Transcriptome Profiling Reveals Novel BMI- and Sex-specific Gene Expression Signatures for Human Cardiac Hypertrophy

Mackenzie S. Newman¹, Tina Nguyen¹, Michael J Watson², Robert W Hull³, Han-Gang Yu¹,*

¹Physiology and Pharmacology, West Virginia University, Morgantown, WV, USA; ²Department of Surgery, Duke University, Durham, NC, USA; ³Department of Cardiology, West Virginia University, Morgantown, WV, USA

Short title: Novel gene expression signature for cardiac hypertrophy

*Corresponding author:

Han-Gang Yu
Department of Physiology and Pharmacology
School of Medicine
West Virginia University, Morgantown, WV 26506-9229
Telephone: 304-293-2324
Email address: hyu@hsc.wvu.edu
Abstract

Background: How obesity or sex may affect the gene expression profiles of human cardiac hypertrophy is unknown. We hypothesized that body-mass index (BMI) and sex can affect gene expression profiles of cardiac hypertrophy.

Results: Human heart tissues were grouped according to sex (male, female), BMI (lean<25 kg/m², obese>30 kg/m²), or left ventricular hypertrophy (LVH) and non-LVH non-failed controls (NF). We identified 24 DE genes comparing female to male samples. In obese subgroup, there were 236 DE genes comparing LVH to NF; in lean subgroup, there were 7 DE genes comparing LVH to NF. In female subgroup, we identified 1320 significant genes comparing LVH to NF; in male subgroup, there were 1383 significant genes comparing LVH to NF. There were seven significant genes comparing obese LVH to lean NF; comparing male obese LVH with male lean NF samples we found 106 significant genes; comparing female obese LVH to male lean NF, we found no significant genes. Using absolute value of log₂ fold-change > 2 or extremely small p-value ($10^{-20}$) as a criterion, we identified 9 significant genes (HBA1, HBB, HIST1H2AC, GSTT1, MYL7, NPPA, NPPB, PDK4, PLA2G2A) in LVH, also found in published dataset for ischemic and dilated cardiomyopathy in heart failure.

Conclusions: We identified a potential gene expression signature that distinguishes between patients with high BMI or between men and women with cardiac hypertrophy. Expression of established biomarkers atrial natriuretic peptide A (NPPA) and B (NPPB) were already significantly increased in hypertrophy compared to controls.

Keywords: RNA-Seq, gene expression signature, cardiac hypertrophy, atrial natriuretic peptide A (NPPA, ANP), brain-type natriuretic peptide B (NPPB, BNP), heart failure
**Background**

In a recent global BMI mortality collaboration study from data collected from 3.9 million adults, the risk of dying before 70 years of age was 19% for men and 11% for women of normal weight (12). For obese men and women, that risk increased to 30% for men and 15% for women, thus, obesity caused an absolute increased risk of 11% for men and 4% for women (12). While this large-scale study confirmed the obesity-mortality causal link, it did not address the question, “why does obesity cause nearly three times more premature death in men than in women?”

While heart failure is frequently the final state of cardiovascular disease, cardiac hypertrophy is a major independent predictor of progressive heart disease and increased mortality (11). Cardiac hypertrophy is also one of the most common independent features in obesity, even in the absence of hypertension or diabetes mellitus (1, 3, 20, 34, 45, 48). Cardiomyocyte hypertrophy has been found to be the most common cause of sudden cardiac death in morbid obese patients (14). Advances in studies of signaling pathways in both physiological and pathological hypertrophies have led to a recent proposal that aims to treat cardiac hypertrophy as a new therapeutic target (6, 17).

Numerous studies from animal models, mostly rodents, have yielded at least 26 "key signaling molecules or processes" critical in hypertrophy and heart failure and thus are potential targets for new treatment of heart failure (38). However, clinical trials for new drugs have seldom been successful (22, 38). While finding new therapeutic targets in heart failure remains important, understanding genetic and molecular mechanisms of cardiac hypertrophy has recently gained increasing interest due to early-stage presentation during the time course of heart failure development (6, 17).
Studies in molecular signaling pathways have revealed different responses of several key signaling proteins to physiological and pathological hypertrophic stimuli (4). Notably, the expression levels of atrial natriuretic peptide (ANP) and beta-myosin heavy chain (β-MHC) protein increased only by receiving aortic banding compared to sham in an experimental mouse cardiac hypertrophy model (4).

Adding to the complexity of understanding the underlying mechanisms of cardiac hypertrophy is the potential contribution of obesity and sex. Obesity caused higher rates of cardiac hypertrophy, reduced quality of life, and shorter life expectancies compared to age-matched lean individuals (29, 35). A recent study in 2.3 million adolescents from 1967 to 2010 found that overweight and obese individuals (measured by BMI) were strongly associated with increased cardiovascular mortality in adults (43). A high rate of sudden cardiac death in individuals with morbid obesity has been recognized for centuries (9). A high prevalence of sudden cardiac death has also been found in young obese people (5). For every 1 kg/m² increase in body mass index (BMI), heart failure risk increases by 5% in men and 7% in women (23). In ventricular biopsy samples from obese patients, the number of adipocytes increases as the ejection fraction decreases (30). Comparatively, sex differences in cardiovascular physiology are well known, but sex-specific manifestations in human cardiovascular disease have only been recently recognized (19, 21, 32). In the meantime, most mechanistic studies of cardiac hypertrophy have only been conducted in male animal models.

Methodologically, previous studies used Northern blotting, real-time PCR, and microarray cDNA for cardiac gene expression profiling under various hypertrophic conditions (27). Recent advances in next-generation sequencing (NGS) such as RNA-Seq (or
transcriptome analysis) offer a unique opportunity to provide an overall snapshot of mRNA expression of all cardiac genes with high accuracy. Advantages of RNA-Seq over other sequencing methods such as cDNA microarrays are a combination of high-throughput sequencing, single-base resolution, low background noise, and a wide dynamic range for quantification of gene expression levels (46).

In this work, we used RNA-Seq to investigate the potential effects of BMI and sex on gene expression profiles of human heart with left ventricular hypertrophy.

**Methods**

*Human heart samples*

Acquisition of human heart samples was approved by the Institutional Review Board (IRB) for the protection of human subjects at both West Virginia University and Duke University. De-identified frozen human heart samples with pathological characterization were provided by the Department of Surgery at Duke University School of Medicine. Whole heart tissue was snap-frozen in liquid nitrogen immediately after collection from surgical procedures. Left ventricles were dissected and stored in -80°C freezer until use. Patient characterizations of the samples are provided in table 1. Average age of patients is 47.21±2.65 years (ranging from 19 to 67 year). The control group, in which heart has no hypertrophy or failure, designated non-failed (NF) group, the mean age is 46.00±3.69 years (n=12). In the hypertrophy group, the mean age is 48.42±2.65 years (n=12). Left ventricular hypertrophy (LVH) (n=12, 6 female, 6 male) samples were verified by echocardiograph (echo) measurement and interpreted by a cardiologist. NF hearts (n=12, 6 female, 6 male) with echo data showing the absence of LVH
were used as controls. Information of patients’ age, sex, and BMI was obtained from pathological reports. BMI<25 was considered “lean” and BMI>30 was considered “obese”.

Total RNA isolation, Next-Generation Sequencing (RNA-Seq), and bioinformatics analysis

Total RNA was isolated using an RNA Fibrous Tissue Miniprep Kit (Qiagen). Quality of RNA was verified with an Agilent 2100 Bioanalyzer and RNA 6000 Pico Kit. Only samples that had RNA Integrity Number (RIN)>7.0 were submitted for sequencing. The samples were then subjected to polyA enrichment followed by fragmentation, first and second strand synthesis, adenylation of 3’ ends, adapter ligation, DNA fragment enrichment, and real-time PCR quantification.

Sequencing was performed using NextSeq 500 (Illumina). Bcl sequencing data was converted to FastQ using onboard instrument software. Reads were mapped to human reference genome (hg38) using Spliced Transcripts Alignment to a Reference (STAR) (13).

Differential expression analysis was performed with NOISeq (v.2.14.1) (41) using RStudio version 0.99.879 (37). NOISeq is a newly-developed tool for differential expression analysis. Compared to the commonly-used DeSeq (2), NOISeq offered a set of tools for better quality control to avoid false positive discoveries (41). Gene annotation information was obtained from the Ensembl Biomart database, release 85 (50). Gene expression levels are indicated by FPKM (fragments Per Kilobase of transcript per Million mapped reads) (42). FPKM was then normalized for batch effect using the ARSyNseq module included with the NOISeq package. Data were analyzed using the noiseqbio method under default conditions. The CPM filtering method was used for differential analyses where at least one group contained five or fewer replicates; otherwise, the Wilcoxon test was used for filtering.
The heart failure dataset was extracted from a recent publication by Liu et al. (28). We used this heart failure dataset against our LVH dataset to explore the potential significance of newly-identified DE genes as “a gene expression signature” for prediction during the course of heart failure.

**Gene Ontology Enrichment and Pathway Analysis**

Gene Ontology (GO) enrichment analysis was carried out by using a comprehensive gene set enrichment tool, Enrichr (10, 25). This web-based tool contains 180,184 annotated gene sets from 102 gene set libraries (25). It calculates four parameters: p-value, q-value or adjusted p-value, z-score, and a combinational score – higher indicates larger significance. Interaction Network Analysis of Differentially Expressed Genes (GeneMANIA) (47) was used for co-expression and association of significant differentially expressed genes.

**Immunoblotting**

Tissues sections were submerged in minimal lysis buffer (fresh protease and phosphatase inhibitors [Sigma], 20mM Tris, 150mM NaCl, 10mM EGTA and 10mM EDTA at pH 7.4) on ice and homogenized briefly at high speed. Samples were then centrifuged for 15 minute increments at 10,000 x g to pellet debris. Supernatants were placed into new tubes and protein concentration was recorded using Bradford’s method on an Eppendorf Biophotometer.

For Western blotting procedures, protein concentrations were normalized between samples to 10-30 μg and mixed with Non-Reducing Lane Marker (Thermo Scientific) with 5% β-mercaptoethanol. After heating in a water bath to 95°C for five minutes, samples were cooled
to 4°C then loaded into a 4-12% bis-tris gel (Invitrogen). Electrophoresis was carried out at 80V for 30 minutes then 140V for the remainder.

Proteins were transferred to 0.45 micron nitrocellulose membranes (Thermo Fisher) at 30V for one hour. Blots were blocked with 3% BSA-V in TBS-T for one hour before primary antibody (1:1000 dilution; Cell Signaling) was added on a shaker at 4°C overnight. Primary antibody solution was replaced with fresh 3% BSA-V in TBS-T containing secondary antibodies at 1:10,000 dilution for one hour at room temperature on a shaker. After five washes with TBS-T, blots were developed with a standard ECL kit (Life Technologies) or ECL Prime (Amersham) on x-ray film or using a G:BOX digital imaging system (Syngene).

Statistics

For Western blots, data are shown as mean ± S.E.; student’s t test was used for statistical analysis with \( p < 0.05 \) being considered as statistically significant, marked with the symbol *. For gene expression, gene size adjusted p-value (FDR – false discovery rate) less than 0.05 was used (\( p_{\text{adj}} < 0.05 \)) to identify significant genes.

We used differentially expressed (DE) gene data (FPKM) and statistics in ischemic cardiomyopathy (ISCH) and dilated cardiomyopathy from a recent publication (28). It is possible to obtain the test statistic based on a single pair of objects (one disease, one non-disease control) due to the availability of multiple reads per subject in RNA-Seq methodology.

Results

**Human heart sample characteristics**
Table 1 summarizes the characteristics of human hearts used in the study. Average age of patients is 47.21±2.65 years (ranging from 19 to 67 years, n=24), 48.42±3.93 years for LVH group (n=12), and 46.00±3.69 years for NF group (n=12). Left ventricular hypertrophy (LVH) (n=12, 6 female, 6 male) samples were diagnosed by echocardiograph (echo) measurement and interpreted by a cardiologist. Non-failed without LVH (NF) hearts (n=12, 6 female, 6 male) confirmed with echo were used as controls for LVH. Information of patients’ age, sex, and BMI were obtained from pathological reports.

Sex-specific LVH gene expression profiles

Comparing LVH to NF samples (n=9 for each group), we found only one significant gene, NPPA (Figure 1A). NPPA was increased by 11.6-fold in LVH (p_adj=0.004). This result contradicts previous gene expression reports on human cardiac hypertrophy, which have identified at least 76 significant genes using conventional techniques such as PCR, Southern blotting, and Northern blotting (27). We wondered whether sex might play a role in this unexpected result.

When we compared gene expression profiles of females versus males, independent of LVH and BMI, we found there are 24 significantly differentially expressed (DE) genes (Figure 1B, Supplemental table 1). A heatmap generated from these 24 DE genes showed different patterns of cardiac gene expression between females and males (Figure 1C). Furthermore, female and male samples can be clearly separated using the 24 DE genes, illustrated by the principle component analysis (PCA) (Figure 1D).

Informed by sex influence in gene expression, we next examined the effect of LVH on gene expression profiles in female and male groups separately. When comparing female LVH
(n=4) to female NF (n=3) samples, we found 1,320 DE genes (Figure 1E, Supplemental table 2). In male LVH over NF comparison, we identified 1,383 DE genes (Figure 1F, Supplemental table 3).

**BMI- and sex-specific LVH gene expression profiles**

To investigate potential effects of obesity on cardiac gene expression, we compared obese (BMI $\geq$ 30) to lean (BMI < 25) groups (n=9 for each group) and found no significant DE genes. However, in the obese group, we found 236 significant genes in LVH compared to NF samples (n=4 for each group) (Figure 2A, Supplemental table 4). In lean group, we found seven significant genes in LVH compared to NF samples (n=3 for each group) (Figure 2B, Supplemental table 5).

Next, when we compared obese LVH to lean NF samples (n=4 for each group), we found seven significant genes (Figure 2C, also see Supplemental table 6). Considering sex factor, we compared male obese LVH to male lean NF samples (n=3 for each group) and found 106 significant genes (Figure 2D, Supplemental table 7). However, comparing female obese LVH to female lean NF samples (n=3 for each group) yielded no significant DE genes. One possibility is that LVH and obesity can independently alter the expression levels of DE genes but in opposite directions.

**Distribution of Sex- and BMI-specific significant cardiac gene expression**

Figure 3 summarizes the distribution of DE genes under different conditions. We identified a total of 23,521 genes in human hearts. No significant DE genes were found in
obesity over lean samples and in female lean NF compared to female obese LVH. One gene was found to be upregulated in LVH compared to NF. Seven DE genes were identified in lean LVH (six upregulated and one downregulated). Seven DE genes were found in lean NF versus obese LVH (five upregulated and two downregulated). Among the 24 DE genes found in females versus males, five were upregulated and nineteen are downregulated. Among 106 DE genes found in male lean NF compared to male obese LVH, 61 were upregulated and 45 were downregulated. Among the 236 DE genes found in obese group, 38 were upregulated and 198 were downregulated. Among 1,320 DE genes found in female LVH, 330 were upregulated and 990 were downregulated. Among 1,383 DE genes found in male LVH, 137 were upregulated and 1246 were downregulated.

**Sex- and BMI-specific significant gene expression signature**

To identify sex-specific LVH DE genes, we compared male LVH and female LVH DE genes. Figure 4A shows a scatterplot of DE genes shared by LVH-M and LVH-F. Quadrant (I) displays 80 genes that are upregulated in female LVH but downregulated in male LVH; Quadrant (II) displays six genes that are upregulated in both female and male LVH; Quadrant (III) displays 141 genes that are downregulated in both female and male LVH; Quadrant (IV) displays 15 genes that are downregulated in female LVH but upregulated in male LVH. Figure 4B shows a heatmap generated from the 80 DE genes from quadrant (I) of 4A, demonstrating the sex-specific modulation of gene expression in LVH.

Using abs(log2FC)>1 as a criterion, we identified 213 sex- and BMI-specific significant genes (Supplemental table 8). Using abs(log2FC)>2 as a criterion, we identified 27 sex- and BMI-specific significant genes (Supplemental table 9).
Significance of LVH DE genes – implication in ischemic and/or dilated cardiomyopathy in heart failure patients

To explore the potential significance of these findings, we compared LVH DE genes to those recently identified in ischemic cardiomyopathy (ISCH) and dilated cardiomyopathy (DCM) (28). **Supplemental Figure 1A** shows a scatterplot of DE genes found in obese LVH and in the previously-published ISCH dataset (28). There are 37 LVH DE genes found in ISCH (**Supplemental table 10**). Quadrant (I) displays 16 genes that are upregulated in obese LVH but downregulated in ISCH; Quadrant (II) displays four gene that is upregulated in both obese LVH and ISCH; Quadrant (III) displays 14 genes that are downregulated in both obese LVH and ISCH; Quadrant (IV) displays 3 genes that are downregulated in obese LVH and upregulated in ISCH. **Supplemental Figure 1B** shows a scatterplot of DE genes found in obese LVH and in published the DCM dataset (28). There are 58 LVH DE genes found in DCM (see **Supplemental table 11**). Quadrant (I) displays 27 genes that are upregulated in obese LVH but downregulated in DCM; Quadrant (II) displays three genes that are upregulated in both obese LVH and DCM; Quadrant (III) displays 17 genes that are downregulated in both obese LVH and DCM; Quadrant (IV) displays 11 genes that are downregulated in obese LVH and upregulated in DCM. **Supplemental Figure 1C** shows a scatterplot of DE genes found in obese LVH and in the published ISCH/DCM dataset (28). There are 31 LVH DE genes found in ISCH/DCM dataset (see **Supplemental table 12**). Quadrant (I) displays 16 genes that are upregulated in obese LVH but downregulated in ISCH/DCM; Quadrant (II) displays three genes that are upregulated in both obese LVH and ISCH/DCM; Quadrant (III) displays eight genes that are downregulated in both obese LVH and ISCH/DCM; Quadrant (IV) displays four genes that are downregulated in obese LVH and upregulated in ISCH/DCM.
Supplemental Figure 2A shows a scatterplot of DE genes found in female LVH and in the published ISCH dataset (28). There are 111 female LVH DE genes found in ISCH (Supplemental table 13). Quadrant (I) displays 41 genes that are upregulated in obese LVH but downregulated in ISCH; Quadrant (II) displays 14 genes that are upregulated in both obese LVH and ISCH; Quadrant (III) displays 46 genes that are downregulated in both obese LVH and ISCH; Quadrant (IV) displays 10 genes that are downregulated in obese LVH and upregulated in ISCH. Supplemental Figure 2B shows a scatterplot of DE genes found in female LVH and in the published DCM dataset (28). There are 181 female LVH DE genes found in DCM (Supplemental table 14). Quadrant (I) displays 63 genes that are upregulated in obese LVH but downregulated in DCM; Quadrant (II) displays 20 genes that are upregulated in both obese LVH and DCM; Quadrant (III) displays 76 genes that are downregulated in both obese LVH and DCM; Quadrant (IV) displays 22 genes that are downregulated in obese LVH and upregulated in DCM. Supplemental Figure 2C shows a scatterplot of DE genes found in female LVH and in the published ISCH/DCM dataset (28). There are 98 LVH DE genes found in ISCH/DCM (Supplemental table 15). Quadrant (I) displays 56 genes that are upregulated in obese LVH but downregulated in ISCH/DCM; Quadrant (II) displays six genes that are upregulated in both obese LVH and ISCH/DCM; Quadrant (III) displays 26 genes that are downregulated in both obese LVH and ISCH/DCM; Quadrant (IV) displays 10 genes that are downregulated in obese LVH and upregulated in ISCH/DCM. Supplemental Figure 2D shows a scatterplot of DE genes found in male LVH and in published ISCH dataset (28). There are 121 LVH DE genes found in ISCH (Supplemental table 16). Quadrant (I) displays 46 genes that are upregulated in obese LVH but downregulated in ISCH; Quadrant (II) displays 16 genes that are upregulated in both obese LVH and ISCH; Quadrant (III) displays 44 genes that are
downregulated in both obese LVH and ISCH; Quadrant (IV) displays 15 genes that are downregulated in obese LVH and upregulated in ISCH. **Supplemental Figure 2E** shows a scatterplot of DE genes found in male LVH and in the published DCM dataset (28). There are 196 LVH DE genes found in DCM (**Supplemental table 17**). Quadrant (I) displays 98 genes that are upregulated in obese LVH but downregulated in DCM; Quadrant (II) displays 17 genes that are upregulated in both obese LVH and DCM; Quadrant (III) displays 46 genes that are downregulated in both obese LVH and DCM; Quadrant (IV) displays 35 genes that are downregulated in obese LVH and upregulated in DCM. **Supplemental Figure 2F** shows a scatterplot of DE genes found in male LVH and in the published ISCH/DCM dataset (28). There are 80 LVH DE genes found in ISCH/DCM (see **Supplemental table 18**). Quadrant (I) displays 33 genes that are upregulated in obese LVH but downregulated in ISCH/DCM; Quadrant (II) displays 15 genes that are upregulated in both obese LVH and ISCH/DCM; Quadrant (III) displays 21 genes that are downregulated in both obese LVH and ISCH/DCM; Quadrant (IV) displays 11 genes that are downregulated in obese LVH and upregulated in ISCH/DCM.

**Gene Expression Signatures**

To explore potential implications of LVH DE genes for future development of heart failure, we selected 10 DE genes using three criteria: abs(log2FC)>2, extremely small p_adj value (10^{-20}), and also have been found in the published ISCH and DCM heart failure dataset (28) (**Table 2**). **Figure 5A** shows the heatmap of male LVH (LVH-M) and female LVH (LVH-F) compared to their respective NF controls. In male LVH, among expression levels of ten DE genes, four (HBB, PLA2G2A, HBA1, PLXDC2) are changed by less than one standard deviation, five (NPPA, NPPB, PDK4, HIST1H2AC, GSTT1) are increased, and one (MYL7) is
decreased. In female LVH, among expression levels of ten DE genes, eight (HBB, NPPA, NPPB, PDK4, PLA2G2A, HBA1, HIST1H2AC, PLXDC2) are increased and two (MYL7, GSTT1) are decreased. **Figure 5B** shows the heatmap of ISCH and DCM compared to NF using the published data (28). Among the expression levels of these ten genes, seven (HBB, NPPA, NPPB, MYL7, PDK4, HIST1H2AC, PLXDC2) are increased in ISCH, seven (HBA1, HBB, NPPA, NPPB, MYL7, PDK4, HIST1H2AC) are increased in DCM, and two (PLA2G2A, GSTT1) are decreased, compared to controls.

**Validation of 10 DE genes**

1. **Expression of NPPA (ANP) and NPPB (BNP) in LVH, ISCH, and DCM**

   Atrial natriuretic peptide (ANP) and brain-type natriuretic peptide (BNP) are biomarkers for heart failure with left ventricular dysfunction (8, 16). We found that the transcripts of NPPA (gene that encodes ANP) were increased in LVH by 24-fold in males (LVH_M: 697 FPKM, NF_M: 29 FPKM; p_adj = 0.01825), 7.4-fold in females (LVH_F: 148 FPKM, NF_F: 20 FPKM; p_adj = 0.0324), 13.3-fold in BMI25 (LVH_BMI25: 601 FPKM, NF_BMI25: 45 FPKM; p_adj = 7x10^{-15}), and 16.4-fold in BMI30 (LVH_BMI30: 327 FPKM, NF_BMI30: 23 FPKM; p_adj = 1.4x10^{-14}) subgroup, respectively ([Figure 6A](#), upper). Its expression was also increased by 19-fold in ISCH (ISCH 234: 876 FPKM, NF-ISCH: 46 FPKM; p_adj = 0) and 5.4-fold in DCM (DCM 333: 251 FPKM, NF-DCM: 46 FPKM; p_adj = 4.4x10^{-7}) ([Figure 6A](#), upper) (28).

   Immunoblotting experiments confirmed that ANP protein expression was increased by 90% in LVH compared to NF after being normalized to α-actin (LVH: 1.10±0.15, n=8, NF: 0.58±0.05, n=5) ([Figure 6A](#), middle and lower).
Similarly, we found that the transcripts of NPPB (gene that encodes BNP) were increased in LVH by 7.5-fold in male (LVH_M: 121 FPKM, NF_M: 16 FPKM; p_adj = 0.0136), 5-fold in female (LVH_F: 130 FPKM, NF_F: 28 FPKM; p_adj = 0.0146), and 3.4-fold in BMI30 (LVH_BMI30: 121 FPKM, NF_BMI30: 36 FPKM; p_adj = 0.04) subgroups, respectively (Figure 6B, upper). Its expression was also increased by 11-fold in ISCH (ISCH 234: 1772 FPKM, NF-ISCH: 159 FPKM; p_adj = 1.79x10^-13) and 4-fold in DCM (DCM 333: 617 FPKM, NF-DCM: 159 FPKM; p_adj = 8.96x10^-7) (28) (Figure 6B, upper). BNP protein expression was increased by 151% in LVH compared to NF control (NF) after being normalized to α-actin (LVH: 1.31±0.52, n=9, NF: 0.52±0.11, n=9) (Figure 6B, middle and lower).

(2) Expression of HBA1 and HBB in LVH, ISCH, and DCM

Figure 7 shows the protein expression of HBA1 (7A) and HBB (7D). Both were increased in female LVH over NF (HBA1: LVH_F=0.46±0.029, NF_F=0.26±0.04, n=3, p<0.05; HBB: LVH_F=0.36±0.42, NF_F=0.23±0.02, n=3, p<0.05), but were not changed in male LVH compared to NF (HBA1: LVH_M=0.17±0.07, NF_M=0.17±0.06, n=6, p>0.05; HBB: LVH_M=0.98±0.14, NF_M=0.94±0.13, n=6, p>0.05) (7B, 7E). These female-LVH-specific increases in protein expression of HBA1 and HBB are consistent with the corresponding increase in transcripts in female LVH (~11-fold increase of HBA1, 7C; ~44-fold increase of HBB, 7F). HBA1 transcripts were reported to increase by 3.8-fold in ischemic and 6.9-fold in dilated cardiomyopathy, respectively (7C) (28); Similarly, HBB transcripts were reported to increase by 6-fold in ischemic and 11.6-fold in dilated cardiomyopathy, respectively (7F) (28).

(3) Expression of GSTT1 and PLA2G2A in LVH, ISCH, and DCM
**Figure 8** shows the protein expression of GSTT1 and PLA2G2A (8A – male, 8B - female). GSTT1 levels were increased by 10-fold in male LVH (LVH_M=0.70±0.22, NF_M=0.07±0.06, n=3, p<0.05), but no significant changes were detected in female LVH (LVH_F=0.94±0.57, NF_F=0.97±0.61, n=3, p>0.05) (8C). PLA2G2A levels were increased in female LVH by 5-fold (LVH_F=0.25±0.20, NF_F=0.05±0.01, n=3, p<0.05) and in male LVH by 7-fold (LVH_M=0.07±0.02, NF_M=0.01±0.005, n=3, p<0.05) (8D). PLA2G2A levels are noticeably lower in male (middle lane, 10A) than in female (middle lane, 8B) left ventricles.

Changes in protein expression levels of GSTT1 are consistent with that in transcripts, 13.7-fold increase in male LVH for GSTT1 (8E). However, for PLA2G2A, there is a higher increase in transcripts in female LVH (6.4-fold) than in male LVH (1.8-fold) (8F). In ischemic and dilated cardiomyopathy GSTT1 transcripts were reported to increase by 40% and 260%, respectively (28), whereas PLA2G2A transcripts were increased by 4.2-fold and 5.6-fold, respectively (28).

**(4) Expression of PDK4 and MYL7 in LVH, ISCH, and DCM**

**Figure 9** shows the protein expression of PDK4 and MYL7 (9A – female, 9B – male). PDK4 levels were increased by 2.2 – fold in male LVH over NF (LVH_M = 1.335 ± 0.307, NF_M = 0.514 ± 0.107, n= 3, p<0.05), but not significantly altered in female LVH compared to NF (LVH_M = 1.282 ± 0.3594, NF_M = 0.5801 ± 0.08030, n= 3, p>0.05) (9C). On the other hand, MYL7 levels were decreased by 27% in female LVH over NF (LVH_F =0.8958 ± 0.1007, NF_F = 1.225 ± 0.04868, n= 3, p<0.05), but not significant change in male LVH compared to NF (LVH_M = 1.687 ± 0.2885, NF_M = 1.646 ± 0.2225, n= 3, p>0.05) (9D).
PDK4 transcript levels were increased in male LVH by 2.3-fold and in female LVH by 1.4-fold (9E). PDK4 transcripts levels were reported to increase (by 2.2-fold) only in ischemic heart failure (28) (9E).

MYL7 transcript levels were increased in male LVH by 74% and in female LVH by 60% (9F). MYL7 transcripts levels were reported to increase by 3.2-fold in both ischemic and dilated cardiomyopathy (28) (9F).

(5) Expression of HIST1H2AC in LVH, ISCH, and DCM

Figure 10 shows the protein expression of HISTH2AC (10A). The expression levels were increased by 154% in female LVH over NF (LVH_F=1.55±0.29, NF_F=0.61±0.09, n=3, p<0.05) and by 254% in male LVH over NF (LVH_M=2.49±0.41, NF_M=0.98±0.37, n=6, p<0.05), respectively (10B), consistently with the increased transcripts in female LVH (~5-fold) and male-LVH (~2-fold) (10C). HIST1H2AC transcripts were reported to increase by 2.9-fold in ischemic and 5.4-fold in dilated cardiomyopathy, respectively (10C) (28).

Finally, protein expression and re-examination of gene expression for PLXDC2 showed insignificant changes in LVH over NF. Thus, it was removed from DE genes for further data analysis.

Gene Ontology (GO) enrichment and pathway analysis

We performed GO enrichment analysis for nine DE genes (NPPA, NPB, HBB, HBA1, PDK4, MYL7, HIST1H2AC, GIST1, PLA2G2A). In biological process, the top two most significant processes are receptor guanylyl cyclase signaling pathway (q-value = 0.0004) (involving NPPA, NPPB) and oxygen transport (q-value = 0.0005) (involving HBB, HBA1)
(Supplemental table 19). In cellular component, the top two most significant components are endocytic vesicle lumen and hemoglobin complex (q-value=0.0004), both involving HBB and HBA1 (Supplemental table 20). In molecular function, HBB and HBA1 are involved in the top five most significant functions, including oxygen transporter activity (q-value = 0.0003), oxygen binding (q-value = 0.0008), peroxidase activity (q-value = 0.0008), oxidoreductase activity (q-value = 0.0008), and antioxidant activity (q-value = 0.002) (Supplemental table 21).

**DE genes interaction network analysis**

To further explore potential interactions among nine DE genes, we performed gene interaction network analysis using GeneMANIA (47). Network interaction in terms of predicted physical interaction and co-expression was analyzed. Among our nine DE genes, we found three clusters: NPPA, NPPB, HBB, HBA1, HIST1H2AC, and MYL7 form the largest cluster, PDK4 and PLA2G2A are co-expressed together, GSTT1 is not associated with other eight DE genes (Supplemental Figure 3). Within the largest cluster, NPPA, NPPB, and MYL7 are co-expressed. HBB is co-expressed and associated with HBA1 and HIST1H2AC. NPPB also is associated with HBB and HIST1H2AC.

**Discussion**

In the present work, we used transcriptome sequencing to explore the potential effects of BMI and sex on gene expression profiles of human hearts with and without left ventricular hypertrophy. We found both BMI and sex can unmask a large set of genes whose expression levels are significantly affected by LVH.
We explored the implications of BMI- and sex-specific LVH DE genes in heart failure. Previously, sex-specific differences in gene expression profiles of heart failure were investigated using cDNA microarray. In new-onset heart failure, 35 upregulated and 16 downregulated transcripts were identified in men versus women (21). At end-stage dilated cardiomyopathy, there were 55 and 31 differentially regulated genes in female and male, respectively (19). Nineteen DE genes were shared by both males and females (19). Most recently, RNA-Seq was used on six patients to identify 983 DE genes in ISCH vs NF (union of three pairs, table 1 in (28)), 1109 DE genes in DCM vs NF (union of six pairs, table 1 in (28)), and 825 DE genes (union of two pairs, table 1 in (28)) in which 476 genes were overexpressed in ISCH and 349 genes were overexpressed in DCM. Sex and whether the patients were obese were not specified in the article. We performed correlation studies of our LVH data and the published data in both types of heart failure, ISCH and DCM (28). Our scatterplots display numerous downregulated (quadrant II) and upregulated (quadrant III) DE genes in obese LVH, females, and male LVH, which are also found in ISCH and DCM.

We selected nine DE genes which were significantly changed in multiple LVH vs NF analyses within our dataset and were shared by LVH, ISCH, and DCM, to provide a gene-expression signature. There were five overexpressed genes (HBB, NPPA, NPPB, PDK4, HIST1H2AC), one downregulated gene, GSTT1, three regulated in opposite directions (MYL7 decreased in LVH, increased in cardiomyopathy; PLA2G2A increased in female LVH, but decreased in cardiomyopathy; HBA1 increased in female LVH and DCM, but decreased in ISCH). For male LVH, the expression levels of three genes (HBB, PLA2G2A, HBA1) were not changed by more than one standard deviation.
Roles of 9 DE genes in human cardiac hypertrophy and failure

Using Genechip and TagMan PCR, the first gene expression fingerprint of heart failure revealed 103 genes in 10 functional groups between NF and HF samples (40). ANP and BNP were two upregulated genes in heart failure. Using quantitative PCR, NPPA transcripts were found to be increased in dilated heart failure patients of both sexes (7). “Expression profiling-based biomarkers” was proposed in a transcriptome analysis of endomyocardial biopsies from 48 heart failure patients, which used 96 DE genes to predict cardiomyopathy etiology accurately (ischemic vs non-ischemic) (24). A clinical study in 2008 on 3,580 patients found that women had new-onset acute heart failure (AHF) more frequently and less dilated cardiomyopathy compared with men (33).

In both male and female samples from end-stage dilated cardiomyopathy, microarrays identified upregulated NPPA and downregulated PLA2G2A expression, respectively (19). NPPA and NPPB overexpression were also detected in new-onset heart failure patients (both male and female) with dilated cardiomyopathy using microarrays (21). Most recently, RNA-Seq was used to show overexpressed NPPA and NPPB as well as decreased expression of PLA2G2A in both DCM and ischemic cardiomyopathy (Figure 7) using a much smaller sample size (one disease vs. one control) (28).

ANP is mainly produced in the atria, whereas BNP is primarily produced in the ventricles, in response to myocardial stress. Both peptides have been reported as valuable diagnostic markers in heart failure (16). N-terminal pro-BNP (NT-proBNP) and mid-regional pro-ANP (MR-proANP) are inactive precursors of BNP and ANP, respectively, with long half-lives. These peptides are both current standards of care for patients with acute or chronic heart failure (31, 49). Increased NT-proBNP has also been found to be closely associated with heart
failure patients with cachexia (BMI<20) (18). NT-proBNP has recently been confirmed as a reliable risk biomarker for cardiovascular events on death of type 2 diabetes patients (44).

Increased transcripts and protein expression of ANP and BNP were readily detected in LVH compared to NF in our study (Figure 6). However, we found much higher NPPA expression than NPPB in male LVH (NPPA: 697 FPKM, NPPB: 121 FPKM) but not in female LVH (NPPA: 148 FPKM, NPPB: 130 FPKM). NPPA expression was increased more in male (24-fold) than in female (7.4-fold) LVH. In addition, the strongest association of LVH with increased NPPA expression was found between lean and obese groups, and such an association was not found for NPPA. In the previously-published heart failure gene expression data (28) we found both NPPA and NPPB expression levels are higher in ISCH (NPPA: 876 FPKM, NPPB: 1772) than in DCM (NPPA: 251 FPKM, NPPB: 617).

In a 2014 report that examined mRNA expression of left ventricles with sudden cardiac death, HBA1 and HBB mRNA expressions were found to be increased, while PDK4 levels were downregulated (39). PDK4 is one of the key regulators of metabolism (36). Its expression levels in human hearts are decreased during development and tend to be reduced in heart failure (36). We found the gene expression levels for HBA1 and HBB were upregulated only in female LVH, while PDK4 levels were increased only in male LVH.

MYL7, one of the myosin light chains that plays a key role in cardiogenesis (15), is increased in human hypertrophic cardiomyopathy (26). We found its gene expression levels were increased only in female LVH.

Roles of GSTT1, PLA2G2A, and HIST1H2AC in human cardiac hypertrophy or failure are unknown. We found that GSTT1 gene expression levels were upregulated in male, but not
Gene expression levels for PLA2G2A and HIST1H2AC were upregulated in LVH of both sexes with a greater increase in female LVH.

Limitations of the study

1) Small sample size: we used a total of 18 samples. For subgroups such as obese female LVH, there were three samples for each group. Although it fulfills the minimal requirement for statistical analysis, it may lead to underestimates of the amount of significant genes in each subgroup as well as large variations in expression levels. However, small samples sizes have demonstrated impact of the identified significant genes, particularly with very small p_adj values, such as PDK4 (p_adj < 10^-6). In a recent study, the gene signatures identified using RNA-Seq from only six heart failure patients were used to accurately classify a large set of 313 patients with microarray data (28). It illustrated use of RNA-Seq as an effective approach to discover novel gene expression signature based on an extremely small sample size.

2) Samples variations: intrinsic individual differences exist at genetic levels. Clinical diagnoses of LVH were based on echocardiography. As shown in patient sample characteristics (Table 1), there are some samples with borderline LVH that were deemed as NF in pathological reports.

Despite of these limitations our data provides an argument for using BMI- and sex-specific gene-expression signature containing multiple significant genes, rather than a single gene, to offer a more accurate prediction for future progression to heart failure.

Conclusions
We identified nine differentially expressed genes in left ventricular hypertrophy that are BMI- and sex-sensitive. Validation of these nine genes (forming a “gene expression signature”) in a large cardiac hypertrophy population may have potential to help for early diagnose of heart failure, which occurs at a significantly high rate in obese population.

Declarations

Ethics approval and consent to participate

Use of human heart samples in this research has been approved by West Virginia University Institutional Review Board and the Duke University Institutional Review Board.

Consent for publication

Not applicable

Availability of data and material

All data generated or analyzed during this study are included in this published article and its supplementary files.

Competing interests

The authors declare that they have no competing interests

Funding
Research reported in this work was supported by National Institute of General Medical Sciences of the National Institutes of Health (U54GM104942, P20 GM103434), American Heart Association (13GRNT16420018), and the Office of Research and Graduate Programs/Health Sciences Center at West Virginia University. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health and American Heart Association. Mackenzie Newman is a recipient of the STEM Mountains of Excellence Fellowship at West Virginia University.

Authors’ contributions
Mackenzie Newman performed experiments and data analysis, and provided critical reading and editing the manuscript. Tina Nguyen performed experiments and data analysis and provided critical reading and editing the manuscript. Michael J. Watson provided human samples and characteristics of samples and critical reading and editing the manuscript. Robert W. Hull helped echocardiography characteristics of samples and provided critical reading and editing the manuscript. Han-Gang Yu developed the project, designed the experiments, and wrote the manuscript.

Acknowledgements
The authors are grateful for initial RNA-Seq data analysis by Genomics Facility at West Virginia University.


38. Simpson PC. Where are the new drugs to treat heart failure? Introduction to the special issue on "key signaling molecules in hypertrophy and heart failure". *J Mol Cell Cardiol* 51: 435-437, 2011.


<table>
<thead>
<tr>
<th>Human sample</th>
<th>Sex</th>
<th>BMI</th>
<th>Age</th>
<th>LV mass (g)</th>
<th>Path report (LVH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1</td>
<td>M</td>
<td>29.3</td>
<td>57</td>
<td>126</td>
<td>no</td>
</tr>
<tr>
<td>NF2</td>
<td>M</td>
<td>17.2</td>
<td>54</td>
<td>175</td>
<td>borderline</td>
</tr>
<tr>
<td>NF3</td>
<td>M</td>
<td>30.1</td>
<td>41</td>
<td>194</td>
<td>none to mild</td>
</tr>
<tr>
<td>NF4</td>
<td>M</td>
<td>22.5</td>
<td>67</td>
<td>97</td>
<td>no</td>
</tr>
<tr>
<td>NF5</td>
<td>M</td>
<td>34.9</td>
<td>38</td>
<td>-</td>
<td>none-mild</td>
</tr>
<tr>
<td>NF6</td>
<td>M</td>
<td>21.6</td>
<td>36</td>
<td>97</td>
<td>no</td>
</tr>
<tr>
<td>NF7</td>
<td>F</td>
<td>21.1</td>
<td>66</td>
<td>-</td>
<td>no</td>
</tr>
<tr>
<td>NF8</td>
<td>F</td>
<td>23.0</td>
<td>33</td>
<td>-</td>
<td>no</td>
</tr>
<tr>
<td>NF9</td>
<td>F</td>
<td>29.7</td>
<td>54</td>
<td>104</td>
<td>no</td>
</tr>
<tr>
<td>NF10</td>
<td>F</td>
<td>22.6</td>
<td>43</td>
<td>103</td>
<td>no</td>
</tr>
<tr>
<td>NF11</td>
<td>F</td>
<td>34.3</td>
<td>64</td>
<td>-</td>
<td>no</td>
</tr>
<tr>
<td>NF12</td>
<td>F</td>
<td>38.1</td>
<td>28</td>
<td>162</td>
<td>no</td>
</tr>
<tr>
<td>LVH1</td>
<td>M</td>
<td>23.0</td>
<td>53</td>
<td>378</td>
<td>moderate-severe</td>
</tr>
<tr>
<td>LVH2</td>
<td>M</td>
<td>51.1</td>
<td>54</td>
<td>300</td>
<td>moderate</td>
</tr>
<tr>
<td>LVH3</td>
<td>M</td>
<td>33.2</td>
<td>51</td>
<td>248</td>
<td>mild</td>
</tr>
<tr>
<td>LVH4</td>
<td>M</td>
<td>19.0</td>
<td>53</td>
<td>347</td>
<td>severe</td>
</tr>
<tr>
<td>LVH5</td>
<td>M</td>
<td>39.1</td>
<td>59</td>
<td>276</td>
<td>mild</td>
</tr>
<tr>
<td>LVH6</td>
<td>M</td>
<td>21.1</td>
<td>19</td>
<td>348</td>
<td>severe</td>
</tr>
<tr>
<td>LVH7</td>
<td>F</td>
<td>42.9</td>
<td>25</td>
<td>-</td>
<td>mild-moderate</td>
</tr>
<tr>
<td>LVH8</td>
<td>F</td>
<td>16.8</td>
<td>38</td>
<td>176</td>
<td>mild</td>
</tr>
<tr>
<td>LVH9</td>
<td>F</td>
<td>22.5</td>
<td>42</td>
<td>176</td>
<td>mild</td>
</tr>
<tr>
<td>Gene</td>
<td>N/25</td>
<td>Y/30</td>
<td>log2FC</td>
<td>p_adj</td>
<td>Protein</td>
</tr>
<tr>
<td>--------</td>
<td>------</td>
<td>---------</td>
<td>--------</td>
<td>-----------</td>
<td>----------------------------------------</td>
</tr>
<tr>
<td>HBB</td>
<td>5.385036</td>
<td>236.2845</td>
<td>-5.45543</td>
<td>0.000788</td>
<td>Hemoglobin subunit beta</td>
</tr>
<tr>
<td>NPPA</td>
<td>28.79361</td>
<td>697.1588</td>
<td>-4.59767</td>
<td>0.018249</td>
<td>Natriuretic peptides A</td>
</tr>
<tr>
<td>HBA1</td>
<td>2.670161</td>
<td>28.20004</td>
<td>-3.4007</td>
<td>0.001351</td>
<td>Hemoglobin subunit alpha</td>
</tr>
<tr>
<td>NPPB</td>
<td>16.17904</td>
<td>120.645</td>
<td>-2.89857</td>
<td>0.013588</td>
<td>Natriuretic peptides B</td>
</tr>
<tr>
<td>PDK4</td>
<td>66.72739</td>
<td>465.2543</td>
<td>-2.80167</td>
<td>4E-15</td>
<td>Pyruvate dehydrogenase kinase isozyme 4</td>
</tr>
<tr>
<td>PLA2G2A</td>
<td>22.78948</td>
<td>142.7502</td>
<td>-2.64705</td>
<td>0.045658</td>
<td>Phospholipase A2</td>
</tr>
<tr>
<td>HIST1H2AC</td>
<td>4.108</td>
<td>20.4747</td>
<td>-2.31733</td>
<td>0.003876</td>
<td>Histone H2A type 1-C</td>
</tr>
<tr>
<td>GSTT1</td>
<td>2.927</td>
<td>13.489</td>
<td>-2.204</td>
<td>0.011703</td>
<td>Glutathione S-transferase theta-1</td>
</tr>
<tr>
<td>PLXDC2</td>
<td>4.073109</td>
<td>16.66727</td>
<td>-2.03282</td>
<td>0.011265</td>
<td>Plexin Domain-Containing protein 2</td>
</tr>
<tr>
<td>MYL7</td>
<td>493.851</td>
<td>205.222</td>
<td>1.267</td>
<td>&lt;10E-20</td>
<td>Myosin light chain 7</td>
</tr>
</tbody>
</table>

LVH: left ventricular hypertrophy; NF: non-LVH non-failed left ventricular tissue.

Table 2: Significant differentially expressed genes in LVH
(abs(log2FC)>2, extreme small p_adj, also found in HF dataset)

**Figure legend:**

**Figure 1: Sex-specific LVH differential expression profiles (volcano).** A) NF versus LVH (n=9 in each group). B) Female versus male samples (n=9 in each group). C) Heatmap generated from 24 differentially expressed genes comparing females to males (1B) using unsupervised k-means clustering. D) Principle component analysis plot of female and male samples from these 24 differentially expressed genes (1B). E) Differential expression profile of female NF (n=4) versus female LVH (n=3). F) Differential expression profile of male NF versus male LVH (n=3 in each group).

Red dashed lines mark p_adj=0.05. Dark dots indicate genes without significant changes in expression levels comparing two individual groups (p_adj>0.05). Red dots: significant genes (p_adj<0.05) with abs(log2FC<1); Green dots with labels: significant genes with p_adj<0.05 and abs(log2FC)>1.

**Figure 2: BMI- and Sex-specific LVH differential expression profiles (volcano).** A) Lean versus obese samples (n=9 in each group). B) Obese NF versus obese LVH (n=4 in each group). C) Lean NF versus lean LVH (n=3 in each group). D) Lean NF versus obese LVH (n=3 in each group). E) Male lean NF versus male obese LVH (n=3 in each group). F) Female lean NF versus female obese LVH (n=3 in each group).

Red dashed lines mark p_adj=0.05. Dark dots indicate genes without significant changes in expression levels comparing two individual groups (p_adj>0.05). Red dots: significant genes (p_adj<0.05) with abs(log2FC<1); Green dots with labels: significant genes with p_adj<0.05 and abs(log2FC)>1.
Figure 3: Distribution of LVH differentially expressed genes under different conditions.

Figure 4: Shared LVH DE genes in both sexes. A) Scatterplot of male versus female LVH DE genes. Quadrant (I) contains DE genes upregulated in male LVH but downregulated in female LVH. Quadrant (II) contains DE genes downregulated in both male and female LVH. Quadrant (III) contains DE genes upregulated in both male and female LVH. Quadrant (IV) contains DE genes upregulated in female LVH but downregulated in male LVH. B) Heatmap from the 80 DE genes from quadrant I of male versus female DE gene scatterplot.

Figure 5: Gene expression signature in LVH and heart failure. A) Heatmap of 10 DE genes in male and female LVH; B) Heatmap of 10 DE genes in ISCH and DCM.

Figure 6: Gene and protein expression of NPPA/ANP (A) and NPPB/BNP (B). Upper: transcript expression of NPPA (A) or NPPB (B) in female and male LVH, ISCH, and DCM; Middle: ANP (A) or BNP (B) immunoblots in LVH and NF, α-actin was used as a loading control; Bottom: ANP (A) or BNP (B) protein expression normalized to α-actin in LVH and NF.

Figure 7: Gene and protein expression of HBA1 and HBB. (A) HBA1 immunoblots in female (upper) and male (lower) samples. α-actin was used as a loading control. (B) HBA1 protein expression normalized to α-actin in LVH and NF. (C) HBA1 transcript changes in LVH over NF. (D) HBB immunoblots in female (upper) and male (lower) samples. α-actin was used as a loading control. (E) HBB protein expression normalized to α-actin in LVH and NF. (F) HBB transcript changes in LVH over NF. * indicates statistically significant difference between the two groups (p<0.05). FC = Fold Change.
Figure 8: Gene and protein expression of GSTT1 and PLA2G2A. GSTT1 and PLA2G2A immunoblots in male (A) and female (B) samples. α-actin was used as a loading control. (C) GSTT1 protein expression normalized to α-actin in LVH and NF. (D) PLA2G2A protein expression normalized to α-actin in LVH and NF. (E) GSTT1 transcript changes in LVH over NF. (F) PLA2G2A transcript changes in LVH over NF. * indicates statistically significant difference between the two groups (p<0.05). FC = Fold Change.

Figure 9: Gene and protein expression of PDK4 and MYL7. PDK4 and MYL7 immunoblots in female (A) and male (B) samples. α-actin was used as a loading control. (C) PDK4 protein expression normalized to α-actin in LVH and NF. (D) MYL7 protein expression normalized to α-actin in LVH and NF. (E) PDK4 transcript changes in LVH over NF. (F) MYL7 transcript changes in LVH over NF. * indicates statistically significant difference between the two groups (p<0.05). FC = Fold Change.

Figure 10: Gene and protein expression of HIST1H2AC. (A) Immunoblots in male (upper) and female (lower) samples. α-actin was used as a loading control. (B) HIST1H2AC protein expression normalized to α-actin in LVH and NF. (C) HIST1H2AC transcript changes in LVH over NF. * indicates statistically significant difference between the two groups (p<0.05). FC = Fold Change.
Figure 1

A. NF vs LVH, All Samples

B. Female vs Male, All Samples

C. Color Key

D. PC 2 10% vs PC 1 98%

E. NF vs LVH, Female Group

F. NF vs LVH, Male Group
Figure 2

A) NF vs LVH, BMI30 Group

B) NF vs LVH, BMI25 Group

C) BMI25NF vs BMI30LVH

D) BMI25NF vs BMI30LVH, Male Group
Figure 4

A

Male vs Female LVH

(I) MT2A

(II) NPPA

(III) NPPB

(IV) PLL4AP

NF vs LVH log2FoldChange, Male

NF vs LVH log2FoldChange, Female

B

LVH-F

LVH-M
Figure 5

A

B

Color Key

Row Z-Score

NF-M

LVH-M

NF-F

LV-HF

HBB

NPPA

NPPB

MYL7

PDK4

PLA2G2A

HBA1

HIST1H2AC

GSTT1

PLXDC2

NF

DCM

ISCH

HBB

NPPA

NPPB

MYL7

PDK4

PLA2G2A

HBA1

HIST1H2AC

GSTT1

PLXDC2
Figure 6

(A) NPPA

(B) NPPB

ANP

Norm to α-actin

p=0.024

BNP

Norm to α-actin

p=0.0022
Figure 8

A

LVH-M  NF-M  LVH-M  NF-M  LVH-M  NF-M

GSTT1
PLA2G2A
α-actin

B

LVH-F  NF-F  LVH-F  NF-F  LVH-F  NF-F

LVH-F  NF-F  LVH-F  NF-F  LVH-F  NF-F

C

GSTT1

Norm to α-actin

0.0  0.5  1.0  1.5
LVH_F  NF_F  LVH_M  NF_M

D

PLA2G2A

Norm to α-actin

0.0  0.1  0.2  0.3  0.4
LVH_F  NF_F  LVH_M  NF_M

E

GSTT1

F, C, FRM

0  5  10  15
LVH_M  LVH_F  ISCH  DCM

F

PLA2G2A

F, C, FRM

0  2  4  6
LVH_M  LVH_F  ISCH  DCM
Figure 10

A

HIST1H2AC

D

-actin

NF-F

LVH-F

LVH-M

B

Norm to c-actin

0

1

2

3

4

LVH-F

NF-F

LVH-M

NF-M

C

HIST1H2AC

HIST1H2AC

HIST1H2AC

HIST1H2AC

FC(FPKM)

0

2

4

6

LVH-M

LVH-F

ISCH

DCM