Journal of Proteomics xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Proteomics

journal homepage: www.elsevier.com/locate/jprot

The cardiac proteome in patients with congenital ventricular septal defect: A comparative study between right atria and right ventricles

Bond A.R.[a], Iacobazzi D.[a], Abdul-Ghani S.[a], Ghorbel M.T.[a], Heesom K.J.[b], George S.J.[a], Caputo M.[a,c], Suleiman M.-S.[a], Tulloh R.M.[a,c,*]

[a] Bristol Heart Institute, Research Floor Level 7, Bristol Royal Infirmary, Marlborough Street, Bristol BS2 8HW, United Kingdom
[b] Proteomics Facility, University of Bristol, Bristol BS8 1TD, United Kingdom
[c] Department of Congenital Heart Disease, King David Building, Upper Maudlin Street, Bristol BS2 8BJ, United Kingdom

ARTICLE INFO

Keywords:
Congenital heart disease
Ventricular septal defect
Right ventricle
Right atria
Proteomics

ABSTRACT

Right ventricle (RV) remodelling occurs in neonatal patients born with ventricular septal defect (VSD). The presence of a defect between the two ventricles allows for shunting of blood from the left to right side. The resulting RV hypertrophy leads to molecular remodelling which has thus far been largely investigated using right atrial (RA) tissue. In this study we used proteomic and phosphoproteomic analysis in order to determine any difference between the proteomes for RA and RV. Samples were therefore taken from the RA and RV of five infants (0.34 ± 0.05 years, mean ± SEM) with VSD who were undergoing cardiac surgery to repair the defect. Significant differences in protein expression between RV and RA were seen. 150 protein accession numbers were identified which were significantly lower in the atria, whereas none were significantly higher in the atria compared to the ventricle. 19 phosphorylation sites (representing 19 phosphoproteins) were also lower in RA. This work has identified differences in the proteome between RA and RV which reflect differences in contractile activity and metabolism. As such, caution should be used when drawing conclusions based on analysis of the RA and extrapolating to the hypertrophied RV.

Significance: RV hypertrophy occurs in neonatal patients born with VSD. Very little is known about how the atria responds to RV hypertrophy, especially at the protein level. Access to tissue from age-matched groups of patients is very rare, and we are in the unique position of being able to get tissue from both the atria and ventricle during reparative surgery of these infants. Our findings will be beneficial to future research into heart chamber malformations in congenital heart defects.

1. Introduction

Ventricular septal defect (VSD), a hole in the septum between the left and right ventricles of the heart, is the most common acyanotic congenital cardiac malformation affecting 30% of babies born with congenital heart disease each year (0.36% of all live births [1,2]). Not all infants with VSD require treatment, many closing by themselves as the child grows. However, larger VSDs cause the flow of oxygenated blood from the left ventricle (LV) back into the right ventricle (RV), causing an increase in right ventricular pressure, and increased blood flow to the lungs via the pulmonary artery; pulmonary hypertension and right ventricular hypertrophy can ensue.

The role of the atria is to fill the ventricles with blood, for subsequent ejection from the heart either to the lungs (RV) or around the systemic circulation (LV). The ventricles therefore contract much more forcefully than the atria. In the right heart, these chamber differences are reflected by the differences in pressures; RA mean pressure < 5 mm Hg and RV systolic pressure often 25 mm Hg, RV diastolic pressure < 5 mm Hg [3]. In patients with a large VSD the RV must generate a much larger systolic pressure (~80 mm Hg; similar to arterial systolic pressure) in response to the increased pressure exerted by the LV, resulting in cardiac remodelling. The cellular ultrastructure of atrial and ventricular cardiomyocytes has many similarities. However, they have very different calcium pattern in response to depolarisation; atrial myocytes have a shorter duration of action potential than ventricular (150 ms vs. 250 ms) [4] and they contract at a faster rate with rapid repolarization, with both chambers showing peak contraction within tens of milliseconds [5]. This is thought to partly be due to differences in transverse (T-tubule) organization in atria [6]; previously thought to be lacking T-tubules [7]. Calcium signals tend to be found at the cell periphery, and as such the atria rely on hormones for the inward movement of Ca^{2+} to the contractile machinery in the adult heart

* Corresponding author at: Professor Robert Tulloh, Bristol Heart Institute, Research Floor Level 7, Bristol Royal Infirmary, Marlborough Street, Bristol BS2 8HW, United Kingdom.
E-mail address: robert.tulloh@bristol.ac.uk (R.M. Tulloh).

https://doi.org/10.1016/j.jprot.2018.03.022
Received 1 November 2017; Received in revised form 8 March 2018; Accepted 19 March 2018

[8]. Recent studies (in a dog model) have now shown that there is a sparse network of T-tubules in the atria but there are differences between the left and right heart [9]. It should be noted that no comparison of cell ultrastructure has been carried out in infants.

Comparisons have been made of individual proteins between the right atria and ventricles from different pathologies (normoxaemic e.g. VSD/ASD (atrial septal defect), and hypoxaemic e.g. tetralogy of fallot (TOF) [10,11]), but these were not performed using current proteomics methods, and as such had to target certain protein groups (e.g. contraction and extracellular matrix). Previous proteomic studies have looked at the proteins implicated in atrial fibrillation (AF) comparing the right and left atria of adult hearts [12], the normal human fetal atria and ventricles [13] and more recently comparing the RV from different pathologies (VSD vs. TOF) [14]. Genomics studies have compared the gene profile from different chambers [15–18] but little has been reported on the differences between the whole proteome of the RA and RV from the same patient, and none from infants with VSD. This is an issue which needs addressing as researchers have in the past drawn conclusions on ventricular remodelling at the molecular level based on atrial data, due to ease of tissue access [19]. Atrium biopsy genomics are useful for a wide array of functions, but are not useful for studying contractility; ventricular biopsies should be used instead [16].

Finally, there is the problem of availability of control/normal cardiac tissue. The only way to obtain fresh “control” cardiac tissue is from age-matched children with a normal heart having just been declared dead in a hospital setting. These situations are extremely rare and have stringent ethical considerations. Tissues from heart transplant or from recent death cannot be considered as control since there will be significant protein turnover due to storage and handling. Therefore, we wished to determine differences between the RA and RV at the protein level in a group of age-matched neonates with VSD, which would impact on future studies.

2. Patients and methods

Infants with RV hypertension who underwent cardiopulmonary bypass were recruited to the RVENCH (Right Ventricle Function in Children) study between 01.07.15 and 31.03.17. The subset of patients described herein (n = 5, Table 1) had right ventricular pressures at systemic levels, and hence high pulmonary artery pressure as a result of a large ventricular septal defect (VSD). One patient had Trisomy 21 but was still included in the analysis as the ventricular function of these patients is not known to be any different. Fully informed parental consent was obtained prior to inclusion in study. Ethical approval was granted by the National Research Ethics Service number 14/NW/1256, IRAS 143683.

Right atrial and right ventricular biopsy samples were taken from the patients at the time of surgery. To maintain protein integrity, all tissue samples were obtained as soon as possible after instituting cardiopulmonary bypass. Biopsies were immersed in Allprotect tissue reagent (Qiagen, UK) overnight, before being stored at −80 °C until protein extraction.

Table 1
Patient demographics and pre-operative data displayed as mean (SEM).

	Mean (SEM)
Age (years)	0.34 (0.05)
Weight (kg)	4.83 (0.22)
Oxygen saturation (%)	97.0 (1.14)
Male:Female	4:1
Systolic blood pressure (mm Hg)	86.4 (4.50)
Fractional shortening (%)	34.6 (0.75)

2.1. Proteomics methods and analysis

Proteomics methods and analysis have been described elsewhere [14]. Briefly, for proteomic analysis (performed by the proteomics facility, BioMedical Sciences Building, University of Bristol), samples were labelled with Tandem Mass Tag (TMT) 10Plex reagents and analysed by LCMS using an Orbitrap Fusion Tribrid mass spectrometer running an SPS-MS3 acquisition. For phospho-proteomic analysis, phosphorylated TMT-labelled peptides were enriched prior to LCMS analysis using titanium oxide-based enrichment. Raw data files were processed and quantified using Proteome Discover software v1.4 (Thermo Scientific) and searched against the Uniprot Human database (downloaded 18/04/16: 134169 sequences) using the SEQUEST algorithm.

For comparison between chambers, proteins not found in both the RA and RV of all patients were excluded from the main analysis. However, proteins only detected in either the RA or RV were also investigated. Fold changes (a fold decrease/increase > 1.3) between chambers were determined and a log-2 transformation applied. Significant changes between the right atria and ventricle were determined by a paired *t*-test, and a –log 10 transformation of the p-value applied. Significant fold-changes ($p < 0.05$) were deemed of biological importance.

Accession numbers were converted to protein symbol (gene name) either using the Uniprot database mapping tool (converting ‘UniprotKB AC/ID’ to ‘Gene name’) or by extracting the relevant name from the protein description assigned by Proteome Discoverer, and these shall be used synonymously with protein name throughout. Gene names were not found by these methods for only ~200 accession numbers. Significantly altered proteins (including cDNA with high similarity sequences to proteins) between pathologies were inputted into Ingenuity Pathway Analysis software (IPA, v39480507, Qiagen) to determine significantly enriched canonical pathways, and diseases and functions (calculated by Fisher's exact test right-tailed). Significantly changed proteins and phosphoproteins were also analysed in the ‘Gene Ontology enRIchment anaLysis and visuaLizAtion’ tool (GOrilla, Database update v.Feb 4 2017, [20]) to determine enriched gene ontology (GO) terms, versus a background list comprised of all proteins detected during the proteomics and phosphoproteomics analysis. A p-value threshold of 0.001 was set, and enrichment false discovery rate q-value threshold < 0.05 (q-value: correction of p-value for testing of multiple gene ontology terms).

3. Results

3.1. Comparison between right atrium and right ventricle in VSD

3605 protein accession numbers (representing 3336 different proteins), were detected in all right atria and right ventricle samples from VSD patients. 150 protein accession numbers were significantly different between heart chambers, all of which were lower in the right atria (Fig. 1A). 414 phosphorylated proteins were identified, each containing at least one translation modification at serine, threonine, or tyrosine (resulting in a total of 700 phosphorylation site matches (as assigned by Proteome Discoverer Software v1.4)) (Fig. 1B). 19 phosphorylation sites, representing 19 unique proteins were significantly lower in the right atria, compared to the RV. Mean (and standard error) values for significantly changed proteins and phosphoproteins are shown in Supplementary Tables A & B, and heat-maps for these are in Supplementary Tables C & D.

Ingenuity pathway analysis of the significantly changed proteins determined that 33 canonical pathways were enriched ($p < 0.05$) (Supplementary Tables E) and the top three were those for tRNA charging, tricarboxylic acid cycle II and dopamine degradation (p-values 3.65E−07, 2.41E−05, and 7.16E−05 respectively). Of the ten most significantly enriched diseases and functions (Table 2) five

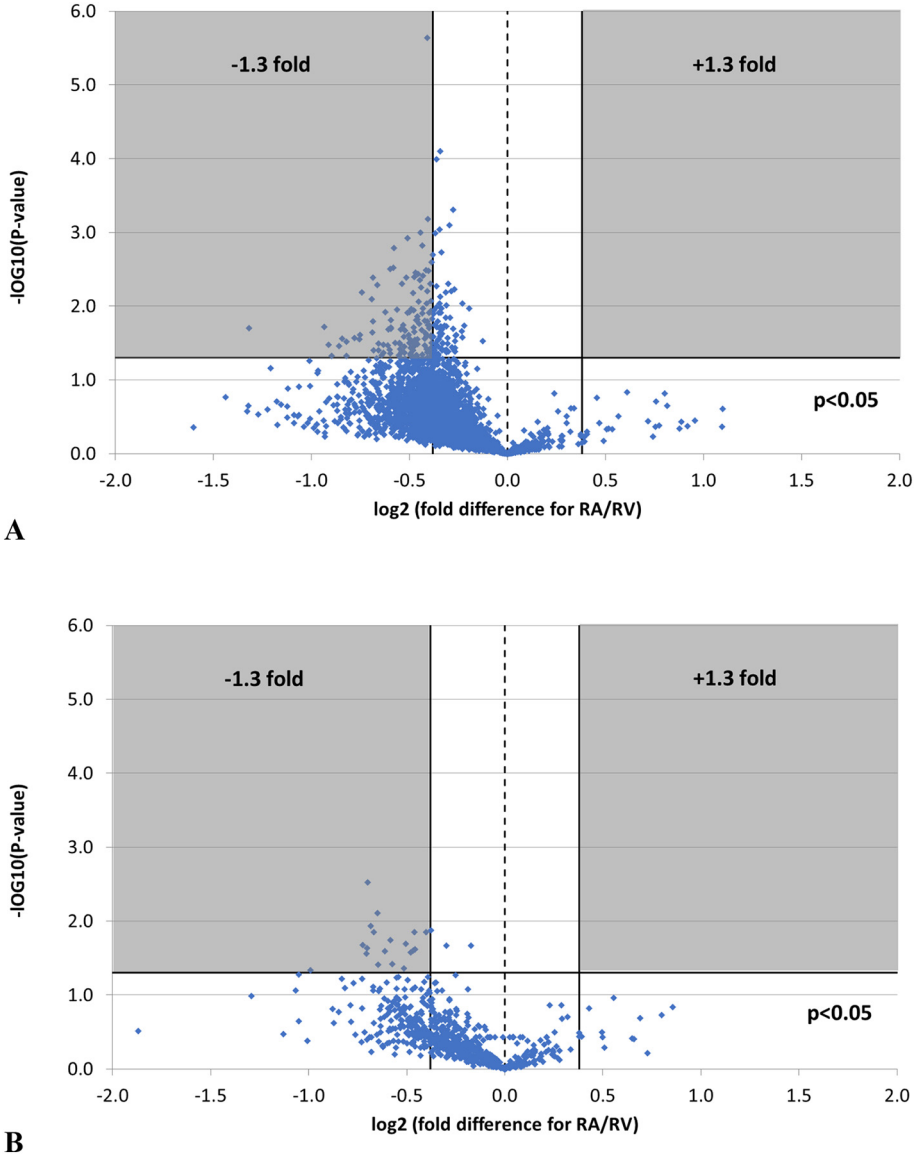

Fig. 1. Volcano plot of entire set of proteins (A) and phosphoproteins (B) quantified in the right atria (RA) versus right ventricle (RV) in patients with ventricular septal defect (VSD) (a negative log2(fold change) indicates higher expression in right ventricular samples). Each point represents the difference in expression (log2 fold difference) between the groups, and the associated significance of this change (independent paired samples *t*-test). Proteins significantly altered (± 1.3-fold, $p < 0.05$) are found within the grey shaded boxes.

Table 2
Diseases or functions with enriched protein expression for significantly higher total protein levels in the right ventricle compared to the right atria, of VSD patients.

Diseases or functions annotation	p-Value	Molecules	# Molecules
Mitochondrial disorder	5.43E−12	BCS1L, CARS2, COA6, DLAT, ECHS1, ETFA, FARS2, IBA57, MIPEP, NDUFS1, PDHB, PDHX, SCO2, TACO1, TSFM	15
Lactic acidosis	5.29E−08	COA6, DLAT, PDHB, PDHX, SCO2, TSFM	6
Oxidative phosphorylation deficiency	2.18E−07	CARS2, COA6, FARS2, MIPEP, SCO2, TSFM	6
Cell spreading of keratinocytes	4.14E−07	ITGA5, ITGAV, ITGB1	3
Enzymopathy	8.71E−07	ALDH3A2, BCS1L, CLPB, CPT1A, DLAT, ETFA, HEXA, MCCC2, NDUFS1, PDHB, PDHX, SCO2, TACO1	13
Pyruvate decarboxylase deficiency	4.09E−06	DLAT, PDHB, PDHX	3
Autosomal recessive disease	4.31E−06	ACO2, CARS2, CLPB, COA6, COCH, CSRP3, DHTKD1, ECHS1, EPS8, ETFA, FARS2, GCSH, HARS2, HEXA, HMGCL, IBA57, ITGB1, KLHL41, MCCC2, MIPEP, MMAB, PDHX, PHYH, PSMB8, SCN5A, SPG7, SUCLA2, TNIK, TSFM, VLDLR	30
Primary dilated cardiomyopthy	5.23E−06	CSRP3, FLNC, LDB3, SCN5A, SCO2, TSFM	6
Hypertrophic cardiomyopathy	1.82E−05	COA6, CPT1A, CSRP3, FLNC, LDB3, SCN5A, SCO2	7
Hereditary myopathy	1.89E−05	BCS1L, CSRP3, DHTKD1, ECHS1, FLNC, IBA57, ITGB1, KLHL41, LDB3, MTM1, NDUFS1, SCN5A, SCO2, SPG7, SUCLA2, TAX1BP3	16

metabolic diseases were identified, of which mitochondrial disorder was the most enriched (the others being lactic acidosis, oxidative phosphorylation deficiency, enzymopathy, and pyruvate decarboxylase deficiency). Cardiomyopathy was identified twice, along with cell spreading of keratinocytes, autosomal recessive disorder and hereditary myopathy.

Analysis of significantly changed phosphorylated proteins between chambers showed that there was significant enrichment of those involved in the regulation of cell communication by electrical coupling (GO:0010649, 3 proteins; HRC-Ser311, CAV1-Tyr42, and CASQ2-Ser339) and the negative regulation of ion transport (GO:0043271, 4 proteins; HRC-Ser311, CAV1-Tyr42, and CASQ2-Ser339, PACSIN3-Ser319).

3.2. Heart contraction

Proteins annotated in the Gene Ontology terms ‘Cardiac Muscle Contraction’ (GO:0060048), ‘Heart Contraction’ (GO:0060047) and ‘Regulation of heart contraction’ (GO:0008016), and including calcium signalling proteins (Supplementary Table F) were compared to our dataset. Of the 274 proteins, 107 accession numbers (representing 99 unique proteins) were detected in our samples, but only 4 (CXADR, CSRP3, GSK3A, and SCN5A) were significantly different between chambers (all lower in RA).

26 phosphorylated proteins were detected (some with multiple phosphorylation sites) but only 3 were significantly higher in RV samples (HRC-Ser311, CAV1-Tyr42, and CASQ2-Ser339).

3.3. Structural and extracellular matrix proteins

Structural proteins in human skeletal muscle [21] were cross-referenced against protein lists found in our samples.

In the RA and RV of patients with VSD, 106 accession numbers (representing 94 proteins) were detected, and these were also found in skeletal muscle. Of these, only five (5.3%) were significantly different between heart chambers (β-endolase (ENO3; involved in calcium signalling) and microtubule-associated protein (MAP4; promotes microtubule assembly), and FLNC, CSRP3 and LDB3), all being lower in the right atria. Phospho-peptide enrichment suggested significantly lower expression of four distinct phosphorylated proteins in the right atria: striated muscle preferentially expressed protein kinase (SPEG) – Serine (Ser)-2448, obscurin (OBSCN) – Ser-5563, EIF5B protein (fragment; EIF5B) – Ser-164 and sarcoplasmic reticulum histidine-rich calcium-binding protein (HRC) – Ser-311, which are involved in myocyte cytoskeletal assembly, sarcoplasmic M-band, protein biosynthesis and calcium signalling respectively.

3.4. Mitochondrial proteins

GOrilla enrichment analysis found that significantly altered proteins were found to be particularly enriched in the mitochondria (GO:0005739-Mitochondrion, 47 proteins; GO:0005759-Mitochondrial matrix, 22 proteins) so the list of proteins and phospho-proteins derived from the analysis was searched using the search terms ‘mitochondria’ and ‘mitochondrial’. The list was also searched using gene lists for ‘Mitochondrial respiratory chain complexes’ and ‘Mitochondrial respiratory chain complex assembly factor’, obtained from the HGNC (HUGO Gene Nomenclature Committee) database [22].

401 mitochondrial proteins were detectable in both the right atria and right ventricle of patients with VSD, of which 30 were significantly different between chambers (Table 3); all being higher in the RV. The majority of these (17 proteins, 57%) have catalytic activity (hydrolase, isomerase, ligase, lyase, oxidoreductase, and transferase activity) [23] (Table 3). However, only seven phosphorylated mitochondrial proteins were detectable (NDUFB4-Ser26, TOMM70A-Ser91, TOMM20-Ser138, MFF-Ser17, CLUH-Ser702, BCKDHA-Ser308 and -Ser318, BCKDK-Ser31, PUS1-Thr133), none of which changed between chambers.

4. Discussion

To our knowledge, this is the first comparison of the proteome of the right atria and right ventricle of infants with ventricular septal defect. It is surprising to discover that there were not more changes in the proteins involved in heart contraction, as the primary function of the ventricles is to pump blood out of the heart, whereas the main mechanical purpose the atria serves is to be a minimally contractile reservoir. The atria of our cohort of patients are mostly normal, however the proteins associated with contraction of the ventricle are probably changed in the presence of the VSD due to marked hypertrophy brought on by exposure to left ventricular pressure in systole. Previous studies have shown that ventricles have a higher expression of genes associated with contractility [24], albeit studies tend to be carried out in adults. Very few studies are carried out in infants, so much of what we know must be extrapolated from adult or animal data, and a lack of healthy control tissue could make interpretation of our results difficult.

Genomics studies have been carried out on heart tissue however these cannot necessarily be directly compared to proteomics. Out of a set of 13 genes described by Barth et al. [24] as known to be expressed differently between atria and ventricular samples, only three were also found in all of our samples (natriuretic peptide A, NPPA; myosin light chain 3, MYL3; cardiac phospholamban, PLN). Of these, none changed significantly, although there was a non-significant > 1.3 fold-change in MYL3 and PLN. They also showed that genes related to metabolism and mitochondria dominated the expression in ventricular myocardium, as we have shown here. A genomics study comparing the RA and LV from adults undergoing aortic valve replacement showed that genes with higher expression in the LV were mainly associated with contractility [16]. Proteome differences between chambers in fetal hearts obtained from elective terminations of healthy pregnancies could perhaps be seen as control healthy hearts [13]. They identified a number of markers higher in atrial (MYL7, NPPA, GJA5, PAM, WBP11, and GNAO1) and ventricular (MYL2, MYL3, MYL5, MYH7, GJA1, RPL3L) tissue. All of these, with the exception of GJA1 and MYL5, were detected in our samples, however none were significantly altered; MYL2, MYH7 and RPL3L (cDNA) were increased non-significantly in our ventricular samples. It is unclear whether the similar expression between chambers is caused by VSD, or due to the small sample size. In the study by Lu et al. [13] they do not appear to have separated the chambers into the left versus right heart, so any differences may be confounded.

Our analysis showed that a large proportion of the proteins which were higher in the right ventricle, were associated with the mitochondria, whose primary function is ATP production, via the oxidative phosphorylation (OXPHOS) pathway. For normal contraction and basal metabolism, the human (adult) heart consumes 30 kg of ATP per day [25], and to maintain this demand, ventricular myocardium contains a large volume of mitochondria (~35% of cardiomyocyte volume in rats) [26]. The number and size of ventricular mitochondria increase with age; a change not seen in atrial cardiomyocytes. Adult atrial myocytes are smaller than ventricular ones, being five times shorter (20 μm and 100 μm respectively) and have a smaller diameter [27], and lower mitochondrial content leading to reduced activity of oxidative enzymes, including succinic dehydrogenase (respiratory Complex II) which participates in the citric acid cycle and electron transport chain [28,29]. The abundance of glycogen in atrial myocytes correlates well with the higher activity of phosphorylase, transglycosidase and glycogen synthetase [29]. Incorporation of ^{3}H-leucine, a measure of the level of protein synthesis at most stages of cardiomyogenesis, is of the same order in both atria and ventricular, except during the perinatal period where ventricular labelling is 10–25% higher [29]. The mitochondrial and nuclear genome are able to produce the ~90 proteins necessary for OXPHOS [30]. Aminoacyl-tRNA synthetases (ARSs) are pivotal substrates in the translation of mRNA to the correct amino acid sequences

Table 3
Significantly increased mitochondrial proteins in the right ventricle (vs. right atria).

Accession	Gene	Description	Enzyme Activity
Q9P1A0	NDUFS1	NDUFS1 protein OS = *Homo sapiens* GN = NDUFS1 PE = 2 SV = 1 - [Q9P1A0_HUMAN]	Oxidoreductase
Q9BQ48	MRPL34	39S ribosomal protein L34, mitochondrial OS = *Homo sapiens* GN = MRPL34 PE = 1 SV = 1 - [RM34_HUMAN]	
P43897	TSFM	Elongation factor Ts, mitochondrial OS = *Homo sapiens* GN = TSFM PE = 1 SV = 2 - [EFTS_HUMAN]	
Q96HY7	DHTKD1	Probable 2-oxoglutarate dehydrogenase E1 component DHKTD1, mitochondrial OS = *Homo sapiens* GN = DHTKD1 PE = 1 SV = 2 - [DHTK1_HUMAN]	Oxidoreductase
Q6UWS5	PET117	Protein PET117 homolog, mitochondrial OS = *Homo sapiens* GN = PET117 PE = 3 SV = 1 - [PT117_HUMAN]	
B2RBJ8	QRSL1	Glutamyl-tRNA(Gln) amidotransferase subunit A, mitochondrial OS = *Homo sapiens* GN = QRSL1 PE = 2 SV = 1 - [B2RBJ8_HUMAN]	Hydrolase/Ligase
P30837	ALDH1B1	Aldehyde dehydrogenase X, mitochondrial OS = *Homo sapiens* GN = ALDH1B1 PE = 1 SV = 3 - [AL1B1_HUMAN]	Oxidoreductase
Q9BT30	ALKBH7	Alpha-ketoglutarate-dependent dioxygenase alkB homolog 7, mitochondrial OS = *Homo sapiens* GN = ALKBH7 PE = 1 SV = 1 - [ALKB7_HUMAN]	
Q6QN92	GCSH	Mitochondrial glycine cleavage system H-protein (Fragment) OS = *Homo sapiens* PE = 2 SV = 1 - [Q6QN92_HUMAN]	
P83111	LACTB	Serine beta-lactamase-like protein LACTB, mitochondrial OS = *Homo sapiens* GN = LACTB PE = 1 SV = 2 - [LACTB_HUMAN]	Hydrolase
Q9HA77	CARS2	Probable cysteine—tRNA ligase, mitochondrial OS = *Homo sapiens* GN = CARS2 PE = 1 SV = 1 - [SYCM_HUMAN]	Ligase
P11177	PDHB	Pyruvate dehydrogenase E1 component subunit beta, mitochondrial OS = *Homo sapiens* GN = PDHB PE = 1 SV = 3 - [ODPB_HUMAN]	Lyase/Oxidoreductase
P82932	MRPS6	28S ribosomal protein S6, mitochondrial OS = *Homo sapiens* GN = MRPS6 PE = 1 SV = 3 - [RT06_HUMAN]	
P27144	AK4	Adenylate kinase 4, mitochondrial OS = *Homo sapiens* GN = AK4 PE = 1 SV = 1 - [KAD4_HUMAN]	
Q96HS1	PGAM5	Serine/threonine-protein phosphatase PGAM5, mitochondrial OS = *Homo sapiens* GN = PGAM5 PE = 1 SV = 2 - [PGAM5_HUMAN]	
Q9NUJ1	ABHD10	Mycophenolic acid acyl-glucuronide esterase, mitochondrial OS = *Homo sapiens* GN = ABHD10 PE = 1 SV = 1 - [ABHDA_HUMAN]	
Q5JTJ3	COA6	Cytochrome c oxidase assembly factor 6 homolog OS = *Homo sapiens* GN = COA6 PE = 1 SV = 1 - [COA6_HUMAN]	
P13804	ETFA	Electron transfer flavoprotein subunit alpha, mitochondrial OS = *Homo sapiens* GN = ETFA PE = 1 SV = 1 - [ETFA_HUMAN]	Oxidoreductase
P82909	MRPS36	28S ribosomal protein S36, mitochondrial OS = *Homo sapiens* GN = MRPS36 PE = 1 SV = 2 - [RT36_HUMAN]	
O43819	SCO2	Protein SCO2 homolog, mitochondrial OS = *Homo sapiens* GN = SCO2 PE = 1 SV = 3 - [SCO2_HUMAN]	Oxidoreductase
Q9HCC0	MCCC2	Methylcrotonoyl-CoA carboxylase beta chain, mitochondrial OS = *Homo sapiens* GN = MCCC2 PE = 1 SV = 1 - [MCCB_HUMAN]	Ligase
Q7Z434	MAVS	Mitochondrial antiviral-signalling protein OS = *Homo sapiens* GN = MAVS PE = 1 SV = 2 - [MAVS_HUMAN]	
P30405	PPIF	Peptidyl-prolyl cis-trans isomerase F, mitochondrial OS = *Homo sapiens* GN = PPIF PE = 1 SV = 1 - [PPIF_HUMAN]	Isomerase
Q5T440	IBA57	Putative transferase CAF17, mitochondrial OS = *Homo sapiens* GN = IBA57 PE = 1 SV = 1 - [CAF17_HUMAN]	
P30084	ECHS1	Enoyl-CoA hydratase, mitochondrial OS = *Homo sapiens* GN = ECHS1 PE = 1 SV = 4 - [ECHM_HUMAN]	Isomerase/Ligase/Lyase/ Oxidoreductase
Q9Y305	ACOT9	Acyl-coenzyme A thioesterase 9, mitochondrial OS = *Homo sapiens* GN = ACOT9 PE = 1 SV = 2 - [ACOT9_HUMAN]	Hydrolase
Q99797	MIPEP	Mitochondrial intermediate peptidase OS = *Homo sapiens* GN = MIPEP PE = 1 SV = 2 - [MIPEP_HUMAN]	Hydrolase
Q7Z4Y4	AK3	GTP:AMP phosphotransferase AK3, mitochondrial OS = *Homo sapiens* GN = AK3 PE = 2 SV = 1 - [Q7Z4Y4_HUMAN]	
A0A0K0K1H7	HEL-S-284	Aconitate hydratase, mitochondrial OS = *Homo sapiens* GN = HEL-S-284 PE = 2 SV = 1 - [A0A0K0K1H7_HUMAN]	
O95363	FARS2	Phenylalanine—tRNA ligase, mitochondrial OS = *Homo sapiens* GN = FARS2 PE = 1 SV = 1 - [SYFM_HUMAN]	Ligase

found in these proteins, by catalysing the direct aminoacetylation of tRNAs with the correct anticodon sequence [31], and were found to be significantly enriched in the RV compared to RA of our patients. Mitochondrial phenylalanine-tRNA synthetase (FARS2), cysteine-tRNA synthase (CARS2), and glutaminyl-tRNA synthase (glutamine-hydrolyzing)-like 1 (QRSL1), two subunits of the cytoplasmic form, FARSA (alpha catalytic subunit) which forms a tetramer with FARSB (beta regulatory subunit), and cDNA with sequence similarity to HARS2 (histidyl-tRNA-synthetase 2) and WARS2 (tryptophanyl tRNA synthetase 2) were all increased in RV. Four further proteins were also up-regulated in the RV which are also known to be involved in mitochondrial translational elongation in protein synthesis (elongation factor Ts, the small mitochondrial ribosomal 28S subunits 6 and 36, and the large 39S subunit 34). The lower levels of these proteins seen in the RA correlates well with the reduced energy demands in this chamber. This is also reflected by the lower protein level of the cytoplasmic enzymes aconitase-1 and -2, dehydrogenase E1 and transketolase, and succinate-CoA ligase ADP-forming (ACO1, ACO2, DHTKD1 and SUCLA2 respectively) which are all involved in the TCA cycle which is at the core of cell metabolism. Of the 30 mitochondrial proteins higher in the RV, 22 have known catalytic activity [32] but the lack of significantly altered phosphorylated mitochondrial proteins suggests there is no change in the activation of these enzymes.

Atrial cardiomyocytes produce a potent natriuretic peptide (NPPA) which increases in response to atrial stretch (increased blood volume) but also increases in the RV in response to hypertrophy [33]. NPPA was detected in our patients, however surprisingly there is no difference between chambers. In the absence of control tissue it is hard to say whether the atrial levels are lower than normal, or the ventricular expression is higher. One patient appears to have much higher levels of NPPA in the right ventricle and lower levels in the atria (RV:RA ratio of 18.9), compared to the other patients (RV:RA ratio of 0.85 ± 0.78; mean ± SEM). When excluded, this leads to levels in the RV being 1.41-fold lower (albeit non-significantly) than those in the atria.

The main analysis performed was on proteins found in all patient samples. However, the data was also interrogated to find whether any proteins were only detected in either the RV or RA of the majority of the patients (detected in either RV or RA of ≥3 patients). Alpha-protein kinase 2 (ALPK2; a kinase that recognises phosphorylation sites in which surrounding peptides have an alpha-helical conformation) was found in the RV, but not the RA, of four patients, and the proteins Cytochrome C Oxidase Subunit 1 (COX1; involved in energy metabolism), Collagen-XXI alpha chain (COL21A1; involved in collagen biosynthesis), Protein FAM81A (FAM81A) and Drebrin-like protein (DBNL; has a role in actin cytoskeleton reorganization) were only found in the RV of three of the five patients (albeit not the same three each time). Gelsolin (GSN) was found only in the RA of three patients. Gelsolin has previously been shown to have increased expression in failing hearts [34].

Despite our patients being age-matched, paired atrial and ventricular samples being taken, and experience of the surgeon, an unknown variable is the exact location within the chamber wall from which the biopsies were taken, with slight discrepancies perhaps leading to unexpected differences. The right atria is made up of three separate sections (a venous component, an appendage and a vestibule) each derived differently embryonically [35] and as such may have very different proteomic characteristics. Increasing the patient sample size in the future would hopefully enable us to statistically minimise the effect of this variation.

5. Conclusions

We can conclude that there are differences in the proteome of the RA and RV of infants with VSD. As discussed, there were large differences in mitochondrial proteins, which are probably linked to the energy demands of the ventricle. We did not see much evidence for changes in proteins associated with hypertrophy in the right ventricle, or for differences in the contractile mechanisms between the two chambers studied. The findings described here will be beneficial to the future study of multiple congenital heart defects, where there are suspected changes in the atrial and ventricular function, such as atrial septal defect, or TOF. It is however clear that the right atria is not a good model of protein expression in the right ventricle, despite its relative ease of access. It is hoped that the findings described here will be beneficial to future research into heart chamber malformations in congenital heart defects.

Funding

Funding was obtained from Sparks, the Childrens charity * 13BTL01. This study was supported by the NIHR Biomedical Research Centre at the University Hospitals Bristol NHS Foundation Trust and the University of Bristol.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jprot.2018.03.022.

References

[1] J.I.E. Hoffmann, S. Kaplan, The incidence of congenital heart disease, J. Am. Coll. Cardiol. 39 (12) (2002) 1890–1900.
[2] M.S. Minette, D.J. Sahn, Ventricular septal defects, Circulation 114 (20) (2006) 2190–2197.
[3] R.A. Bloomfield, H.D. Lauson, A. Cournand, E.S. Breed, D.W. Richards, Recording of right heart pressures in normal subjects and in patients with chronic pulmonary disease and various types of cardio-circulatory disease, J. Clin. Invest. 25 (4) (1946) 639–664.
[4] V.A. Barnett, Cardiac myocytes, in: P.A. Iaizzo (Ed.), Handbook of Cardiac Anatomy, Physiology and Devices, Humana Press Inc., Totowa, NJ, 2005, pp. 113–122.
[5] I. Luss, P. Boknik, L.R. Jones, U. Kirchhefer, J. Knapp, B. Linck, H. Luss, A. Meissner, F.U. Muller, W. Schmitz, U. Vahlensieck, J. Neumann, Expression of cardiac calcium regulatory proteins in atrium v ventricle in different species, J. Mol. Cell. Cardiol. 31 (6) (1999) 1299–1314.
[6] A.W. Trafford, J.D. Clarke, M.A. Richards, D.A. Eisner, K.M. Dibb, Calcium signalling microdomains and the t-tubular system in atrial mycoytes: potential roles in cardiac disease and arrhythmias, Cardiovasc. Res. 98 (2) (2013) 192–203.
[7] M. Frisk, J.T. Koivumaki, P.A. Norseng, M.M. Maleckar, O.M. Sejersted, W.E. Louch, Variable t-tubule organization and Ca2+ homeostasis across the atria, Am. J. Physiol. Heart Circ. Physiol. 307 (4) (2014) H609–20.
[8] M.D. Bootman, D.R. Higazi, S. Coombes, H.L. Roderick, Calcium signalling during excitation-contraction coupling in mammalian atrial myocytes, J. Cell Sci. 119 (Pt 19) (2006) 3915–3925.
[9] R. Arora, G.L. Aistrup, S. Supple, C. Frank, J. Singh, S. Tai, A. Zhao, L. Chicos, W. Marszalec, A. Guo, L.S. Song, J.A. Wasserstrom, Regional distribution of T-tubule density in left and right atria in dogs, Heart Rhythm. 14 (2) (2017) 273–281.
[10] R. Heying, M. Qing, K. Schumacher, M. Sokalska-Duhme, J.F. Vazquez-Jimenez, M.C. Seghaye, Myocardial cardiotrophin-1 is differentially induced in congenital cardiac defects depending on hypoxemia, Futur. Cardiol. 10 (1) (2014) 53–62.
[11] V. Pelouch, M. Milerova, B. Ostadal, B. Hucin, M. Samanek, Differences between atrial and ventricular protein profiling in children with congenital heart disease, Mol. Cell. Biochem. 147 (1995) 43–49.
[12] H. Liu, G. Chen, H. Zheng, H. Qin, M. Liang, K. Feng, Z. Wu, Differences in atrial fibrillation associated proteins between the left and right atrial appendages from patients with rheumatic mitral valve disease: a comparative proteomic analysis, Mol. Med. Rep. 14 (5) (2016) 4232–4242.
[13] Z.Q. Lu, A. Sinha, P. Sharma, T. Kislinger, A.O. Gramolini, Proteomic analysis of human fetal atria and ventricle, J. Proteome Res. 13 (12) (2014) 5869–5878.
[14] A.R. Bond, D. Iacobazzi, S. Abdul-Ghani, M. Ghorbel, K. Heesom, M. Wilson, C. Gillett, S.J. George, M. Caputo, S. Suleiman, R.M.R. Tulloh, Changes in contractile protein expression are linked to ventricular stiffness in infants with pulmonary hypertension or right ventricular hypertrophy due to congenital heart disease, Open Heart 5 (1) (2018) e000716.
[15] M. Tsubakihara, N.K. Williams, A. Keogh, C.G. dos Remedios, Comparison of gene expression between left atria and left ventricles from non-diseased humans, Proteomics 4 (1) (2004) 261–270.
[16] J. Asp, J. Synnergren, M. Jonsson, G. Dellgren, A. Jeppsson, Comparison of human cardiac gene expression profiles in paired samples of right atrium and left ventricle collected in vivo, Physiol. Genomics 44 (1) (2012) 89–98.
[17] P. Ellinghaus, R.J. Scheubel, D. Dobrev, U. Ravens, J. Holtz, J. Huetter, U. Nielsch, H. Morawietz, Comparing the global mRNA expression profile of human atrial and ventricular myocardium with high-density oligonucleotide arrays, J. Thorac. Cardiovasc. Surg. 129 (6) (2005) 1383–1390.
[18] S. Kaab, A.S. Barth, D. Margerie, M. Dugas, M. Gebauer, L. Zwermann, S. Merk, A. Pfeufer, K. Steinmeyer, M. Bleich, E. Kreuzer, G. Steinbeck, M. Nabauer, Global gene expression in human myocardium-oligonucleotide microarray analysis of regional diversity and transcriptional regulation in heart failure, J. Mol. Med. (Berl) 82 (5) (2004) 308–316.
[19] H. Zhang, D.X. Gong, Y.J. Zhang, S.J. Li, S. Hu, Effect of mitochondrial aldehyde dehydrogenase-2 genotype on cardioprotection in patients with congenital heart disease, Eur. Heart J. 33 (13) (2012) 1606–1614.
[20] E. Eden, R. Navon, I. Steinfeld, D. Lipson, Z. Yakhini, GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists, BMC Bioinformatics 10 (2009) 48.
[21] K. Hojlund, B.P. Bowen, H. Hwang, C.R. Flynn, L. Madireddy, T. Geetha, P. Langlais, C. Meyer, L.J. Mandarino, Z. Yi, In vivo phosphoproteome of human skeletal muscle revealed by phosphopeptide enrichment and HPLC-ESI-MS/MS, J. Proteome Res. 8 (11) (2009) 4954–4965.
[22] H.D. [Internet]. HGNC Database, HUGO Gene Nomenclature Committee (HGNC), EMBL Outstation - Hinxton, European Bioinformatics Institute, Wellcome Trust Genome Campus, Hinxton, Cambridgeshire, CB10 1SD, UK.
[23] H. Mi, X. Huang, A. Muruganujan, H. Tang, C. Mills, D. Kang, P.D. Thomas, PANTHER version 11: expanded annotation data from gene ontology and reactome pathways, and data analysis tool enhancements, Nucleic Acids Res. 45 (D1) (2017) D183–D189.
[24] A.S. Barth, S. Merk, E. Arnoldi, L. Zwermann, P. Kloos, M. Gebauer, K. Steinmeyer, M. Bleich, S. Kaab, A. Pfeufer, P. Uberfuhr, M. Dugas, G. Steinbeck, M. Nabauer, Functional profiling of human atrial and ventricular gene expression, Pflugers Arch. 450 (4) (2005) 201–208.
[25] R. Ferrari, S. Censi, F. Mastrorilli, A. Boraso, Prognostic benefits of heart rate reduction in cardiovascular disease, Eur. Heart J. Suppl. 5 (G) (2003) G10–G14.
[26] E. Page, L.P. McCallister, Quantitative electron microscopic description of heart muscle cells. Application to normal, hypertrophied and thyroxin-stimulated hearts, Am. J. Cardiol. 31 (2) (1973) 172–181.
[27] M.J. Legato, Ultrastructure of the atrial, ventricular, and Purkinje cell, with special reference to the genesis of arrhythmias, Circulation 47 (1) (1973) 178–189.
[28] H. Plattner, F. Tiefenbrunner, W. Pfaller, Cytomorphometric and biochemical differences between the muscle cells in atria and ventricles of the guinea pig heart, Anat. Rec. 167 (1) (1970) 11–16.
[29] P.P. Rumyantsev, B.M. Carlson, Growth and Hyperplasia of Cardiac Muscle Cells, Harwood Academic, London, 1991.
[30] P. Smits, J. Smeitink, L. van den Heuvel, Mitochondrial translation and beyond: processes implicated in combined oxidative phosphorylation deficiencies, J Biomed Biotechnol 2010 (2010) 737385.
[31] M. Ibba, D. Soll, Aminoacyl-tRNA synthesis, Annu. Rev. Biochem. 69 (2000) 617–650.

[32] D. Szklarczyk, A. Franceschini, S. Wyder, K. Forslund, D. Heller, J. Huerta-Cepas, M. Simonovic, A. Roth, A. Santos, K.P. Tsafou, M. Kuhn, P. Bork, L.J. Jensen, C. von Mering, STRING v10: protein-protein interaction networks, integrated over the tree of life, Nucleic Acids Res. 43 (database issue) (2015) D447–52.

[33] H. Ruskoaho, J. Leppaluoto, Immunoreactive atrial natriuretic peptide in ventricles, atria, hypothalamus, and plasma of genetically hypertensive rats, Circ. Res. 62 (2) (1988) 384–394.

[34] J. Yang, C.S. Moravec, M.A. Sussman, N.R. DiPaola, D. Fu, L. Hawthorn, C.A. Mitchell, J.B. Young, G.S. Francis, P.M. McCarthy, M. Bond, Decreased SLIM1 expression and increased gelsolin expression in failing human hearts measured by high-density oligonucleotide arrays, Circulation 102 (25) (2000) 3046–3052.

[35] R.H. Anderson, A.C. Cook, The structure and components of the atrial chambers, Europace 9 (Suppl. 6) (2007) vi3–9.