Transcriptomics of heart biopsies reveals differences in patients with stable coronary artery disease with or without diagnostic parameters for heart failure with preserved ejection fraction.

Sarbashis Das Ph.D.[1],*, Christoffer Frisk M.Sc.[1],*, Maria J Eriksson M.D. Ph.D.[2,3], Matthias Corbascio M.D. Ph.D.[3,4], Camilla Hage Ph.D.[5,6], Chanchal Kumar Ph.D.[7], Anna Walentinsson Ph.D.[7], Michaela Asp M.Sc.[8], Joakim Lundeberg Ph.D.[8], Eva Maret Ph.D.[2,3], Hans Persson M.D. Ph.D.[9,10], Cecilia Linde M.D. Ph.D.[5,6] and Bengt Persson M.D. Ph.D.[1,11]

[1] Department of Cell and Molecular Biology, Science for Life Laboratory, Uppsala University, S-751 24 Uppsala, Sweden; [2] Karolinska University Hospital, Department of Clinical Physiology, S-171 76 Stockholm, Sweden; [3] Karolinska Institutet, Department of Molecular Medicine and Surgery, S-171 77 Stockholm, Sweden; [4] Karolinska University Hospital, Department of Thoracic Surgery, S-171 76 Stockholm, Sweden; [5] Karolinska Institutet, Department of Medicine, S-171 77 Stockholm, Sweden; [6] Karolinska University Hospital, Heart and Vascular Theme, S-171 76 Stockholm, Sweden; [7] AstraZeneca R&D, S-431 83 Mölndal, Sweden; [8] Science for Life Laboratory, Royal Institute of Technology, S-171 21 Stockholm, Sweden; [9] Karolinska Institutet, Department of Clinical Sciences, Danderyd Hospital, S-182 88 Stockholm, Sweden; [10] Danderyd Hospital, Department of Cardiology, S-182 88 Stockholm, Sweden; [11] Department of Medical Biochemistry and Biophysics, Science for Life Laboratory, Karolinska Institutet, S-17177 Stockholm, Sweden

*These authors contributed equally to the work.

Address for correspondence:
Bengt Persson
Dept of Cell and Molecular Biology
Science for Life Laboratory
Uppsala University
Box 596
S-751 24 Uppsala
Sweden
E-mail: bengt.persson@icm.uu.se

Short title: Transcriptomics of heart biopsies

Total number of words: 7640

Subject codes: heart failure, coronary artery disease, gene expression and regulation, echocardiography, computational biology

Abstract

Rationale. Heart failure affects 2–3 % of the adult Western population and its prevalence increases, in particular the proportion of heart failure with preserved left ventricular (LV) ejection fraction (HFpEF). Studies suggest that patients with HFpEF have altered myocardial structure and functional changes such as incomplete relaxation and increased cardiac stiffness. Translational research is scarce. We hypothesised that patients undergoing elective coronary bypass surgery (CABG) with HFpEF characteristics will show distinctive gene expression compared to patients with normal LV physiology.

Objective. To investigate differences in gene expression between patients with HFpEF characteristics and patients with normal LV physiology.

Methods and Results. Sixteen patients undergoing elective CABG were included in this study provided they had LV ejection fraction ≥ 45%. Myocardial biopsies were obtained from the LV during CABG for analysis of mRNA expression. Five out of 16 patients (31%) had echocardiographic characteristics and increased NTproBNP levels indicative of HFpEF and this group was used as HFpEF proxy, while 11 patients had Normal LV physiology. Utilizing principal component analysis on batch corrected normalized gene expression data, the samples clustered into two groups, corresponding to HFpEF proxy and Normal physiology. A total of 743 differentially expressed genes were identified and analysed to find functional correlations and regulatory properties. We found that the top biological functions associated with down-regulated genes in the HFpEF proxy group were cardiac muscle contraction, oxidative phosphorylation, cellular remodelling and matrix organization. In addition, genes regulated by the transcription factor *STAT4* and the tumour suppressor *TP53* were downregulated in samples from patients in the HFpEF proxy group.

Conclusions. This exploratory study could confirm our hypothesis that patients undergoing CABG with HFpEF characteristics compared to patients with Normal physiology had distinctive gene expression in cardiac biopsies with downregulated genes for myocardial contraction, energy supply, remodelling and fibrosis.

Keywords: cardiac biopsies, coronary artery disease, bioinformatics, LV function, RNA-Seq

Non-standard Abbreviations and Acronyms

CABG
DEG, differentially expressed gene
HFpEF
PREFERS

Introduction

Coronary artery disease and hypertension are the most common etiologic factors for heart failure (HF). Heart failure affects 2–3 % of the adult Western population[1, 2] and prevalence increases with age[3, 4]. In particular, the proportion of HF with preserved left ventricular (LV) ejection fraction (EF) – HFpEF – is increasing[5], with comparable poor prognosis[6, 7] as HF with reduced EF (HFrEF). There is currently no available evidence based therapy for HFpEF[8, 9]. There may be different pathophysiology in HFpEF compared to HFrEF[10]. It has been proposed that co-morbidities such as ischemia, hypertension and diabetes may be the drivers of disease progression in HFpEF[11, 12] through diverse mechanisms involving coronary microvascular inflammation, endothelial dysfunction leading to intra- and extracellular rearrangements. This has been demonstrated in incomplete relaxation of myocardial strips[13] and increased passive cardiac stiffness by titin changes and increased interstitial fibrosis[11, 14].

At the molecular level, gene expression programs are systematically regulated by transcription factors, chromatin regulators and other factors that are important for the establishment and maintenance of the cell state. Dysregulation of these programs can result in different diseases[15]. Studies of gene expression in heart tissue has a great potential to uncover the underlying molecular mechanisms in the evolution of HF.

In this study, we obtained myocardial biopsies from patients undergoing elective coronary bypass surgery (CABG) who all had LVEF ≥45%[16]. We hypothesise that patients with HFpEF characteristics will show distinctive gene expression compared to patients with Normal physiology.

Methods

Patients

Patients enrolled were scheduled for elective coronary artery bypass operation (CABG) without concomitant valve surgery and LVEF ≥45% reflecting a preserved LVEF as defined at the time of study design[16]. They all had angina pectoris with or without a previous myocardial infarction. Cardiac biopsies were obtained during CABG for analysis of mRNA expression in the myocardial tissue. All patients were assessed at a baseline visit four–eight weeks prior to CABG by clinical characteristics, echocardiography and blood sampling including natriuretic peptides. From the ongoing study CABG-PREFERS[16] we now report data from the initial patients.

Definitions

For the purpose of this paper, preserved LVEF was defined as LVEF ≥45%[16]. The patients were divided into two groups according to echocardiography, NTproBNP levels and HF guidelines definitions[3, 4, 17]. The group with echocardiographic characteristics and increased NTproBNP levels indicative of HFpEF[4, 16, 17] was called *HFpEF proxy* for the purpose of this study and was used as a representative for HFpEF even when not showing signs or symptoms of heart failure. The *Normal physiology* group had LVEF ≥45% and no signs of HFpEF[8, 18, 19].

The HFpEF proxy definition was based on the combination of the following five criteria; LVEF ≥45%, left atrial volume indexed for body surface area (LAVI) >34 ml/m^2 ratio of early mitral inflow wave velocity (E) to myocardial tissue early diastolic wave velocity (é) defined as E/e'>8 or é septal < 0.07 m/s or e' lateral < 0.09 m/s; tricuspid regurgitation velocity >2.8 m/s and additionally NTproBNP >125 ng/L. Three of five abnormal criteria were required to be abnormal to fulfil the definition (majority rule). In equivocal cases, classification was performed by consensus of two experts (M.J.E. and H.P.) in line with previous experiences from the CHARM echocardiographic substudy[20].

Information on patient characteristics and co-morbidities was collected at the baseline visit, recorded and extracted from the patient records[16].

Transthoracic Doppler echocardiography was performed according to guidelines as previously reported[16]. A Vivid 9 ultrasound system (Vingmed-General Electric, Horten, Norway) equipped with a phased array 3.5 MHz transducer (Doppler frequency 5.0 to 3.5 MHz) was used in all studies. Images were digitally stored on a dedicated server, and data analysis was performed offline on the EchoPAC workstation (GE EchoPAC sw only, Norway) by one experienced sonographer. The mean value of 3 cardiac cycles was calculated for each variable.

Tissue collection

From patients undergoing coronary artery bypass surgery, two core needle biopsies were taken from the lateral wall of the left ventricle before initiation of cardiac arrest and stored in –70° C as previously described[16] and used for mRNA analysis. Patients were prepared for surgery according to standard clinical routines with placement of a central venous line in the internal jugular vein, an arterial line in the distal radial artery and a peripheral venous line in the brachial vein. A midline sternotomy was performed and one or two mammary arteries are procured for usage as conduits.[21]
Matthias: please adjust

RNA extraction and sequencing

Total RNA was extracted using the RNeasy Fibrous Tissue Mini Kit (#74704, Qiagen). RNA libraries for sequencing were prepared using TruSeq Stranded mRNA Sample prep kit with 96 dual indexes (Illumina, CA, USA) according to the manufacturers instructions with the following changes. The protocols were automated using an Agilent NGS workstation (Agilent, CA, USA) using purification steps as described.[22, 23]

Clustering was done by 'cBot' and samples were sequenced on HiSeq2500 (HiSeq Control Software 2.2.58/RTA 1.18.64) with a 2x125 setup using 'HiSeq SBS Kit v4' chemistry. The Bcl to FastQ conversion was performed using bcl2fastq-v2.17.1.14 from the CASAVA software suite. The quality scale used is Sanger / phred33 / Illumina 1.8+.

Analysis of transcriptome (RNA-Seq) data

Whole transcriptome sequencing was performed for each biopsy. Initial quality checking of the sequencing raw reads was performed to identify potential outliers before doing further analysis using FastQC. Sequencing paired-end reads were mapped towards the human reference genome (version GR38) using Star Aligner[24] with default options, and Ensembl genome annotation (version 37) was used for subsequent analysis. Reads that mapped to the exons of the coding genes were counted using HTSeq[25]. Genes with count values of zero (i.e. no read detected) in all samples were filtered out before further analysis. Count data was also investigated for several potential biases such as RNA-degradation, GC content etc. using NOISeq[26] package in R (http://www.R-project.org). Normalization of the count data was performed using the Trim mean of M-values (TMM) approach[24].

Batch correction of the data was performed using ARSyNseq function of the NOISeq package[26]. Differential gene expression analysis was done using the function noiseqbio with parameters k=0.5, norm='n', lc=0, r=20, adj=1.5, a0per=0.9, which is recommended for human RNAseq analysis[26, 27]. For NOISeq, we set the parameter 'q' to 0.95, corresponding to a false discovery rate (FDR) < 0.05. Analysis and plots were generated in R environment using ggplot2 and VennDiagram. Principal component analysis was performed on the normalized batch corrected expression values using "princomp" function in R environment.

Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) was performed using SIMCA v14.1 (Umetrics, Umeå, Sweden) on the already normalized and batch corrected data to identify genes showing high variation in a pairwise manner. The Pareto scaling method was used in this case, since we wanted to detect small to medium feature differences.

Clustering Analysis

First, Spearman correlations of the gene expressions were calculated across all samples. Then dissimilarity measures were derived using 1-correlation, which was used as the distance for the subsequent clustering. Clustering of the differentially expressed genes was performed using unsupervised hierarchical clustering with agglomeration method as complete. Normalized expression data were standardized before plotting heatmap. Heatmap was plotted using the ComplexHeatmap package for R environment.

Functional analysis of the dysregulated genes

Gene set enrichment analysis for Gene Ontology (GO) terms with focus on biological processes (BP) and cellular component (CC) was performed for the DE genes using Enrichr[28] with the probability density function as p-value model. The DEGs in each comparison were used as foreground data or input and all the genes that were expressed in at least one sample in our transcriptome data were used as background data. The enrichment

was tested using Fisher's exact test with corrected p-value < 0.05. The DEGs were further analysed using the Ingenuity Pathways Analysis tool (IPA, http://www.ingenuity.com, Qiagen). This tool uses the information in the Ingenuity® Knowledge Base to assess signalling and metabolic pathways, upstream regulators, regulatory effect networks, and disease and biological functions that are likely to be perturbed based on a data set of interest (in our case, the DEGs). The IPA upstream analysis was performed to predict which regulators (i.e. any gene, protein or miRNA) that are activated or inhibited based on a calculated Activation Z-score, to explain the observed DEG changes in HFpEF proxy group vs Normal physiology group.

The IPA regulatory effect network analysis generated hypotheses for how a phenotype, function or disease is regulated by activated or inhibited upstream regulators. In our study, regulatory effect network analysis was used to specifically study the impact of the identified upstream regulators on downstream heart disease functions, given the observed gene expression changes in the two groups.

Ethics statement

This study was conducted according to the Declaration of Helsinki and approved by the local Ethics Committee. All patients were included following oral and written informed consent.

Results

Patients

From a total of 16 patients, 5 were classified as HFpEF proxy and 11 as Normal physiology. The clinical characteristics of all patients are summarized in Table 1. Majority were male with a median age of 68 years. Many had a history of hypertension (88%) and diabetes (50%) but only one had a previous myocardial infarction and revascularization reflecting that they had been subject to elective CABG procedures. Three patients had a diagnosis of heart failure, all in the HFpEF proxy group. Patient details including echocardiographic variables revealing HFpEF proxy or Normal physiology are given in Table 2.

Gene expression profiles of left ventricular tissue in HFpEF proxy and Normal physiology groups

The transcriptome sequencing resulted in an average of 26 million reads per biopsy sample. The initial quality of the sequenced reads was checked using FASTQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and subsequently mapped to the human reference genome (GRCh38). Number of mapped reads was between 17 and 30 million with an average of 24 million (Fig. S1) which covered more than 85% of the sequenced reads. Normalization and batch correction was performed using Trim mean of the M-values (TMM)[24] and ARSyNseq respectively after filtering out lowly expressed genes with values less than 1 CPM (counts per millions).

Principal Component Analysis

Principal Component Analysis (PCA) using batch corrected normalized gene expression of the 500 most highly variable genes revealed two clusters, corresponding to HFpEF proxy and Normal physiology along the first principal component 1 (PC1). The PCA model revealed that the largest variation of the PC1 could explain 22% of the variation while principal component 2 explained 14%. Using normalized batch corrected data, a validation of the correlation between these groups and sequencing batches was performed and found no batch effect contributing to this clustering (Fig. 1a).

Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA)

The OPLS-DA model depicted in Fig. 1b distinguished HFpEF proxy from Normal physiology using the first predictive component. An S-plot was generated to identify signature genes in both conditions (Fig. 1c). In the S-plot, the magnitude of the contribution of each gene to the OPLS-DA model (p[1]) was plotted against significance of the corresponding genes (p(corr)[1]). As depicted in Fig. 1c genes (marked in red) contributing most to the discrimination of HFpEF proxy were *MYH7, MYBPC3, TCAP, PTGDS, BSG, ALDOA, EEF2, IDH2, CRIP2, EEF1A2, PLN, CYCS, MYOZ2, MDH1* and *DCN*. Several of these genes were also found among the differentially expressed genes (below).

Genes dysregulated in HFpEF proxy patients

Differentially expressed genes (DEGs) were identified using NOIseqbio[26] with a false discovery rate (FDR) < 0.05. The NOIseqbio analysis identified 743 DEGs discriminating between HFpEF proxy and Normal physiology whereof the majority were down-regulated in HFpEF proxy samples compared with Normal physiology samples (**Fig. 2a**). The distribution of fold change as a function of significance level in all the cases revealed that more than 90% of significant DE genes had fold changes ranging from 0.38 to –1.4 in log2 scale (**Fig. 2a,b**). We observed that the vast majority of the DEGs were down-regulated in HFpEF proxy samples compared with Normal physiology samples (**Fig. 2a**). Among the 743 DEGs, 69 genes were transcriptional regulators, of which 67 were down- and 2 up-regulated. We also performed OPLS-DA and

plotted the OPLS-DA component using S-plot which results were generally in agreement with the NOIseqbio analysis. The complete list of up- and down-regulated genes is given in the Supplemental Table S1.

Functional analysis of the dysregulated genes in PEF

The top biological functions associated with down-regulated genes in HFpEF physiology were cardiac muscle contraction and extracellular matrix assembly/organisation (Fig. 2c, Supplemental Table S2). Enriched GO terms reflecting molecular function revealed genes for cadherin binding, kinase binding, actin filament and integrin binding (Fig. 2d, Supplementary Table S2). We found down-regulation of genes involved in cardiac muscle contraction: myosin heavy chain 6 and 7 (*MYH6, MYH7*), cardiac myosin-binding protein C (*MYBPC3*), cardiac troponin T2 (*TNNT2)*, titin cap (*TCAP*) and two potassium voltage-gated channels (*KCNH2, KCNQ1*).

Matrix-related genes that were down-regulated in PEF physiology include *LAMA5*, *LAMB2* coding for two laminin subunits (α5 and β2), *SPARC* coding for secreted protein acidic and rich in cysteine, and a number of collagens – collagens Iα1, IIIα1, IVα, IVβ1, VIα1, VIβ1 and XVIIIα1.

Among the up-regulated genes we identified lumican (*LUM*), phospholamban (*PLN*), myozenin 2 (*MYOZ2)*, and cytochrome c (*CYCS)*.

Upstream regulators and regulatory effect networks

Our analysis, using the Ingenuity Pathways Analysis (IPA) tool, suggested that out of the total set of 736 DE genes (7 Ensembl gene IDs were not recognized by IPA and hence excluded from this analysis), 381 DE genes (52 %) belong to known upstream regulators predicted to be activated or inhibited (Activation Z-score $\geq \pm 2$), at a Fisher's Exact test p-value≤ 0.05[29]. A comprehensive list of 52 upstream regulators along with predicted activation states is found in Supplemental Table S3. Of these, 34 were predicted to be inhibited and 18 were predicted to be activated.

The most significant upstream regulator identified in our analysis as being downregulated in patients with HFpEF proxy group was tumor suppressor *TP53,* which regulates a total of 96 DE genes (13 % of the DE gene set). Additional predicted inhibited upstream regulators included specific transcription factors *(SRF, MYOD1, HIF1A, STAT4, FOS,* and *GLI1*), receptor tyrosine kinases (*ERBB2, IGF1R,* and *INSR*) as well as growth factors (*VEGFA* and *TGFB1*). Among the predicted activated upstream regulators were Fas cell surface death receptor (*FAS*), histone demethylase *KDM5A*, estrogen receptor 1 (*ESR1*), mTOR2 subunit *RICTOR*, Notch signaling transcriptional repressor *HEY2* as well as specific microRNAs *(miR-296, mir-122, miR-199a, miR-124,* and *miR-125b)* among others.

A total of 16 regulatory effect networks were identified (Supplemental Table S4). The most causally consistent and densely connected network is shown in Figure 3, illustrating paths leading to heart failure and other heart diseases.

Co-expression based module analysis

Work ongoing – to be included in next version

Discussion

In summary, we found that patients in the HFpEF proxy group undergoing elective CABG displayed distinctive gene expression compared to patients with Normal physiology in this exploratory translational study of perioperative myocardial biopsies. The top biological functions associated with down-regulated genes in HFpEF proxy patients were cardiac muscle contraction, oxidative phosphorylation, endocytosis/cell remodelling, matrix organization and fibrosis. Further, genes regulated by transcription factor *STAT4* and tumor suppressor *TP53* were found to be down-regulated.

Avvakta placering: The down-regulated genes may lead to increased stiffness of cardiomyocytes and pathways associated with XXXXX seems to be downregulated and pathways indicating XXXX upregulated.

Patients

The patients investigated in this study were the initial group in whom the myocardial biopsies were obtained within the ongoing CABG-PREFERS study[16]. The myocardial left ventricular biopsies were acquired and prepared according to a standard method utilized to attain cardiac biopsies[21]. They represent patients with a clinical indication for CABG surgery but stable enough to undergo an elective procedure. Hence, very few had a previous myocardial infarction or coronary intervention and few had a previous HF diagnosis. The three patients with a clinical HF diagnosis were all in the HFpEF proxy group. Assessment of current HF symptoms with New York heart Association class (NYHA) is difficult in patients with angina pectoris before elective CABG but was grade II in more than half of the patients. Dyspnoea may be a symptom linked to angina pectoris and reversible cardiac dysfunction causing both chest pain and dyspnoea. Reversible ischemia as a cause for symptoms of dyspnoea is further reflected by low mean NT-pro BNP levels (range 127–367 ng/L). In contrast, hypertension and diabetes, co-morbidities identified as potential drivers of HFpEF, were very common.

HFpEF

HFpEF is more frequent today, which may be due to increasing life span of the population, improved survival after myocardial infarction and increasing rates of HF risk factors like hypertension, overweight, diabetes. However, the pathophysiology of this disease in not well understood at the transcriptome level. Already in the 1980s, it was recognized that ischemia might lead to diastolic dysfunction. We identified HFpEF characteristics in 31% of the group of patients planned for CABG, and yet all patients had significant ischemic heart disease by angiography and clinical symptoms, implying that other prevalent comorbidities like hypertension and diabetes may also play a role for development of HFpEF and possibly linked to microvascular dysfunction[30].

Imaging

HFpEF has diagnostic challenges and in an individual there are overlaps and grey zones that cannot be overlooked. Poor echocardiographic windows, tachyarrhythmias and atrial fibrillation makes measurements more difficult. In contrast to HFrEF, we need a number of 4–8 objective variables to describe the complex of structural and diastolic abnormalities that characterize HFpEF. The present guidelines advocate the use of at least 4 parameters for diagnosis [Ref EACVI/ASE guidelines] and risk prediction, some of these parameters may be used interchangeably (CHARMES, KaRen abstr/paper). In summary, more abnormal variables increase the precision of the HFpEF diagnosis and with the current study we have been able to use the majority criteria for HFpEF diagnosis according to present guidelines. If we in an individual patient have access to 5 parameters at least 3 should be abnormal to render HFpEF proxy. We have also used a consensus method by 2 reviewers of the data in few patients where uncertainty still was present. Thus, the five HFpEF proxy patients fulfil objective criteria for HFpEF but in general they have mild echocardiographic dysfunction and low NTBNPs. However, early stages of HFpEF may be more sensitive to treatment as has been shown in

studies with RAAS blockade (IPRESERVE and TOPCAT ref). Thus, RNA sequencing may be of particular value in these early or mild HFpEF patients for finding new pathophysiologic translational mechanisms.

Genes dysregulated in HFpEF proxy patients

To get an overview of the gene expression profiles, we performed PCA which distinguished PEF proxy patients from those with Normal physiology (Fig. 1a,b), suggesting that the two groups have different gene expression profiles. The downregulated genes (Supplemental Table 1) were analysed with respect to enrichment of GO terms (Fig. 2c,d), revealing that cardiac muscle contraction, oxidative phosphorylation, endocytosis and extracellular matrix organisation were associated with the dysregulated genes (Fig. 2c, Supplemental Table S2).

Cardiac contraction

Downregulated cardiac contraction genes may correlate with impaired systolic pump function in patients of the HpEF proxy group and several of the genes (Table 3) have been described in previous studies [refs]. The gene *TNNT2* encodes for the tropomyosin binding subunit in the troponin complex which is located in the thin filament of the striated muscle and regulates muscle contraction in response to alteration in the intracellular calcium ion concentration[31]. However, we found no differences between the 2 groups for measures of systolic function at rest, EF and global longitudinal strain (GLS) were similar in the two groups. The described downregulated genes for myocardial contraction may be related to more long-term changes caused by passive cardiac stiffness by fibrosis or titin changes[14]. Short-time adaptations of systolic function, cardiac relaxation and diastolic function may counteract long-term adaptations and are caused by other regulatory physiological functions rather than transcriptome proliferative changes. (kommentar). Our study shows that *RASD1*, coding for ras-related dexamethasone-induced protein 1, is downregulated in HFpEF proxy patients. *RASD1* is a modulator of cardiac endocrine function in response to volume overload underlying anti-natriuretic peptide excretion such as ANP and BNP [ref?]. This protein has also been shown to be downregulated in volume overloaded rat hearts[32].
Furthermore, *PLN*, coding for phospholamban was also up-regulated in HFpEF physiology. PLN is a pentamer and a major substrate for the c-AMP dependent kinase in cardiac muscle [ref]. Phospholamban modulates the activity of the sarcoplasmatic reticulum ATPas type 2 (SERCA 2a), which in turn modulates Ca^{2+} handling by the sarcoplasmatic reticulum and increases contractility at least in studies of CCM therapy[33]. These genes have been seen to be down-regulated in HFrEF [Tschöpe K in press].

Oxidative phosphorylation

During the cardiac cycle there is an active coupling between contractility during systole and and relaxation during early diastole. Cardiac contraction and relaxation are both active energy dependent processes that are closely linked, i e in the ischemic cascade. The finding of downregulated genes in HFpEF for oxidative phosphorylation and energy supply may thus be considered important both for systolic and diastolic function and may be crucial for development for HFpEF as regards both systolic and diastolic cardiac function and in relation to ischemia in these patients. Further, transcriptional changes may develop more slowly and be counteracted by short-term alterations of contractility and relaxation by changes in neurohormonal and sympathetic tone, calcium fluxes, and ischemia (Katz A Circulation 2000).

Extracellular matrix organisation

Our results show several downregulated genes involved in extracellular matrix assembly, which may intuitively influence remodelling and dilatation of the heart rather than increasing passive myocardial stiffness as is shown typical for HFpEF[14] due to increased synthesis of collagens typically with predominance for collagen I and III in the myocardium. This finding is therefore surprising and counterintuitive. However, the process of myocardial fibrosis is complex and involves a dynamic process of fibrous tissue turnover including active synthesis and degrading of collagen [refGonzales, JACC 2018, april]. The genes *LAMA5* and *LAMB2*, coding for two laminin subunits (α5 and β2), were also down-regulated in HFpEF proxy patients. The secreted

protein acidic and rich in cysteine (*SPARC*) is a matrix-cellular collagen binding protein serving a key role in collagen assembly into the extracellular matrix. Recent studies demonstrated that disruption of the *SPARC* gene, is associated with decreased capacity to generate organized, mature collagen fibres[34, 35].

ANKRD1 is a transcription factor known to interact with sarcomeric proteins in the myofibrillar stretch sensor system[36]. It has been observed that the expression of *ANKRD1* in both transcription and protein levels increased in failing heart[37, 38]. In our data we also see a trend of increasing mRNA expression in PEF physiology.

COL1A1 and *COL3A1* are fibrosis markers [ref] and showed decreased expression in HFpEF proxy, while other fibrosis markers (*MMP2*, *TIMP1*) did not show any significant difference.

We find that the gene *LUM* coding for lumican is among the most up-regulated genes in the HFpEF proxy group compared to Normal physiology. Lumican is an extracellular matrix localized proteoglycan associated with inflammatory conditions known to bind collagen. In a recent study, cardiac lumican was increased in experimental and clinical HF[39]. This study also indicated that inflammatory and mechanical stimuli induce lumican production by cardiac fibroblasts indicating a role in HF development. However, we are unaware of whether lumican has previously been shown to be up-regulated in CABG patients in the PEF proxy group.

Furthermore, the inflammatory markers *IL6ST* and *IL6R* are upregulated in the HFpEF proxy group. This is of interest since inflammation is believed to be one important driver for HFpEF (ref needed).

Upstream regulators and regulatory effect networks

The tumour suppressor *TP53* has recently been described as an important regulatory factor in the heart [ref PNAS]. In this study, we find a total of 96 DE genes (13%) to belong to those that are regulated by *TP53*. It has described to be increased in a mouse model of human dilated cardiomyopathy (DCM) (37), suggesting that elevation of p53 not only reflects the stressed status of the heart, but also plays a key role in the common path toward heart failure.

Conclusion

This exploratory study could confirm our hypothesis that patients undergoing elective CABG with HFpEF characteristics had distinctive gene expression findings in myocardial biopsy studies compared to patients with Normal physiology. One third of the patients showed HFpEF characteristics and we found four highly relevant biological areas that differed between HFpEF and Normal groups: cardiac contraction, energy supply, cell remodelling and matrix collagens. Down-regulation of these functions in patients of the HFpEF proxy group is complex to describe and understand, but our findings lends support to further study a larger patient cohort to find pathophysiological mechanisms that can explain and possibly find treatment for HFpEF.

Acknowledgments

The authors acknowledge support from the National Genomics Infrastructure in Stockholm funded by Science for Life Laboratory, the Knut and Alice Wallenberg Foundation and the Swedish Research Council, and SNIC/Uppsala Multidisciplinary Centre for Advanced Computational Science for assistance with massively parallel sequencing and access to the UPPMAX computational infrastructure.

Sources of Funding

Funding from the Science for Life Laboratory–Astra Zeneca; Mölndal, Sweden collaborative grant No. 1377 is gratefully acknowledged.

Disclosures

None.

References

1. Go AS, Mozaffarian D, Roger VL, et al. Heart disease and stroke statistics--2014 update: a report from the American Heart Association. *Circulation*. 2014;129:e28-e292
2. Paren P, Schaufelberger M, Bjorck L, Lappas G, Fu M, Rosengren A. Trends in prevalence from 1990 to 2007 of patients hospitalized with heart failure in Sweden. *Eur J Heart Fail*. 2014;16:737-742
3. Heidenreich PA, Albert NM, Allen LA, et al. Forecasting the impact of heart failure in the United States: a policy statement from the American Heart Association. *Circ Heart Fail*. 2013;6:606-619
4. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail*. 2016;18:891-975
5. Gerber Y, Weston SA, Redfield MM, Chamberlain AM, Manemann SM, Jiang R, Killian JM, Roger VL. A contemporary appraisal of the heart failure epidemic in Olmsted County, Minnesota, 2000 to 2010. *JAMA Intern Med*. 2015;175:996-1004
6. Owan TE, Hodge DO, Herges RM, Jacobsen SJ, Roger VL, Redfield MM. Trends in prevalence and outcome of heart failure with preserved ejection fraction. *N Engl J Med*. 2006;355:251-259
7. Bhatia RS, Tu JV, Lee DS, Austin PC, Fang J, Haouzi A, Gong Y, Liu PP. Outcome of heart failure with preserved ejection fraction in a population-based study. *N Engl J Med*. 2006;355:260-269
8. Yang J, Xu WW, Hu SJ. Heart failure: advanced development in genetics and epigenetics. *Biomed Res Int*. 2015;2015:352734
9. Butler J, Fonarow GC, Zile MR, et al. Developing therapies for heart failure with preserved ejection fraction: current state and future directions. *JACC Heart Fail*. 2014;2:97-112
10. Lam CS, Lund LH. Microvascular endothelial dysfunction in heart failure with preserved ejection fraction. *Heart*. 2016;102:257-259
11. Paulus WJ, Tschope C. A novel paradigm for heart failure with preserved ejection fraction: comorbidities drive myocardial dysfunction and remodeling through coronary microvascular endothelial inflammation. *J Am Coll Cardiol*. 2013;62:263-271
12. Lund LH, Donal E, Oger E, Hage C, Persson H, Haugen-Lofman I, Ennezat PV, Sportouch-Dukhan C, Drouet E, Daubert JC, Linde C, KaRen I. Association between cardiovascular vs. non-cardiovascular co-morbidities and outcomes in heart failure with preserved ejection fraction. *Eur J Heart Fail*. 2014;16:992-1001
13. van Heerebeek L, Borbely A, Niessen HW, Bronzwaer JG, van der Velden J, Stienen GJ, Linke WA, Laarman GJ, Paulus WJ. Myocardial structure and function differ in systolic and diastolic heart failure. *Circulation*. 2006;113:1966-1973
14. Zile MR, Baicu CF, Ikonomidis JS, Stroud RE, Nietert PJ, Bradshaw AD, Slater R, Palmer BM, Van Buren P, Meyer M, Redfield MM, Bull DA, Granzier HL, LeWinter MM. Myocardial stiffness in patients with heart failure and a preserved ejection fraction: contributions of collagen and titin. *Circulation*. 2015;131:1247-1259
15. Lee TI, Young RA. Transcriptional regulation and its misregulation in disease. *Cell*. 2013;152:1237-1251
16. Linde C, Eriksson MJ, Hage C, Wallen H, Persson B, Corbascio M, Lundeberg J, Maret E, Ugander M, Persson H, Stockholm County/Karolinska Institutet Dhfi. Rationale and design of the PREFERS (Preserved and Reduced Ejection Fraction Epidemiological Regional Study) Stockholm heart failure study: an epidemiological regional study in Stockholm county of 2.1 million inhabitants. *Eur J Heart Fail*. 2016;18:1287-1297
17. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed

with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. 2016;37:2129-2200
18. From AM, Borlaug BA. Heart failure with preserved ejection fraction: pathophysiology and emerging therapies. *Cardiovasc Ther*. 2011;29:e6-21
19. Nagueh SF, Smiseth OA, Appleton CP, et al. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *Eur Heart J Cardiovasc Imaging*. 2016;17:1321-1360
20. Persson H, Lonn E, Edner M, Baruch L, Lang CC, Morton JJ, Ostergren J, McKelvie RS, Investigators of the CES-C. Diastolic dysfunction in heart failure with preserved systolic function: need for objective evidence:results from the CHARM Echocardiographic Substudy-CHARMES. *J Am Coll Cardiol*. 2007;49:687-694
21. Popov S, Takemori H, Tokudome T, et al. Lack of salt-inducible kinase 2 (SIK2) prevents the development of cardiac hypertrophy in response to chronic high-salt intake. *PLoS One*. 2014;9:e95771
22. Lundin S, Stranneheim H, Pettersson E, Klevebring D, Lundeberg J. Increased throughput by parallelization of library preparation for massive sequencing. *PLoS One*. 2010;5:e10029
23. Borgstrom E, Lundin S, Lundeberg J. Large scale library generation for high throughput sequencing. *PLoS One*. 2011;6:e19119
24. Robinson MD, Oshlack A. A scaling normalization method for differential expression analysis of RNA-seq data. *Genome Biol*. 2010;11:R25
25. Anders S, Pyl PT, Huber W. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics*. 2015;31:166-169
26. Tarazona S, Furio-Tari P, Turra D, Pietro AD, Nueda MJ, Ferrer A, Conesa A. Data quality aware analysis of differential expression in RNA-seq with NOISeq R/Bioc package. *Nucleic Acids Res*. 2015;43:e140
27. Tarazona S, Garcia-Alcalde F, Dopazo J, Ferrer A, Conesa A. Differential expression in RNA-seq: a matter of depth. *Genome Res*. 2011;21:2213-2223
28. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, Koplev S, Jenkins SL, Jagodnik KM, Lachmann A, McDermott MG, Monteiro CD, Gundersen GW, Ma'ayan A. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res*. 2016;44:W90-97
29. Kramer A, Green J, Pollard J, Jr., Tugendreich S. Causal analysis approaches in Ingenuity Pathway Analysis. *Bioinformatics*. 2014;30:523-530
30. Sandesara PB, O'Neal WT, Kelli HM, Samman-Tahhan A, Hammadah M, Quyyumi AA, Sperling LS. The Prognostic Significance of Diabetes and Microvascular Complications in Patients With Heart Failure With Preserved Ejection Fraction. *Diabetes Care*. 2018;41:150-155
31. Liew CC, Dzau VJ. Molecular genetics and genomics of heart failure. *Nat Rev Genet*. 2004;5:811-825
32. McGrath MF, Ogawa T, de Bold AJ. Ras dexamethasone-induced protein 1 is a modulator of hormone secretion in the volume overloaded heart. *Am J Physiol Heart Circ Physiol*. 2012;302:H1826-1837
33. Imai M, Rastogi S, Gupta RC, Mishra S, Sharov VG, Stanley WC, Mika Y, Rousso B, Burkhoff D, Ben-Haim S, Sabbah HN. Therapy with cardiac contractility modulation electrical signals improves left ventricular function and remodeling in dogs with chronic heart failure. *J Am Coll Cardiol*. 2007;49:2120-2128
34. McCurdy S, Baicu CF, Heymans S, Bradshaw AD. Cardiac extracellular matrix remodeling: fibrillar collagens and Secreted Protein Acidic and Rich in Cysteine (SPARC). *J Mol Cell Cardiol*. 2010;48:544-549
35. Bradshaw AD. The role of secreted protein acidic and rich in cysteine (SPARC) in cardiac repair and fibrosis: Does expression of SPARC by macrophages influence outcomes? *J Mol Cell Cardiol*. 2016;93:156-161
36. Mikhailov AT, Torrado M. The enigmatic role of the ankyrin repeat domain 1 gene in heart development and disease. *Int J Dev Biol*. 2008;52:811-821

37. Zolk O, Frohme M, Maurer A, Kluxen FW, Hentsch B, Zubakov D, Hoheisel JD, Zucker IH, Pepe S, Eschenhagen T. Cardiac ankyrin repeat protein, a negative regulator of cardiac gene expression, is augmented in human heart failure. *Biochem Biophys Res Commun*. 2002;293:1377-1382
38. Herrer I, Rosello-Lleti E, Rivera M, Molina-Navarro MM, Tarazon E, Ortega A, Martinez-Dolz L, Trivino JC, Lago F, Gonzalez-Juanatey JR, Bertomeu V, Montero JA, Portoles M. RNA-sequencing analysis reveals new alterations in cardiomyocyte cytoskeletal genes in patients with heart failure. *Lab Invest*. 2014;94:645-653
39. Engebretsen KV, Lunde IG, Strand ME, Waehre A, Sjaastad I, Marstein HS, Skrbic B, Dahl CP, Askevold ET, Christensen G, Bjornstad JL, Tonnessen T. Lumican is increased in experimental and clinical heart failure, and its production by cardiac fibroblasts is induced by mechanical and proinflammatory stimuli. *FEBS J*. 2013;280:2382-2398

NOT CITED – to be removed if not needed

1. ESC Guidelines for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012. *Revista Española de Cardiología (English Edition)*. 2012;65:938.

30. Walter W, Sánchez-Cabo F, Ricote M. GOplot: an R package for visually combining expression data with functional analysis. *Bioinformatics (Oxford, England)*. 2015;31:2912–2914.

43. Desta L, Jernberg T, Löfman I, Hofman-Bang C, Hagerman I, Spaak J, Persson H. Incidence, temporal trends, and prognostic impact of heart failure complicating acute myocardial infarction. The SWEDEHEART Registry (Swedish Web-System for Enhancement and Development of Evidence-Based Care in Heart Disease Evaluated According to Recommended Therapies): a study of 199,851 patients admitted with index acute myocardial infarctions, 1996 to 2008. *JACC Heart Fail*. 2015;3:234–242.

48. Donal E, Lund L,Oger E, Reynaud A, Hage C, Haugen Lovfman I, Ennezat PV, Persson H, Bauer F, Sportouch-Dukhan C, Drouet E, Daubert JC, Linde C, Heart failure with preserved ejection fraction: predictors of short-term clinical evolution of disease state in patients hospitalized for acute heart failure. Data from the KaRen study. Archives of cardiovascular disease 2014;107:112-121.

70 Lang RM, Bierig M, Devereux RB, Flachskampf FA, et al. Recommendations for chamber quantification: a report from the American Society of Echocardiography's Guidelines and Standards Committee and the Chamber Quantification Writing Group, developed in conjunction with the European Association of Echocardiography, a branch of the European Society of Cardiology. J Am Soc Echocardiogr (2005); 18: 1440–1463.

71 Nagueh SF, Appleton CP, Gillebert TC, et al. Recommendations for the evaluation of left ventricular diastolic function by echocardiography. J Am Soc Echocardiogr. 2009;22(2):107-33

72 Nagueh SF, Smiseth OA, Appleton CP, et al. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2016;29(4):277-314.

Tables

Table 1: Patient characteristics. Data are expressed as median and quartiles (Q1;Q3) or number (%)

Variable	**Patients (n=16)**
Age (years) (median (Q1;Q3))	68 (63;73)
Sex Men/Women ((n (%))	15/1 (94%/6%)
Smoking Current ((n (%))	1 (6%)
Previous	10 (67%)
Concomittant disease	
Heart failure	3 (19%)
Atrial fibrillation (permanent or paroxysmal)	4 (25%)
Myocardial infarction	1 (6%)
Percutanious coronary intervention	3 (19%)
CABG	0 (0%)
Stroke/TIA	1 (6%)
PAD	1 (6%)
Hypertension	14 (88%)
Diabetes	8 (50%)
type 1	1 (6%)
type 2	7 (44%
COPD	0 (0%)
Anemia	0 (0%)
Cancer	1 (6%)
At enrolment	
BMI (kg/m2)	27 (24;30)
Systolic blood pressure (mmHg)	135 (130;145)
Diastolic blood pressure (mmHg)	80 (75;80)
Heart rate (beats/minute)	66 (57;77)
Creatinine (µmol/L)	85 (71;92)
Hemoglobin (g/L)	144 (137;153)
Sodium (mmol/L)	140 (138;141)
Potassium (mmol/L)	4.0 (3.8;4.3)
Troponin T (ng/L)	10 (7;17)
NT-proBNP (pmol/L)	205 (127;367)
LDL (mmol/L)	3.0 (2.5;3.6)
HbA1c (mmol/mol)	41 (37;46)
Urate (µmol/L)	365 (263;423)
Treament ((n (%))	
Nitrates (long standing)	8 (50%)
Antiplatelet	13 (81%)
Anticoagulants	2 (13%)
Betablocker	14 (88%)
ACE inhibitor	7 (44%)
ARB	10 (63%)

Statins	15 (94%)
Loop diuretics	0 (0%)
Tiazide diuretics	4 (25%)

ACE = Angiotensin converting enzyme
ARB = Angiotensin II receptor blocker
BMI=Body Mass Index
COPD=Chronic Obstructive Pulmonary Disease
CABG= Coronary Artery Bypass Surgery
NT-proBNP=N-Terminal pro-Brain Natriuretic Peptide
PAD=Peripheral Artery Disease
TIA= Transient Ischemic Attack

Table 2: List of patients included in this study with notations of disease status (PEF/Normal), age, gender, echocardiographic parameters, and comorbidities.

Patient nr	**Group**	**Age**	**Gender**	**Hypertension**	**Diabetes type I**	**Diabetes type II**	**Atrial fibrillation**	**Stroke/TIA**	**Peripheral vasc disease**	**LVEF** (%)	**LAVI** (mL/m2)	**E/e'**	**E'sept** (m/s)	**E'lat** (m/s)	**TR_Vmax**(m/s)	**NTproBNP** (ng/L)	**LVGLS %**	**LVMI** (g/ m^2)	**RWT**	**LVDed** (mm)	**IVSTed** (mm)	**PWTed** (mm)
1	PEF	81	M	x			x			68	43.5	10.6	0.060	0.070	2.4	697	-21.1	102	0.38	48	11	9
2	PEF	58	F	x		x				63	52.1	13.1	0.062	0.075	na	161	-20.4	94	0.45	44	13	10
9	PEF	71	M	x						54	40.3	8.8	0.070	0.080	2.5	190	-12.9	107	0.42	52	11	11
11	PEF	77	M	x		x	x		x	49	44.1	11.4	0.064	0.098	2.9	2160	-15.2	89	0.49	45	11	11
15	PEF	75	M	x		x			x	48	37.1	12.0	0.048	0.060	2.8	298	-15.8	121	0.32	56	13	9
3	Normal	67	M	x				x		57	31.5	5.5	0.050	0.070	na	338	-16.6	83	0.44	45	11	10
4	Normal	68	M	x						60	25.0	5.4	0.070	0.140	na	na	-16.8	101	0.39	46	14	9
5	Normal	73	M				x			57	33.1	5.8	0.065	0.120	na	396	-18.4	76	0.33	48	9	8
6	Normal	49	M	x		x				48	32.0	7.6	0.098	0.100	2.0	181	-11.9	84	0.32	50	12	8
7	Normal	64	M	x						56	27.9	9.1	0.082	0.104	na	147	-17.6	80	0.42	43	14	9
8	Normal	65	M							60	27.3	7.8	0.080	0.100	na	262	-17.0	58	0.33	43	10	7
10	Normal	60	M	x	x					66	33.5	8.0	0.072	0.124	na	219	-19.5	77	0.36	45	10	8
12	Normal	67	M	x		x	x			53	25.5	8.7	0.056	0.047	na	77	-18.5	103	0.36	50	12	9
13	Normal	61	M	x		x				65	30.0	6.8	0.092	0.116	na	107	-20.0	100	0.38	47	12	9
14	Normal	63	M	x		x				59	40.6	6.8	0.097	0.099	na	66	-19.9	84	0.37	49	11	9
16	Normal	68	M	x						61	35.8	7.6	0.094	0.113	na	84	-22.0	117	0.44	50	11	11

ID – study number, LVEF – left ventricular ejection fraction, LVGLS –left ventricular global longitudinal strain, LAVI – left atrial volume index, E – early mitral inflow velocity, e – early diastolic tissue velocity, sept – septal, lat – lateral, TR_Vmax - tricuspid regurgitation maximal velocity, LVMI - left ventricular mass index, RWT – relative wall thickness.

Bara pat 74 har AFib vid eko

Figures with Figure legends

Figure 1: Gene expression profiles of left ventricle tissues discriminate PEF physiology from Normal physiology. (a) Principal component analysis (PCA) score plot with the two principal components (PC1 and PC2) plotted on the x- and y-axis, respectively. Each data point represents one sample, which is color-coded according to the condition and form-coded according to the batch. Red corresponds to HFpEF while dark green red indicates Normal (b) Orthogonal projections to latent structures discriminant analysis (OPLS-DA) score plot for HFpEF and Normal. (c) S-plot of the OPLS-DA data showing the magnitude of each gene's contribution to the separation, p[1], in relationship to its significance, p(corr)[1]. Genes with most significant contribution are highlighted in red.

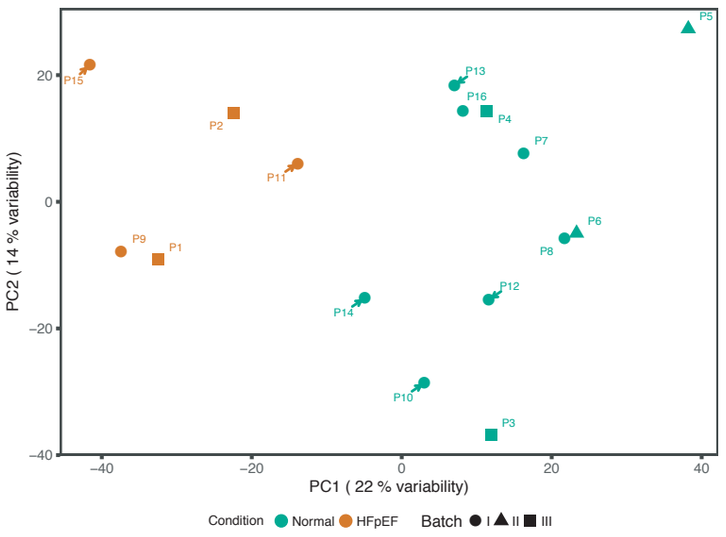
Figure 2: Identification and annotation of the dysregulated genes in HFpEF versus Normal. (a) Bar plot showing number of differentially expressed genes between HFpEF and Normal. The x-axis represents number of significantly dysregulated genes and y is the log2 fold change of the corresponding genes. (b) Volcano plot of the differentially expressed genes. The x-axis represents log2 fold change of HFpEF versus Normal while the y-axis indicates the differences of mean expression between HFpEF and Normal. Each point represents a gene and significantly differentially expressed genes are highlighted in green. A selection of significantly expressed genes are labelled with gene symbols. (c,d) GO annotations of biological process and molecular function, respectively, of the down-regulated genes. The horizontal bars show percentage of the down-regulated genes with the corresponding GO annotations (scale at bottom x axis), The red lines represent significance of the corresponding GO annotations (scale at top x axis).

Figure 3: Transcription factor regulatory effect networks identified using Ingenuity Pathway Analysis (IPA).
Description of network shapes and color coding:
Network nodes; upper panel; transcription factors, middle panel; differentially expressed genes, lower panel; biological functions and diseases. Network edges; solid line; direct interaction, dashed line; indirect interaction. Node colors (upper and lower panels); orange; predicted activation, blue; predicted inhibition. Node colors (middle panel); green; downregulated in data set, red; upregulated in data set (not represented in this network). Edge colors; orange; predicted activation, blue; predicted inhibition, yellow; findings inconsistent with state of downstream node, grey; effect not predicted.

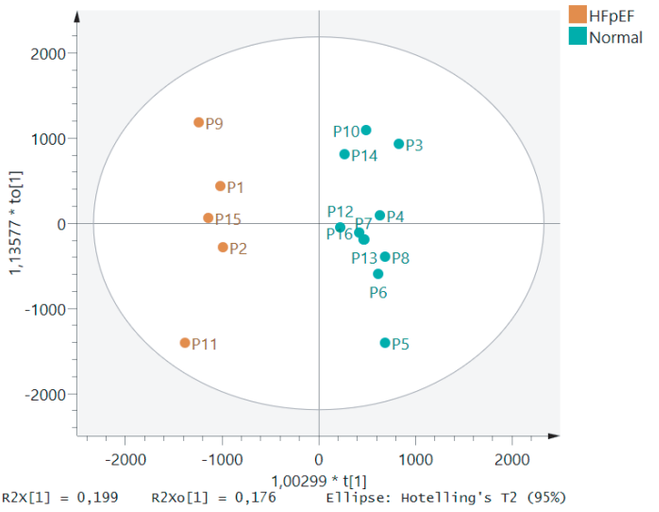
Figure 4: Predicted upstream regulators and their regulation. (a) Bar plot showing activation or inhibition scores of the upstream regulators. (b--f) Heat maps showing the gene expression of the genes affected by five transcription factors from panel a in PEF (colored red in Condition; left) compared to Normal (colored green in Condition; right). Patient numbers and conditions are shown at the bottom of each heatmap.

Figure-1:

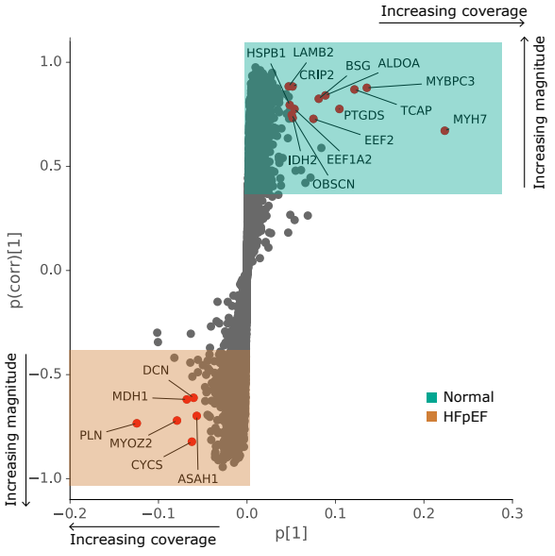
a.



b.



c.



d.

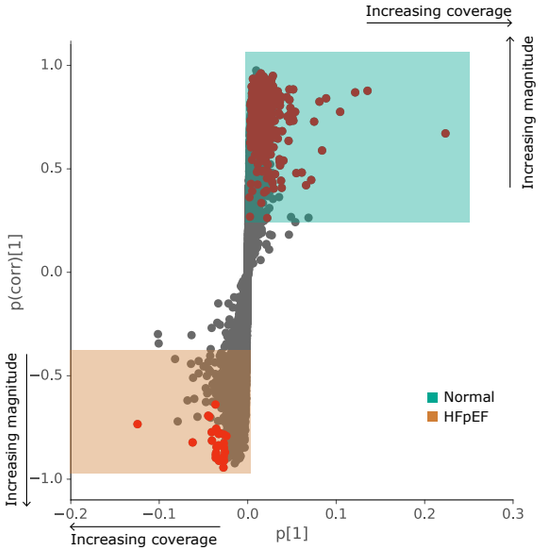
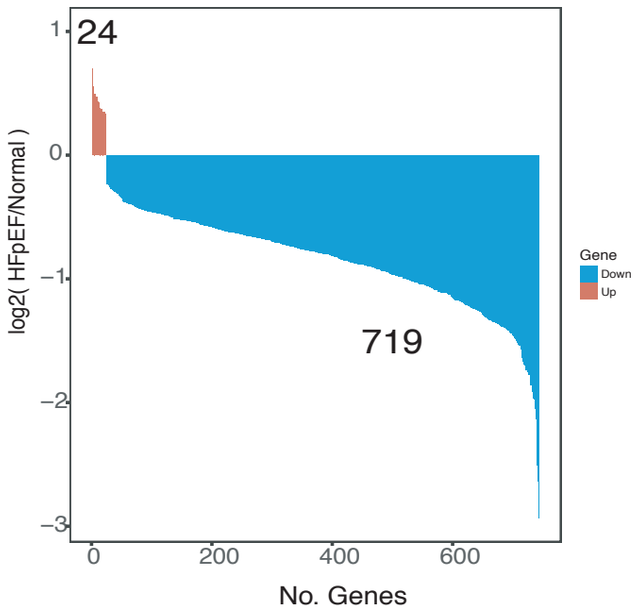
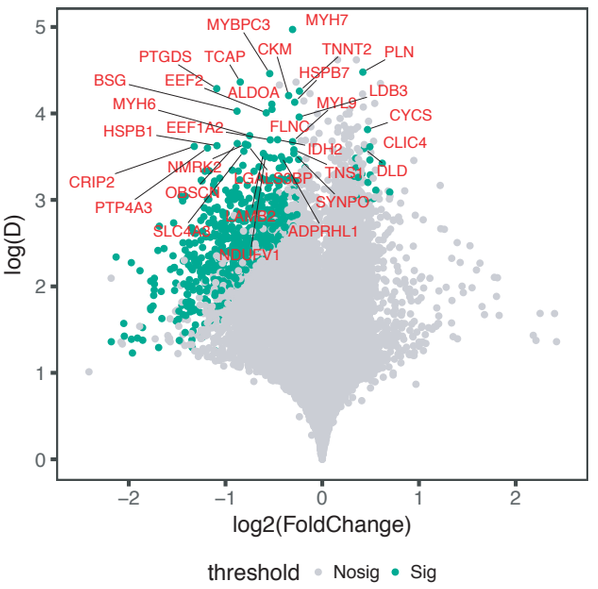


Figure-2:

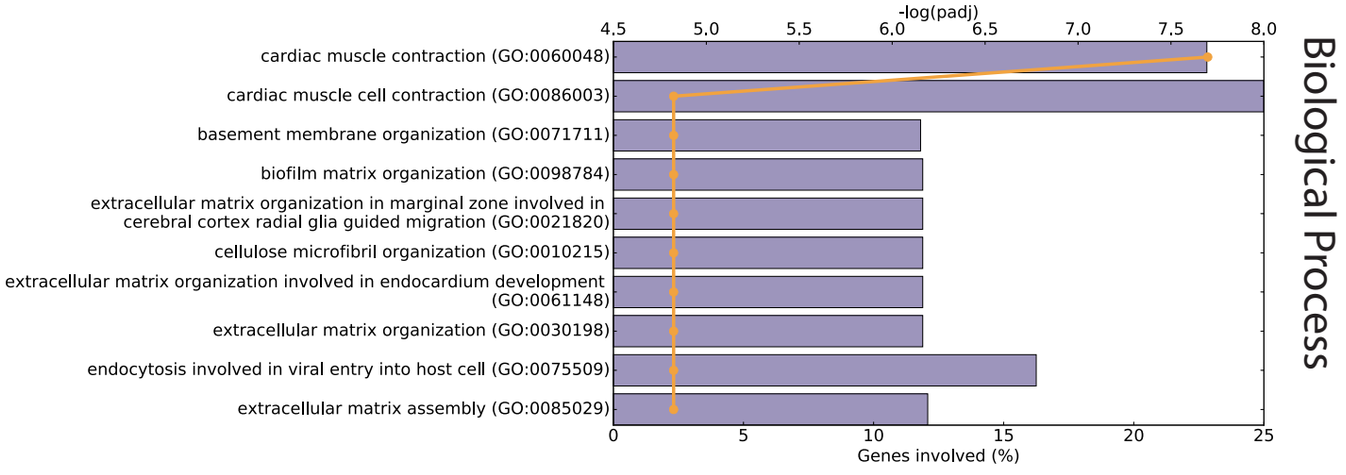
a.



b.



c.



d.

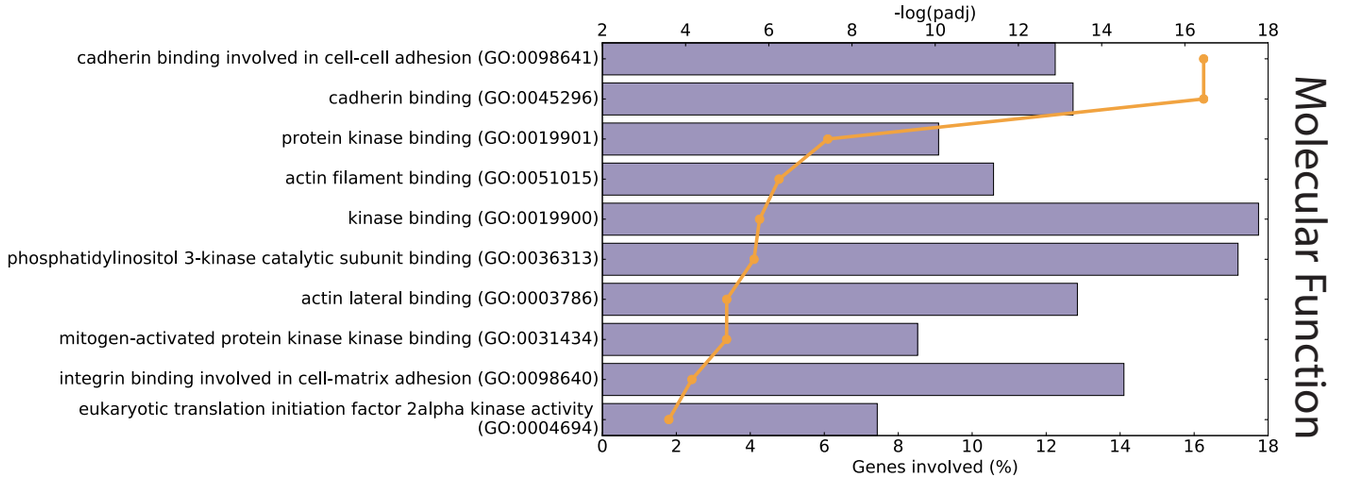
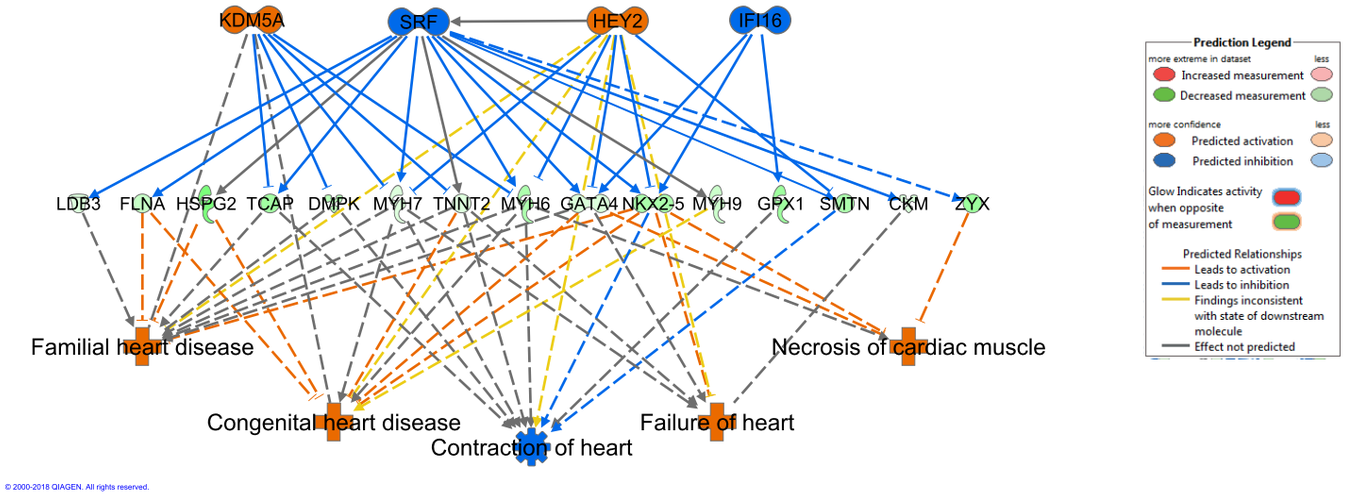


Figure-3:

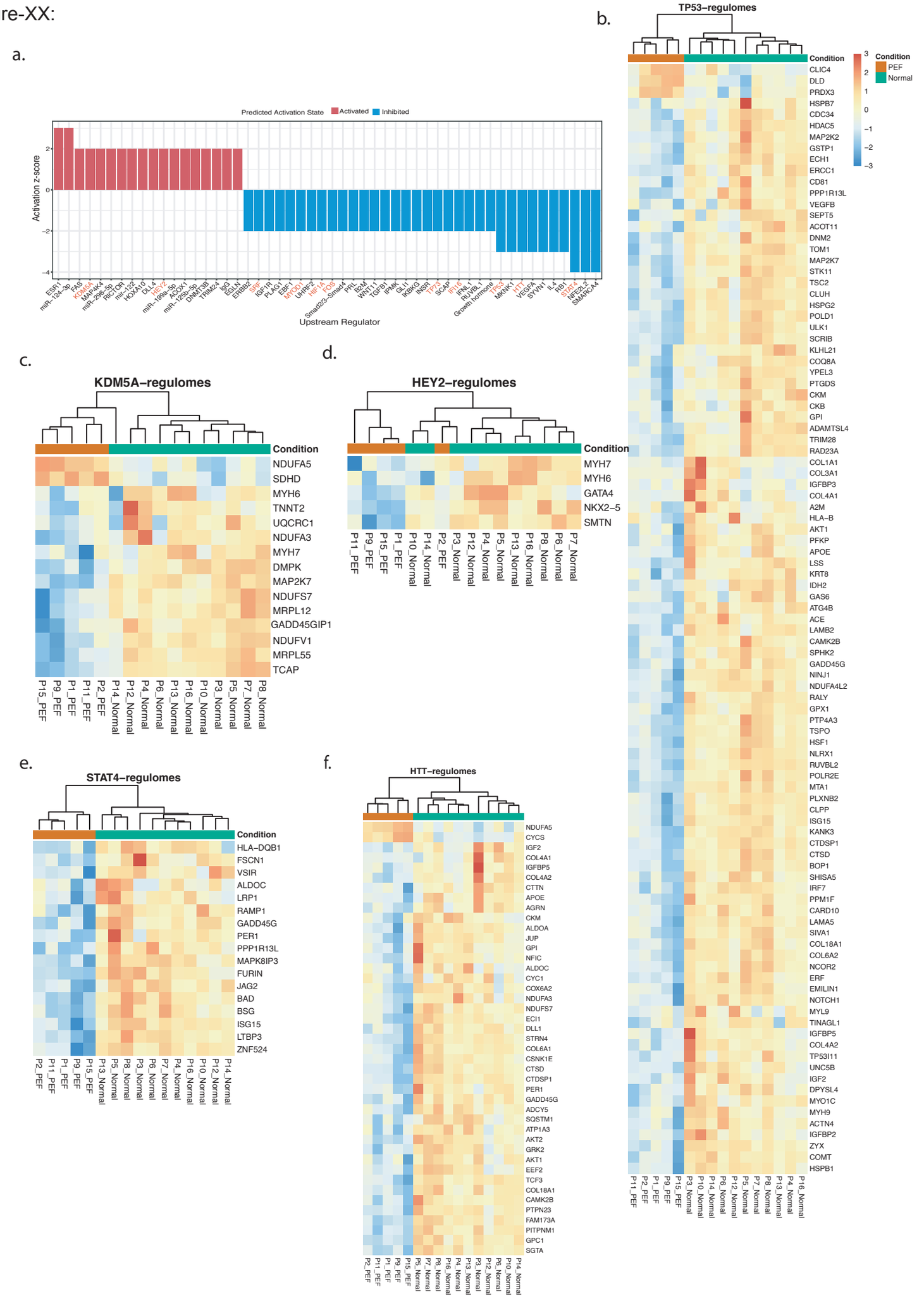

KDM5A
SRF
HEY2
IFI16
LDB3
FLNA
HSPG2
TCAP
DMPK
MYH7
TNNT2
MYH6
GATA4
NKX2-5
MYH9
GPX1
SMTN
CKM
ZYX
Familial heart disease
Necrosis of cardiac muscle
Congenital heart disease
Failure of heart
Contraction of heart
Prediction Legend
more extreme in dataset
less
Increased measurement
Decreased measurement
more confidence
less
Predicted activation
Predicted inhibition
Glow Indicates activity when opposite of measurement
Predicted Relationships
Leads to activation
Leads to inhibition
Findings inconsistent with state of downstream molecule
Effect not predicted
© 2000-2018 QIAGEN. All rights reserved.

OLD Legends to Suppl Tables

Supplemental Table S3. Predicted upstream regulators identified using Ingenuity Pathway Analysis (Activation Z-score ≥±2, p-value of overlap≤0.05)

Supplemental Table S4. Regulatory effect networks identified using Ingenuity Pathway Analysis.

Figure-XX:


a.
Predicted Activation State
Activated
Inhibited
Activation z-score
Upstream Regulator
b.
TP53−regulomes
Condition
PEF
Normal
c.
KDM5A−regulomes
NDUFA5
SDHD
MYH6
TNNT2
UQCRC1
NDUFA3
MYH7
DMPK
MAP2K7
NDUFS7
MRPL12
GADD45GIP1
NDUFV1
MRPL55
TCAP
d.
HEY2−regulomes
MYH7
MYH6
GATA4
NKX2−5
SMTN
e.
STAT4−regulomes
HLA−DQB1
FSCN1
VSIR
ALDOC
LRP1
RAMP1
GADD45G
PER1
PPP1R13L
MAPK8IP3
FURIN
JAG2
BAD
BSG
ISG15
LTBP3
ZNF524
f.
HTT−regulomes