Supplementary

Appendix 1

Table of contents:

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Methods

Patients Characteristics

Of all patients with a primary diagnosis of tetralogy of Fallot (TOF) undergoing surgical treatment at the German Heart Center Munich between April 2009 and May 2016, RV tissue and a signed informed consent were available in the institutional biomaterial bank from 31 patients (Figure 1). Surgeries included corrective repair for infants or elective replacement of the right ventricular to pulmonary artery conduit for adult patients, due to long-term consequences of hemodynamic abnormalities. Demographic, clinical, electrocardiographic data, transthoracic echocardiography (TTE), chest x-ray, cardiovascular magnetic resonance imaging (CMR), computed tomography (CT) scans and catheterization data were collected by retrospective chart review and were analyzed. Based on clinical information, clinical status with distinct signs of RVI and RV function was assessed, following the definition of adult and infant patient groups.

The criteria for RVI were selected based on the International Right Heart Foundation Working Group recommendations (1) and the scientific statement of the American Heart Association (2). These included clinical parameters such as functional capacity (New York Heart Association (NYHA) classification), peripheral edema, and engorgement of jugular veins, enlargement of liver, dyspnea and cyanosis. Serum markers like reduced GFR, increased creatinine and blood urea nitrogen were selected, in order to evaluate the cardiorenal abnormalities. For cardiohepatic abnormalities, elevated blood levels of bilirubin, ɣ-glutamyl transpeptidase (ɣ-GT) and alkaline phosphatase (AP) were chosen. The assessment of RV size and function was examined with (TTE) and CMR. Parameters included right ventricular ejection fraction (RVEF), left ventricular ejection fraction (LVEF), pulmonary trunk regurgitation fraction, right ventricular stroke volume (RVSV), right ventricular enddiastolic volume index (RVEDVI), right ventricular endsystolic volume index (RVESVI), evidence of moderate to severe pulmonary regurgitation, maximum velocity over pulmonic valve (PV V max), mean pressure gradient over pulmonic valve (PV mean PG), maximum pressure gradient over pulmonic valve (PV max PG), right ventricular hypertrophy and increased right ventricular pressure (RVP).

RV tissue from infants with TOF obtained at the time of corrective surgery during infancy was selected for comparison due to the similar underlying structural heart defect in this group and due to the lack of long-standing right ventricular hemodynamic stressors. Patients with confirmed genetic diseases, additional syndromes and any other organ failure were excluded.

RNA Isolation and Quality Assessment

RV tissue was collected during surgical procedures. Immediately after tissue removal, all samples were frozen in liquid nitrogen and stored at -80 °C until RNA isolation. Following manufacturer's instructions, total RNA was isolated using miRNeasy Mini Kit and QIAcube robotic workstation (Qiagen, Hilden, Germany) at the Institute of human genetics at Helmholtz Centre Munich, Germany. Quantity of RNA was assessed by measuring the concentration of isolated total RNA, using NanoDrop 2000 spectrophometer (Thermo Fisher Scientific, Waltham, Massachusetts, United States). RNA purity was estimated by examining the 260/280 ratio, as recommended by the manufacturer (Thermo Fisher, Scientific, Waltham Massachusetts, Unites States). Quality of the isolated RNA was estimated by determination of RNA integrity number (RIN), according to manufacturer's specifications.

Total RNA Sequencing

Whole transcriptome analysis was performed by total RNA sequencing of cardiac tissue samples as previously described (3). RNA library was prepared by using 1 µg of RNA, which was poly (A) selected, fragmented and reverse transcribed with Elute, Prime and fragment mix adhered to Illumina's information (Illumina, San Diego, California United Stated). Subsequently, tailing, adaptor ligation and library enrichment was done, following manufacturer's recommendation of TruSeq Stranded mRNA Sample Prep Guide (Illumina, San Diego, California, United States). Quality and Quantity of the RNA library were estimated by Agilent 2100 Bioanalyzer and Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, Massachusetts, United States). RNA sequencing of 100 bp paired-end runs was performed with Illumina HiSeq 4000 platform (Illumina, San Diego, California, United States). A proximate alignment against human genome assembly hg19 (GRCh37) and UCSC known gene annotation was done by using STAR aligner (v2.4.2a) (4). Quantification of the number of reads mapping to annotated genes was accomplished by using HT-seq count (v0.6.0), in which fragments per kilobase of transcript per million fragments mapped (FPKM) were selected as the unit

 http://dx.doi.org/10.21037/cdt-20-894

of measurement (5). Utilizing R Bioconductor package DESeq2 differential gene expression analysis was completed (6), followed by pathway- and Gene Set Enrichment Analysis with R Bioconductor package gage (7), pathview (8) and goseq (9). Tools like Gene Ontology (GO) (Department of Genetics, Stanford University School of Medicine, Stanford, California, United States.) (10,11), Kyoto Encyclopedia of Genes and Genomes (KEGG) (Institute for Chemical Research, Kyoto University, Uji, Kyoto, Japan) (12), ToppFun (Division of Biomedical Informatics, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, United States) (13) and PANTHER Classification System (14) were applied to validate pathway analysis, biological activity and allocation of individual genes to Gene Ontology categories (Figure 2 and Figure S2).

The total of 3,010 differentially expressed genes were compared to 50 most significant genes, regulating cardiac development and heart maturation, which were identified by RNA sequencing of murine cells during embryonic and postnatal period. Overlapping genes were not included in further interpretation of results (15) (Figure S1).

Validation of selected genes with Western blot

Total proteins, isolated from RV myocardial tissue from patients, were separated by 8-12% SDS polyacrylamide gel electrophoresis using Mini-Protean 3 system (Biorad), as previously described (16,17). Following separation, proteins were transferred to nitro-cellulose membranes, and incubated with one of the following primary antibodies: alcohol dehydrogenase 1B (ADH1B, Abcam, Cambridge, UK; ab175515, RRID: N/A), monoamine oxidase B (MAOB, Merck, Darmstadt, Germany; ST1582, RRID: AB_10617089), peroxisome proliferator-activated receptor gamma (PPARγ, Cell Signaling, Frankfurt, Germany; S4946, RRID: AB_2166051), superoxide dismutase 3 (SOD3, Merck, Darmstadt, Germany; S4946, RRID: AB_532286), interleukin 6 receptor (IL6R, R&D Systems, Wiesbaden-Nordenstadt, Germany; MAB227, RRID: AB_2127908), or β-actin (Santa Cruz, Heidelberg, Germany, SC-1616, RRID: AB_630836). Goat anti-rabbit, goat anti-mouse or rabbit anti-goat secondary antibodies were used (Merck, 401253, RRID: AB_437779; 401393, RRID: AB_437797; 401515, RRID: AB_437816). Following enhanced chemiluminescence reaction, bands were quantified using ImageJ. Two-tailored student's test was used for statistical evaluation. Data are presented as a mean ± standard deviation.

Ethical Approval

The study was approved by the institution's ethical committee at the Technical University of Munich (approval 10/16/2017, number 242/17S, and approval 01/11/2017, number 592,16S). The study protocol conforms to the ethical standards of the Declaration of Helsinki 1975. All patients or parents gave written informed consent before enrollment.

Statistics

Statistic evaluation was implemented by applying R Bioconductor package DEseq2 to transcriptome profiles and tested for differential gene expression between adult and infant patients. The P values were corrected for the purpose of multiple testing by Benjamini and Hochberg procedure. The level of significance was set at a P value of less than 0.01 and a fold-change value of greater than 2 or less than –2. Based on that, all significant differentially expressed genes were selected for further analysis. The results of Gene Ontology and Pathway analysis with an adjusted P value less than 0.01 (GO) and a P value less than 0.05 (KEGG) were assigned as significant.

http://dx.doi.org/10.21037/cdt-20-894

Online Figures:

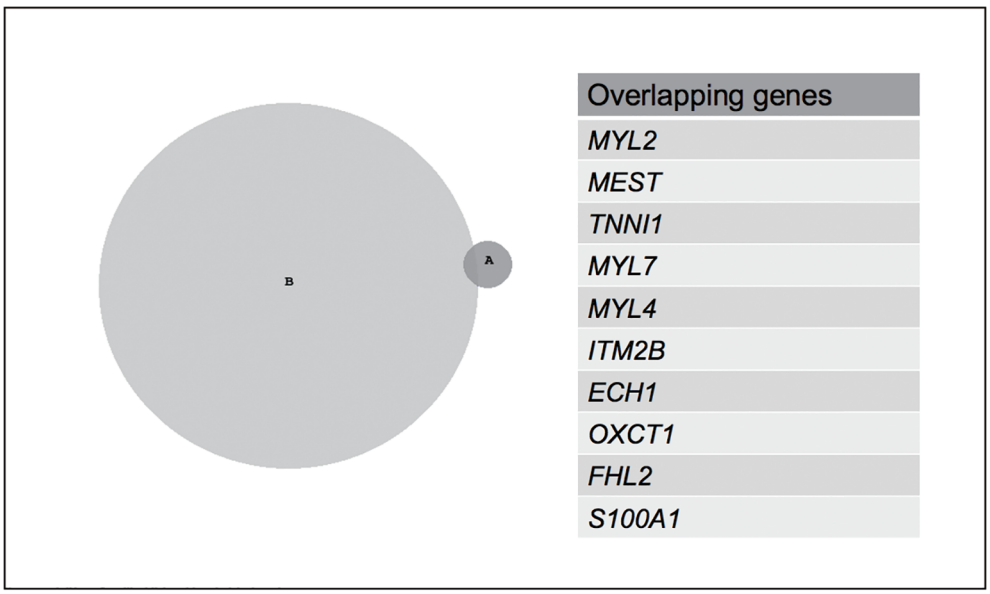

Figure S1 Venn diagram demonstrating the intersection of differentially expressed genes. The total of 3,010 genes differentially expressed between infants and adults with right ventricular impairment are represented by B. The top 50 genes being potential markers for cardiac development and heart maturation are illustrated by A. Genes identical expressed in both studies are listed on the right. These 10 genes were identified as overlapping genes playing a role during heart development and were therefore excluded for further analysis. The complete list of the 50 developmental markers is shown in Table S9.

http://dx.doi.org/10.21037/cdt-20-894

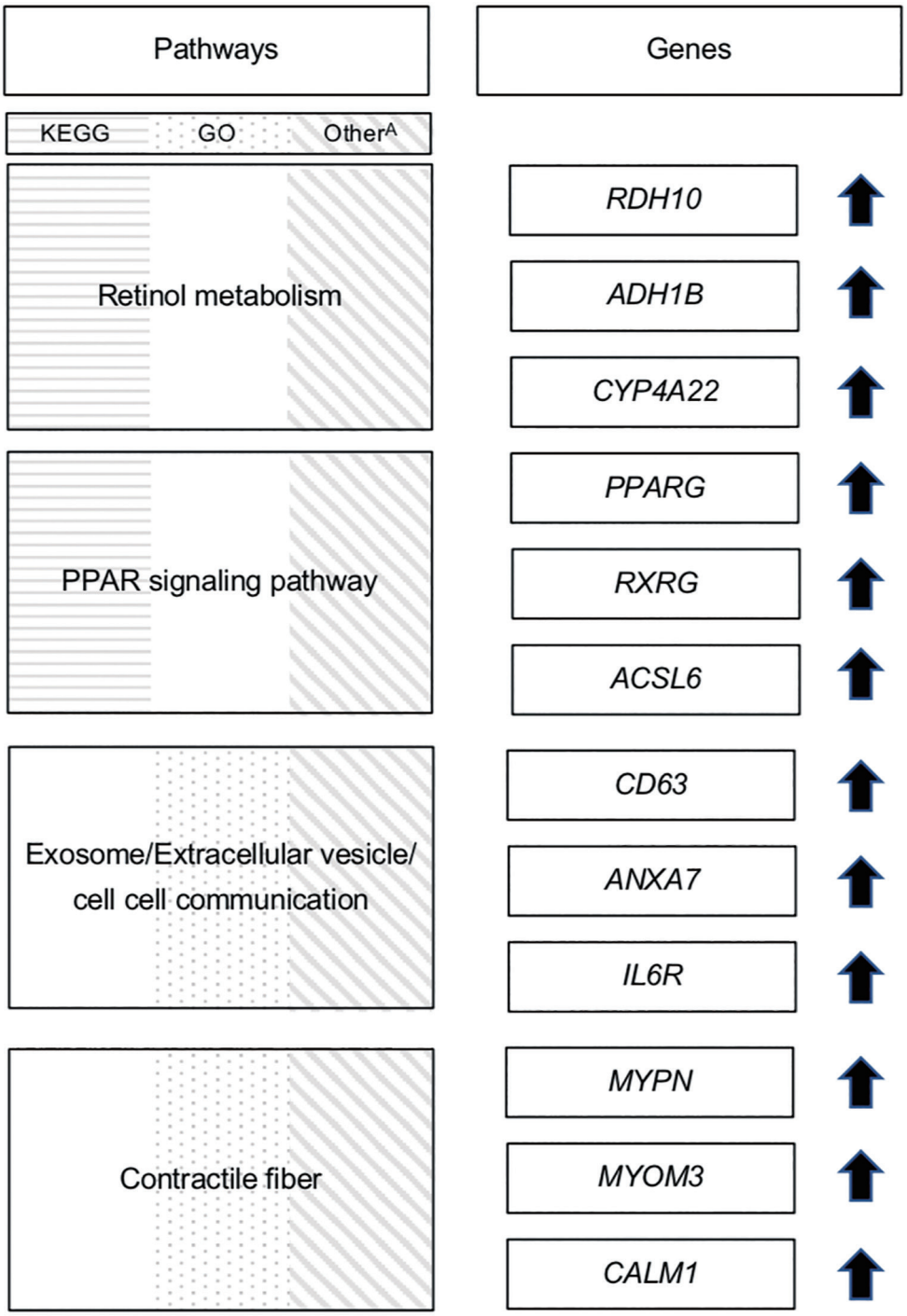

Figure S2 Summary of the results in the form of an overview of the modified signaling pathways with associated genes and their regulation. A, used validation tools like PANTHER Classification, ToppFun. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; PPAR, peroxisome proliferator-activated receptor.

http://dx.doi.org/10.21037/cdt-20-894

Online Tables:

Table S1 Presentation of the 10 most significant upregulated GO terms for cellular component in adult patients with CCHD and RVI

Category	Term	P value	Adjusted P value
GO:0044444	Cytoplasmic part	3.74E-33	8.36E-29
GO:0005737	Cytoplasm	1.23E-29	1.37E-25
GO:0030016	Myofibril	2.61E-18	1.95E-14
GO:0043292	Contractile fiber	2.36E-17	1.32E-13
GO:0030017	Sarcomere	1.24E-16	5.53E-13
GO:0044449	Contractile fiber part	2.68E-16	1.00E-13
GO:0005829	Cytosol	4.18E-15	1.34E-11
GO:0070062	Extracellular exosome	3.81E-14	1.06E-10
GO:1903561	Extracellular vesicle	8.00E-14	1.68E-10
GO:0043230	Extracellular organelle	9.03E-14	1.68E-10

GO, Gene Ontology.

Table S2 Presentation of the 10 most significant upregulated GO terms for molecular function in adult patients with CCHD and RVI

Category	Term	P value	Adjusted P value
GO:0008092	Cytoskeletal protein binding	5.69E-14	1.41E-10
GO:0016491	Oxidoreductase activity	8.96E-12	1.34E-08
GO:0003824	Catalytic activity	3.99E-11	3.72E-08
GO:0005515	Protein binding	2.10E-10	1.45E-07
GO:0003674	Molecular_function	8.28E-09	3.63E-06
GO:0061134	Peptidase regulator activity	4.40E-08	1.61E-05
GO:0003779	Actin binding	1.18E-07	3.66E-05
GO:0005488	Binding	1.36E-07	4.11E-05
GO:0004857	Enzyme inhibitor activity	2.11E-07	6.05E-05
GO:0061135	Endopeptidase regulator activity	2.39E-07	6.69E-05

GO, Gene Ontology.

http://dx.doi.org/10.21037/cdt-20-894

Table S3 Presentation of the 10 most significant upregulated GO terms for biological processes in adult patients with CCHD and RVI

Category	Term	P value	Adjusted P value
GO:1901564	Organonitrogen compound metabolic process	8.61E-14	1.68E-10
GO:0044281	Small molecule metabolic process	7.13E-12	1.14E-08
GO:0055114	Oxidation-reduction process	1.44E-11	2.02E-08
GO:0002283	Neutrophil activation involved in immune response	1.72E-11	2.26E-08
GO:0002446	Neutrophil mediated immunity	1.82E-11	2.26E-08
GO:0043312	Neutrophil degranulation	2.33E-11	2.60E-08
GO:0042119	Neutrophil activation	2.69E-11	2.86E-08
GO:0036230	Granulocyte activation	3.56E-11	3.46E-08
GO:0016192	Vesicle-mediated transport	6.82E-11	6.10E-08
GO:0006887	Exocytosis	1.03E-10	8.85E-08

GO, Gene Ontology.

Table S4 Presentation of the 10 most significant downregulated GO terms for cellular component in adult patients with CCHD and RVI

Category	Term	P value	Adjusted P value
GO:0005694	Chromosome	8.57E-28	1.92E-23
GO:0005634	Nucleus	1.05E-26	1.17E-22
GO:0044427	Chromosomal Part	6.44E-24	3.60E-20
GO:0044815	DNA packaging complex	2.95E-23	9.35E-20
GO:0000786	Nucleosome	1.82E-21	3.71E-18
GO:0032993	Protein-DNA complex	4.64E-21	8.66E-18
GO:0044428	Nuclear part	7.83E-19	1.35E-15
GO:0031981	Nuclear lumen	3.36E-18	5.37E-15
GO:0005654	Nucleoplasm	2.77E-17	3.65E-14
GO:0000228	Nuclear chromosome	1.48E-15	1.58E-12

GO, Gene Ontology; DNA, deoxyribonucleic acid.

http://dx.doi.org/10.21037/cdt-20-894

Table S5 Presentation of the 10 most significant downregulated GO terms for molecular function in adult patients with CCHD and RVI

Category	Term	P value	Adjusted P value
GO:0003677	DNA binding	5.29E-22	1.18E-18
GO:0003676	Nucleic acid binding	1.45E-13	9.80E-11
GO:0097159	Organic Cyclic compound binding	2.43E-09	6.63E-07
GO:1901363	Heterocyclic compound binding	3.36E-09	8.73E-07
GO:0003700	DNA binding transcription factor activity	1.14E-07	2.16E-05
GO:0043142	Single-stranded DNA-dependent ATPase activity	7.75E-07	0.00013
GO:0140110	Transcription regulator activity	1.52E-06	0.00023
GO:0000981	RNA Polymerase 2 transcription factor activity, sequence-specific DNA binding	2.39E-06	0.00035
GO:0042393	Histone binding	2.43E-06	0.00036
GO:0031492	Nucleosomal DNA binding	3.18E-06	0.00044

GO, Gene Ontology; DNA, deoxyribonucleic acid; ATP, adenosine triphosphate; RNA, ribonucleic acid.

Table S6 Presentation of the 10 most significant downregulated GO terms for biological processes in adult patients with CCHD and RVI

Category	Term	P value	Adjusted P value
GO:0007049	Cell Cycle	4.93E-26	3.60E-20
GO:1903047	Mitotic cell cycle process	1.33E-23	5.54E-20
GO:0022402	Cell cycle process	1.49E-23	5.54E-20
GO:0051276	Chromosome organization	2.29E-22	6.40E-19
GO:0000278	Mitotic cell cycle	2.88E-22	7.16E-19
GO:0090304	Nucleic acid metabolic process	3.76E-18	5.61E-15
GO:0006259	DNA metabolic process	1.15E-17	1.61E-14
GO:0006261	DNA-dependent DNA replication	1.20E-16	1.49E-13
GO:0140014	Mitotic nuclear division	3.79E-16	4.47E-13
GO:0051301	Cell division	6.13E-16	6.86E-13

GO, Gene Ontology; DNA, deoxyribonucleic acid.

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Table S7 Upregulated genes matching to the selected differentially expressed signaling pathways in adult patients with CCHD and RVI

	P value	Gene	P value	Adjusted P value	Regulation
Gene Ontology (PANTHER)					
Extracellular exosome; GO:0070062	3.81E-14	APOB	3.69E-25	2.27E-22	Up
		SERPINB6	6.42E-21	2.35E-18	Up
		ACSL4	5,14E-09	2.18E-07	Up
		GPA33	4.73E-09	2.03E-07	Up
		DSC1	2.80E-11	2.00E-09	Up
		MYL12A	1.42E-62	1.66E-58	Up
		SERPINA5	3.32E-09	1.48E-07	Up
		CAB39	2.33E-12	2.16E-10	Up
		CD59	6.34E-19	1.79E-16	Up
		DSTN	8.09E-13	8.49E-11	Up
		SYNC	5.10E-13	5.55E-11	Up
		PPM1L	2.96E-19	8.65E-17	Up
		RNF11	4.70E-15	7.23E-13	Up
		MME	3.12E-17	7.51E-15	Up
		CLIC5	7.40E-22	3.09E-19	Up
		ANXA7	8.92E-18	2.27E-15	Up
		RRAS	6.83E-12	5.63E-10	Up
		RRAS2	2.50E-12	2.30E-10	Up
		RAB5A	3.32E-09	1.48E-07	Up
Extracellular vesicle and cell cell communication; GO:1903561	8.00E-14	APOB	3.69E-25	2.27E-22	Up
		CD59	6.34E-19	1.79E-16	Up
		LGMN	6.89E-05	0.00086	Up
		LGALS3	1,73E-07	4.87E-06	Up
		SERPINB6	6,42E-21	4.87E-06	Up
		CD63	0.0012	0.0092	Up
		DES	5.10E-14	6.67E-12	Up
		HSPA4	5,37E-15	8,21E-13	Up
		PPM1L	2.96E-19	8.65E-17	Up
		RNF11	4.70E-15	7.23E-13	Up
		MME	3.12E-17	7.51E-15	Up
		MYL12A	1.42E-62	1.66E-58	Up
		CLIC5	7.40E-22	3,09E-19	Up
		ANXA7	8.92E-18	2.27E-15	Up

Table S7 (*continued*)

http://dx.doi.org/10.21037/cdt-20-894

Table S7 (*continued*)

	P value	Gene	P value	Adjusted P value	Regulation
		SCN3A	1.07E-25	7.34E-23	Up
		SYT13	5.97E-20	2.00E-17	Up
		GRIK2	4.46E-23	2.05E-20	Up
		BDNF	9.87E-16	1.79E-13	Up
		GRIN2A	2.40E-21	9.19E-19	Up
		IL6R	9.89E-13	9.98E-11	Up
		DGKG	1.61E-27	1.21E-24	Up
		DOCK5	4.44E-30	4.72E-27	Up
		PRKG1	8.56E-20	2.82E-17	Up
		MYH7	9.04E-11	5.86E-09	Up
		RCAN2	5.08E-22	2.16E-19	Up
		HOMER2	4.05E-23	1.90E-20	Up
		C1orf168	1.69E-24	8.99E-22	Up
		ITGBL1	6.94E-21	2.50E-18	Up
		SARS	9.46E-06	0.000155	Up
		CHP1	3.64E-08	1.24E-06	Up
		SERPINB1	3.50E-08	1.20E-06	Up
		STAMBP	0,00023	0.0024	Up
Response to oxidative stress; GO:0006979	8.67E-05	HBA2	3.88E-12	3.42E-10	Up
		TPO	7.76E-15	1.16E-12	Up
		BECN1	2.20E-12	2.05E-10	Up
		HBB	8.34E-13	8.67E-11	Up
		MAOB	2.27E-18	6.11E-16	Up
		KPNA4	3.76E-29	3.39E-26	Up
		SOD3	0.00034	0.0033	Up
		TXN2	0.00058	0.0052	Up
Contractile Fiber; GO:0043292	2.36E-17	HABP4	4.01E-16	7.57E-14	Up
		DUSP27	1.55E-27	1.21E-24	Up
		CALM1	3.56E-05	0.00049	Up
		MYBPC1	6.86E-13	7.23E-11	Up
		CMYA5	1.77E-13	2.13E-11	Up
		KLHL41	4.98E-42	1.46E-38	Up
		SYNM	5.47E-16	1.02E-13	Up
		SYNC	5.10E-13	5.55E-11	Up

Table S7 (*continued*)

http://dx.doi.org/10.21037/cdt-20-894

Table S7 (*continued*)

	P value	Gene	P value	Adjusted P value	Regulation
		FHL5	2.19E-37	3.42E-34	Up
		FHL1	9.46E-35	1.30E-31	Up
		MYH7	9.04E-11	5.86E-09	Up
		MYPN	1.35E-15	2.36E-13	Up
		MYOM3	9.39E-15	1.38E-12	Up
Signaling pathway (KEGG analysis)					
Retinol metabolism (hsa00830)	0.0017	ADH1B	4.93E-11	3.36E-09	Up
		ADH1C	0.00037	0.0035	Up
		RDH10	0.00076	0.0064	Up
		PNPLA4	2.61E-10	1.58E-08	Up
		CYP1A1	1.01E-05	0.00016	Up
		CYP4A22	0.00098	0.0079	Up
		RETSAT	1.18E-08	4.61E-07	Up
PPAR signaling pathway (hsa03320)	0.021	RXRG	0,0051	0,03	Up
		PPARG	2.03E-08	7.46E-07	Up
		APOA1	0.0019	0.014	Up
		ACSL6	1.82E-21	7.33E-19	Up
		ACSL4	5.14E-09	2.18E-07	Up
		CYP4A22	0.00098	0.0079	Up
		AQP7	8.07E-05	0.00099	Up
		ACADM	5.53E-07	1.36E-05	Up

KEGG, Kyoto Encyclopedia of Genes and Genomes; has, Homo sapiens (human); GO, Gene Ontology; PANTHER, Protein Analysis Through Evolutionary Relationships.

http://dx.doi.org/10.21037/cdt-20-894

Table S8 Downregulated genes matching to the selected differentially expressed signaling pathways in adult patients with CCHD and RVI

	P value	Gene	P value	Adjusted P value	Regulation
Gene Ontology (PANTHER)					
Cell Cycle; GO:0007049	4.92E-24	CDT1	5.37E-05	0.00069	Down
		CDC7	2.69E-11	1.94E-09	Down
		MCM2	1.14E-05	0.00018	Down
		PRIM1	3.61E-06	6.85E-05	Down
		ORC6	7.67E-08	2.39E-06	Down
		BCAT1	1.16E-05	0.00018	Down
		MCM3	4.74E-09	2.04E-07	Down
		CDK6	2.20E-09	1.03E-07	Down
		MCM5	4.20E-15	6.55E-13	Down
		RCC1	6.30E-05	0.0008	Down
		E2F7	9.65E-08	2.92E-06	Down
		NASP	8.50E-06	0.00014	Down
		CDK14	0.00021	0.0022	Down
Cell division; GO:051301	6.13E-16	CDT1	5.37E-05	0.00069	Down
		CENPF	3.34E-08	1.15E-06	Down
		NCAPG	1.02E-08	4.03E-07	Down
		STAG2	8.89E-08	2.72E-06	Down
		CDC7	2.69E-11	1.94E-09	Down
		FBXL7	6.42E-08	2.03E-06	Down
		PARD6G	5.22E-05	0.00068	Down
		SPDL1	3.99E-06	7.46E-05	Down
		CASC5	4.48E-11	3.08E-09	Down
		TPX2	5.89E-08	1.88E-06	Down
Mitotic cell cycle; GO:0000278	2.88E-22	DNMT3A	6.29E-09	2.60E-07	Down
		SOX4	8.12E-21	2.88E-18	Down
		WDR62	7.06E-06	0.00012	Down
		NCAPG	1.02E-08	4.03E-07	Down
		AJUBA	6.43E-06	0.00011	Down
		TOP2A	9.27E-14	1.17E-11	Down
		POLA1	3.10E-06	5.98E-05	Down
		SNX30	3.65E-13	4.11E-11	Down
DNA replication; GO:0006260	4.56E-15	POLD1	5.57E-11	3.73E-09	Down
		GINS1	1.59E-07	4.55E-06	Down
		ING4	1.58E-11	1.21E-09	Down

Table S8 (*continued*)

http://dx.doi.org/10.21037/cdt-20-894

Table S8 (*continued*)

	P value	Gene	P value	Adjusted P value	Regulation
		HELB	3.29E-07	8.62E-06	Down
		DTL	1.08E-06	2.42E-05	Down
		POLQ	3.04E-05	0.00043	Down
		RBBP4	5.68E-06	0.0001	Down
		FAM111A	7.36E-06	0.00013	Down
Signaling pathway (KEGG)					
Cell cycle (hsa04110)	0.00098	CDK6	2.20E-09	1.03E-07	Down
		ORC6	7.67E-08	2.39E-06	Down
		SKP2	6.68E-08	2.11E-06	Down
		CDC7	2.69E-11	1.94E-09	Down
		MCM5	4.20E-15	6.55E-13	Down
DNA replication (hsa03030)	0.0019	LIG1	5.68E-14	7.38E-12	Down
		POLA1	3.10E-06	5.98E-05	Down
		PRIM1	3.61E-06	6.85E-05	Down
		POLE	2.85E-10	1.67E-08	Down
		RNASEH2C	1.10E-06	2.45E-05	Down
Ribosome (hsa03010)	0.012	RPL18	5.85E-05	0.00075	Down
		MRPS6	0.0086	0.045	Down
		RPS8	0.0004	0.0038	Down

KEGG, Kyoto Encyclopedia of Genes and Genomes; has, Homo sapiens (human); GO, Gene Ontology; PANTHER, Protein Analysis Through Evolutionary Relationships; DNA, deoxyribonucleic acid.

http://dx.doi.org/10.21037/cdt-20-894

Table S9 Results of a separate study representing the top 50 genes identified as potential markers for cardiac development and heart maturation (15). The 50 developmental markers listed below were used for identification of genes with the same expression in our study, symbolizing developmentally regulated genes. The detected overlapping genes were not included for further analysis (Figure S1)

Cluster of the top 50 genes	Developmental markers	Expression
Cluster 1	Myh6	Increased
Cluster 1	Atp2a2	Increased
Cluster 1	Pln	Increased
Cluster 1	Cox6a2	Increased
Cluster 1	Cox7b	Increased
Cluster 1	Ndufa1	Increased
Cluster 1	Uqcrq	Increased
Cluster 1	Atp5e	Increased
Cluster 1	Cox7a1	Increased
Cluster 1	Cox6c	Increased
Cluster 1	Tnni3	Increased
Cluster 1	Fabp3	Increased
Cluster 1	Myl2 *	Increased
Cluster 2	Pgam1	Decreased
Cluster 2	Tubb5	Decreased
Cluster 2	Nme1	Decreased
Cluster 2	Gm5506	Decreased
Cluster 2	Eif5a	Decreased
Cluster 2	Ngfrap1	Decreased
Cluster 2	Cks1b	Decreased
Cluster 2	Cdkn1c	Decreased
Cluster 2	Mest *	Decreased
Cluster 2	Gpc3	Decreased
Cluster 2	H2afz	Decreased
Cluster 2	Tnni1 *	Decreased
Cluster 2	Mif	Decreased
Cluster 2	Hmgn2	Decreased
Cluster 2	Gyg	Decreased
Cluster 2	Myl7 *	Decreased
Cluster 2	Myl4 *	Decreased
Cluster 3	Hadha	Early low expression, then increasing
Cluster 3	Ryr2	Early low expression, then increasing
Cluster 3	Srl	Early low expression, then increasing

Table S9 (*continued*)

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Table S9 (*continued*)

Cluster of the top 50 genes	Developmental markers	Expression
Cluster 3	Nfib	Early low expression, then increasing
Cluster 3	Nfia	Early low expression, then increasing
Cluster 3	Klf6	Early low expression, then increasing
Cluster 3	Itm2b *	Early low expression, then increasing
Cluster 3	Ech1 *	Early low expression, then increasing
Cluster 3	Phyh	Early low expression, then increasing
Cluster 3	Oxct1 *	Early low expression, then increasing
Cluster 3	Gpc1	Early low expression, then increasing
Cluster 3	Fhl2 *	Early low expression, then increasing
Cluster 3	Mt1	Early low expression, then increasing
Cluster 3	Mgst3	Early low expression, then increasing
Cluster 3	Acadl	Early low expression, then increasing
Cluster 3	Lpl	Early low expression, then increasing
Cluster 3	Brp44l	Early low expression, then increasing
Cluster 3	D830015G02Rik	Early low expression, then increasing
Cluster 3	Lars2	Early low expression, then increasing
Cluster 3	S100a1 *	Early low expression, then increasing

*, detected overlapping genes excluded for further analysis.

Table S10 Presentation of overexpressed fetal genes in adult patients with CCHD and RVI

Fetal gene	P value	Adjusted P value	Expression
MEF2C	0.0081	0.043	Increased
NPPA	0.00072	0.0062	Increased
ACTA1	4.55E-09	1.96E-07	Increased
DES	5.10E-14	6.67E-12	Increased
DMD	0.0062	0.035	Increased

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