulate LCOS. When comparing pre-operative samples between patients who developed LCOS versus patients who did not (No-LCOS), there was a down regulation of genes controlling inflammatory pathways (IL signaling, PDGF, NOTCH1, NGF, GPCR) and an up-regulation of genes controlling metabolic pathways (heme metabolism, oxidative phosphorylation, protein metabolism including amino acid and derivatives, fatty acid metabolism, TCA cycle and respiratory electron transport chain). This relationship is reversed in post-operative samples comparing gene profiles in patients who develop LCOS vs those who do not. Specifically, there was an up-regulation in genes controlling inflammatory pathways and a down-regulation of genes controlling metabolic pathways (Fig. 3, Supplemental Table 3).

In the GSEA results for the No-LCOS group, pre-operative and post-operative comparison revealed no changes in inflammatory gene regulation, whereas up-regulation of genes controlling metabolic pathways (TCA cycle, metabolism of proteins, mitochondrial protein import) was observed. On the contrary, comparison of pre-operative and post-operative samples for the patients who developed LCOS revealed an up-regulation of...
genes controlling inflammatory pathways and a downregulation of genes controlling metabolic pathways (Fig. 3, Supplemental Table 3).

Real time PCR validation of data.  We selected 20 genes for qPCR validation of the GSEA analysis (Supplemental Table 4). These genes were determined to be statistically significant in our differential gene analysis and they are members of the enriched inflammation and metabolic pathways as reported above. A detailed link between validation genes and differentially enriched pathways is indicated via a circos plot in Fig. 4. We emphasized two gene groups based on their corresponding pathways: inflammatory genes and metabolic genes. When comparing the pre-operative samples between the LCOS and No-LCOS groups, there was an up-regulation of GYPA (1.76 fold), CYC1 (1.52 fold), ALAS2 (1.8 fold) genes representing the metabolic pathway in patients with LCOS. On analysis of the post-operative samples between the LCOS and No-LCOS groups, this relationship was reversed with downregulation of metabolic genes such as CYC1 (0.76 fold) and up-regulation of inflammatory pathway associated genes like PIK3CB (1.3 fold). (Fig. 5).

When comparing pre-operative with post-operative samples in the No-LCOS group, there was up-regulation of metabolic pathway associated genes such as UQRC1 (1.75 fold), COX8A (1.21 fold), and CYC1 (1.21 fold). In contrast, comparison of post-operative samples over pre-operative samples in patients who developed LCOS revealed down regulation of metabolic pathway associated genes like UQRC1 (0.63 fold), COX8A (0.49 fold), CYC1 (0.22 fold). Furthermore, in patients that developed LCOS there was an increase in STAT5B (2.11 fold) an inflammatory signaling associate gene (Fig. 5).

**Discussion**

To the best of our knowledge, this is the first study that explores the whole blood transcriptome profile in neonates with HLHS undergoing the Norwood procedure. Prior studies exploring gene profile in patients with HLHS have used atrial myocardium and do not capture the evolving and dynamic clinical condition of this high risk group. Whole blood transcriptomic data is potentially more relevant and useful for understanding genetic regulation of evolving clinical condition and finding likely therapeutic targets. Our data suggests that neonates with HLHS who develop post-operative LCOS have differentially regulated genes compared to those neonates who did not develop LCOS. We observed that some genes that were differentially regulated controlled the inflammatory pathway, consistent with known pathophysiological changes contributing to development of LCOS. We observed that genes controlling metabolic pathways were also differentially regulated in patients who developed LCOS compared to those without the LCOS.

A CPB-induced inflammatory response is known to be associated with post-operative morbidity, and in particular post-operative interleukins levels have served as valuable prognostic biomarkers. Transcriptome profiles in adults after CPB have revealed increased expression of genes controlling inflammatory and complement pathways. In our study we found increased expression of STAT5B (> twofold change), which mediates signal transduction triggered by various cell ligands, such as IL2, IL4, CSF1 and different growth hormones. Similarly LYN and PTPN6 which control the interleukin signaling was found to be increased in patients who developed LCOS. In patients who did not develop LCOS, there was down regulation of interleukin cascades (IL7R, IL5RA, IL2RB, PRKACB) and inflammatory cascade which could explain the attenuated SIRS response.
Corticosteroids have been used to attenuate the inflammatory response after cardiopulmonary bypass with mixed results in both adults and pediatric patients. In a recently published randomized controlled trial using corticosteroids in neonates undergoing CPB, steroids were shown to improve outcomes in neonates undergoing palliative surgery but not corrective surgery. Differential gene expression after cardiopulmonary bypass can explain individual variation in response to corticosteroids.

In our cohort we found differential regulation of metabolic pathways both at pre-operative and post-operative phases. There was dysregulation in protein metabolism including amino acid and its derivatives, fatty acid metabolism and respiratory electron transport chain. We hypothesize the altered metabolic response is the result of low cardiac output state leading to hypoperfusion compounded with anaerobic metabolism that our patient population experiences due to baseline low blood oxygen saturation. Metabolomic studies in neonates suffering from hypoxic ischemic insult have demonstrated distinct alteration in protein and fatty acid metabolism. Chu et al. found that neonates with poor outcomes after hypoxic insult have altered metabolic profile related to oxidative stress pathways and tissue damage including increased concentration of glutarate, methylmalonate, 3-hydroxy-butyrate and orotate compared to neonates with good outcome. Similarly Walsh et al. has found

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**Figure 3.** HALLMARK an REACTOME enriched pathways: distribution of Normalized Enrichment Scores (NES) for various pathophysiological pathways. From outside of circle to the inside: outermost circle compares NES scores in Pre-Operative samples of patients who developed LCOS versus No-LCOS, the second circle compares NES scores in Post-Operative samples of patients who developed LCOS vs patients who did not, the third circle compares NES scores between Pre and Post-Operative samples of patients who did not develop LCOS, the innermost circle compares NES scores between Post-Operative and Pre-Operative samples of patients who developed LCOS. (GSEAsoftware version 3.0. https://software.broadinstitute.org/cancer/software/gsea/wiki/index.php/GSEA_v3.0_Release_Notes).
altered metabolic profiles involving amino acids, acylcarnitines and glycerophospholipids in neonates with poor outcomes after hypoxemic ischemic insult\(^3\). These metabolic profile serve as potential diagnostic and prognostic biomarkers for neonates with hypoxemic ischemic encephalopathy.

Metabolic profiling of children undergoing cardiac surgery has identified novel pathways that predict post-operative morbidity and mortality\(^4\). These studies noted shift in metabolic fingerprinting related to protein metabolism from baseline to immediately after cardiac surgery. When comparing non-survivors to survivors, Davidson et al. found differences in nicotinamide and aspartate metabolism to be associated with poor outcomes. Interestingly, tight glycemic control did not alter the metabolic and inflammatory profile in these patients\(^4\).

Our study found similarly distinct metabolic fingerprinting involving protein and fatty acid metabolism. Patients who developed LCOS showed statistically significant down-regulated pathways involving TCA cycle, amino acid metabolism and mitochondrial pathways. Fatty acid metabolism under aerobic condition utilizes acylcarnitine pathways, wherein they enter the electron transport chain to produce ATP. In hypoxemic conditions, fatty acid metabolism is altered energy production and accumulation of metabolites which can be directly toxic\(^3\). In our study there was down-regulation of CYC1 which controlled both protein metabolism and mitochondrial protein import. Down-regulation of UQ_CRC1, which is related to respiratory electron transport chain and ATP synthesis, along COX & which is involved in electron transport chain play an important role in oxidative phosphorylation\(^5\). Altered myocardial function, which is primarily dependent on fatty acid metabolism, play an important role in contributing to normal cardiac output\(^6\). Down regulation of these genes can potentially lead to altered energy kinetics which could potentially contribute to LCOS state. These genes can potentially serve as diagnostic and prognostic biomarkers as well as therapeutic targets in children with congenital heart disease.

**Limitations.** The sample size for this study is small, hence we cannot confidently generalize the data to other neonatal populations. Additionally, our cohort consistent of predominantly male patients. The effect of sex on transcriptome profile in congenital heart disease is not known. However, we developed this study as feasibility experiment. The data show that transcriptome analysis is consistent with published literature related to altered inflammatory homeostasis in children with CHD undergoing surgery with CPB. Future studies including larger numbers of patients would be needed to further validate the results of this study. The metabolic transcriptome dysregulation data was not completely validated with proteomic analysis. However, this will serve as target for
Figure 5. (A) Detailed qPCR analysis of validation genes as identified by HALLMARK and REACTOME. (B) Summary of the results from the qPCR validation indicating statistical significance (p-value < 0.05) and direction (either up or down) for the given comparison. Red indicates genes that were significantly upregulated, blue indicates genes that were significantly downregulated and white indicates genes that were unchanged.

our future studies exploring metabolic alteration in patients with congenital heart disease, specifically related to protein and fatty acid metabolism along with altered mitochondrial function. Finally, a potential source of the increased variability in transcriptomic response of the LCOS samples induced by surgery could be epigenomic differences between LCOS and No-LCOS groups, which is outside the scope of our current study.
Conclusions
Patient with HLHS undergoing Norwood procedure and developing LCOS have altered transcriptome profile related to inflammatory and metabolic pathway when compared to those who did not develop LCOS. Future studies involving transcriptomic, metabolomic and proteomic data can identify novel pathways and potential therapeutic targets to improve outcomes in this high-risk population.

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References
1. Keller, M. D., Strickland, M. J., Riehle-Colarusso, T., Mahle, W. T. & Correa, A. Prevalence of congenital heart defects in metropolitan Atlanta, 1998–2005. J. Pediatr. 153(6), 807–813. https://doi.org/10.1016/j.peds.2008.05.059 (2008).
2. Hoffman, J. I. E. & Kaplan, S. The incidence of congenital heart disease. J. Am. Coll. Cardiol. 39(12), 1890–1900. https://doi.org/10.1016/S0735-1097(02)01886-7 (2002).
3. Moller, J. H., Taubert, K. A., Allen, H. D., Clark, E. B. & Lauer, R. M. Cardiovascular health and disease in children: current status. A special writing group from the task force on children and youth, American Heart Association. Circulation 89(2), 923–930. https://doi.org/10.1161/01.CIR.89.2.923 (1994).
4. Ohye, R. G. et al. Comparison of shunt types in the Norwood procedure for single-ventricle lesions. N. Engl. J. Med. https://doi.org/10.1056/NEJMoa0912461 (2010).
5. Westaby, S. Complement and the damaging effects of cardiopulmonary bypass. Thorac. https://doi.org/10.1136/thx.38.5.321 (1983).
6. Bronicki, R. A. & Hall, M. Cardiopulmonary bypass-induced inflammatory response: Pathophysiology and treatment. Pediatr. Crit. Care Med. https://doi.org/10.1097/PCC.0000000000000759 (2016).
7. Holmes, J. H. IV. The incidence of congenital heart disease. J. Pediatr. 153(6), 807–813. https://doi.org/10.1016/j.peds.2008.05.059 (2008).
8. Wernovsky, G. et al. Postoperative course and hemodynamic profile after the arterial switch operation in neonates and infants: a comparison of low-flow cardiopulmonary bypass and circulatory arrest. Circulation https://doi.org/10.1161/01.CIR.92.8.2226 (1995).
9. Hoffman, T. M. et al. Efficacy and safety of milrinone in preventing low cardiac output syndrome in infants and children after corrective surgery for congenital heart disease. Circulation 107(7), 996–1002. https://doi.org/10.1161/01.CIR.0000051365.81920.28 (2003).
10. Boyle, E. M., Pohlman, T. H., Johnson, M. C. & Verrier, E. D. Endothelial cell injury in cardiovascular surgery: the systemic inflammatory response. Ann. Thorac. Surg. https://doi.org/10.1016/S0003-4975(96)01061-2 (1997).
11. Downing, S. W. & Edmunds, L. H. Release of vasoactive substances during cardiopulmonary bypass. Ann. Thorac. Surg. https://doi.org/10.1003/S0003-4975(92)01131-9 (1992).
12. Hill, G. E. Cardiopulmonary bypass-induced inflammation: is it important? J. Cardiothorac. Vasc. Anesth. 12, 21–25 (1998).
13. Tomic, V. et al. Transcriptomic and proteomic patterns of systemic inflammation in a pump and off-pump coronary artery bypass grafting. Circulation https://doi.org/10.1161/CIRCULATIONAHA.104.531152 (2005).
14. Liangos, O. et al. Whole blood transcriptomics in cardiac surgery identifies a gene regulatory network connecting ischemia reperfusion with systemic inflammation. PLoS ONE 9(10), 1–12. https://doi.org/10.1371/journal.pone.0013658 (2015).
15. Ruel, M. et al. Gene expression profile after cardiopulmonary bypass and cardiopulmonary arrest. J. Thorac. Cardiovasc. Surg. 126(5), 1521–1530. https://doi.org/10.1016/j.jtcvs.2009.08.022 (2009).
16. Cook, S. A. DNA microarrays: implications for cardiovascular medicine. Circ. Res. 91(7), 559–564. https://doi.org/10.1161/01.RES.0000036019.55901.62 (2002).
17. Andropoulos, D. B. et al. Brain immaturity is associated with brain injury before and after neonatal cardiac surgery with high-flow bypass and cerebral oxygenation monitoring. J. Thorac. Cardiovasc. Surg. https://doi.org/10.1016/j.jtcvs.2009.08.022 (2010).
18. Kim, M. S. et al. Differential expression of extracellular matrix and adhesion molecules in fetal-origin amniotic epithelial cells of preeclamptic pregnancy. PLoS ONE https://doi.org/10.1371/journal.pone.0003638 (2006).
19. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBO J. https://doi.org/10.1101/gr.092759.109 (2009).
20. Li, H. et al. The sequence alignment/map format and SAMtools. Bioinformatics https://doi.org/10.1093/bioinformatics/btp352 (2009).
21. Pertea, M., Kim, D., Pertea, G. M., Leek, J. T. & Salzberg, S. L. Transcript-level expression analysis of RNA-seq experiments with HISAT StringTie and Ballgown. Nat. Protoc. https://doi.org/10.1038/nprot.2016.095 (2016).
22. Liao, Y., Smyth, G. K. & Shi, W. FeatureCounts: An efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics https://doi.org/10.1093/bioinformatics/btt656 (2014).
23. Frankish, A. et al. Comparison of GENCODE and RefSeq gene annotation and the impact of reference geneset on variant effect prediction. BMC Genom. https://doi.org/10.1186/1471-2164-16-S8-S2 (2015).
24. Risso, D., Ngai, J., Speed, T. P. & Dudoit, S. Normalization of RNA-seq data using factor analysis of control genes or samples. Nat. Biotechnol. https://doi.org/10.1038/nbt.2911 (2014).
25. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. https://doi.org/10.1186/s13059-014-0550-8 (2014).
26. Krzywinski, M. et al. Circos: An information aesthetic for comparative genomics. Genome Res. https://doi.org/10.1101/gr.092759.109 (2009).
27. Mootha, V. K. et al. PGC-1α-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat. Genet. https://doi.org/10.1038/ng1180 (2003).
28. Subramanian, A. et al. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc. Natl. Acad. Sci. USA. https://doi.org/10.1073/pnas.0506580102 (2005).
29. Robinson, M. D., McCarthy, D. J. & Smyth, G. K. edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics https://doi.org/10.1093/bioinformatics/btp616 (2009).
30. Steen, C. B., Liu, C. L., Alizadeh, A. A. & Newman, A. M. Profiling cell type abundance and expression in bulk tissues with CIBERSORTx. Methods Mol. Biol. https://doi.org/10.1007/978-1-0716-0360-7_7 (2020).
31. Miyamoto, S. D. et al. Gene expression and ß-adrenergic signaling are altered in hypoplastic left heart syndrome. J. Heart Lung Transplant. https://doi.org/10.1016/j.hejlt.2014.02.030 (2014).
32. Allan, C. K. et al. The relationship between inflammatory activation and clinical outcome after infant cardiopulmonary bypass. Anesth. Analg. https://doi.org/10.1213/ANE.0b013e3181f333aa (2010).
33. Mahle, W. T. et al. Inflammatory response after neonatal cardiac surgery and its relationship to clinical outcomes. Ann. Thorac. Surg. https://doi.org/10.1016/j.athoracsur.2013.10.009 (2014).
34. Yu, H., Pardoll, D. & Jove, R. STATs in cancer inflammation and immunity: a leading role for STAT3. Nat. Rev. Cancer https://doi.org/10.1038/nrc2734 (2009).
35. Brady, A. et al. Expression of phosphorylated signal transducer and activator of transcription 5 is associated with an increased risk of death in acute myeloid leukemia. Eur. J. Haematol. https://doi.org/10.1111/j.1600-0609.2012.01825.x (2012).
36. Graham, E. M. The utility of steroids in pediatric cardiac operations. Pediatr. Crit. Care Med. https://doi.org/10.1097/PCC.000000000000139 (2014).
37. Graham, E. M. et al. Corticosteroid therapy in neonates undergoing cardiopulmonary bypass. J. Am. Coll. Cardiol. 74(5), 659–668. https://doi.org/10.1016/j.jacc.2019.05.060 (2019).
38. Chu, C. Y. et al. Metabolic and bioinformatic analyses in asphyxiated neonates. Clin. Biochem. https://doi.org/10.1016/j.clinbiochem.2006.01.006 (2006).
39. Walsh, B. H. et al. The metabolomic profile of umbilical cord blood in neonatal hypoxic ischaemic encephalopathy. PLoS ONE https://doi.org/10.1371/journal.pone.0050520 (2012).
40. Davidson, J. A. et al. Metabolomic fingerprinting of infants undergoing cardiopulmonary bypass: changes in metabolic pathways and association with mortality and cardiac intensive care unit length of stay. J. Am. Heart Assoc. https://doi.org/10.1161/JAHA.118.010711 (2018).
41. Correia, G. D. S. et al. Metabolic profiling of children undergoing surgery for congenital heart disease. Crit. Care Med. https://doi.org/10.1097/CCM.0000000000000982 (2015).
42. Ellinger, J. et al. Systematic expression analysis of the mitochondrial complex III subunits identifies UQRC1 as biomarker in clear cell renal cell carcinoma. Eur. Urol. Suppl. https://doi.org/10.1016/s1569-9056(17)31035-7 (2017).
43. Hallmann, K. et al. Loss of the smallest subunit of cytochrome c oxidase, COX8A, causes Leigh-like syndrome and epilepsy. Brain https://doi.org/10.1093/brain/awv357 (2016).
44. Stanley, W. C., Recchia, F. A. & Lopaschuk, G. D. Myocardial substrate metabolism in the normal and failing heart. Physiol. Rev. https://doi.org/10.1152/physrev.00006.2004 (2005).

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Author contributions
P.N.J., C.C., and M.R. wrote the manuscript text. M.R. conducted the RNA-Seq analysis and prepared all figures. J.L., L.S., P.C. oversaw the project and assisted with writing of the manuscript. D.G. assisted with clinical statistical analysis and edited the manuscript. Y.Z. and K.L. performed the genetic analysis. All authors reviewed the manuscript.

Competing interests
The authors declare no competing interests.

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