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# Transcriptomic Profile of Heart Failure: A Sex-Stratified Analysis of RNA-Sequencing Data

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## Abstract

# Background

Sexual dimorphism in heart failure (HF) is well-recognized. Underlying transcriptomic mechanisms in prior studies that often-treated sex as a covariate, masking fundamental differences in cardiac remodeling. This study aimed to investigate sex-stratified RNA-seq analysis across HF subtypes to reveal distinct transcriptomic programs and provide a comprehensive, etiology-specific, sex-biased gene expression.

# Methods

Left ventricular RNA-sequencing data from 366 samples — non-failing donors (NFD; n = 166), dilated cardiomyopathy (DCM; n = 166), hypertrophic cardiomyopathy (HCM; n = 28), and peripartum cardiomyopathy (PPCM; n = 6) were obtained from the MAGNet consortium (GSE141910), yielding expression data for 20781genes. Sex-stratified analyses integrated differential gene expression (DGE), age- and race-adjusted analysis of covariance (ANCOVA), upstream transcription factor (TF) enrichment, and functional pathway analysis.

# Results

Median age was 55 years (53% male, 66% Caucasian). DCM exhibited greater DGE in males (2,438 upregulated/2,560 downregulated) than females (292/118), whereas HCM showed greater DGE in females (33/9) than males (19 upregulated only). Functional enrichment revealed distinct sex- and etiology-specific profiles: in males, immune/cytoskeletal processes in DCM and vascular/cytokine signaling in HCM; in females, immune regulation in DCM and Cell morphogenesis/post-translational protein modification in HCM. Upstream TF mapping revealed sex-divergent regulators—male networks anchored by SPI1/HIF1A and female networks by SP1, around a conserved, hormone-responsive STAT5/PGR core. SMOC2 (SPARC-related modular calcium-binding protein 2) was consistently upregulated as a sex- and etiology-independent marker of pathological remodeling ($p = 2.2\times10^{-16}$).

# Conclusions

Although the transcriptomic architecture is substantially shared across sexes, HF exhibits etiology-specific sexual dimorphism and divergent biological programs. These sex-biased transcriptomic signatures may inform potential precision cardiovascular therapeutics.

## Plain English Summary

Heart failure (HF) is a common condition where the heart struggles to pump blood properly. Men and women often develop HF differently—men often develop it earlier and experience more severe symptoms, while women may have distinct disease patterns. Yet most studies analyze men's and women's heart tissue together, which can hide important differences between the sexes. In this study, we examined 366 heart tissues from men and women: healthy donor hearts and hearts with two forms of HF. We looked separately at which genes were switched on or off in men versus women. We found that genetic changes linked to HF differed between men and women and depended on the type of HF. Far more genes were exaggerated in men with the enlarged, weakened heart type, while women showed more changes in the thickened-muscle type. In men, genes related to the body's immune system were more affected, while in women, changes were more common in genes tied to the heart's structure. Interestingly, we also identified one gene that showed consistent changes in all types of HF, regardless of sex. These findings suggest that HF may develop through different biological pathways in men and women depending on its type. These findings could explain more personalized, sex-specific treatment approaches in the future, though further research is needed before these findings can change patient care.

## Highlights

1. A comprehensive study leverages RNA-sequencing data from 366 human myocardial samples to reveal sex-stratified molecular profiles in heart failure.
2. In dilated cardiomyopathy (DCM), men show 12 times greater differential gene expression than women, but this trend completely reverses in hypertrophic cardiomyopathy (HCM).
3. Sex-stratified transcription factor analysis shows SP1-associated matrix-stabilizing programs in females and SPI1/HIF1A-associated immune-inflammatory programs in males, highlighting candidate regulators for future precision therapeutic investigation.
4. SMOC2 emerged as a consistent, sex- and etiology-independent marker of pathological cardiac remodeling.

## Introduction

Heart failure (HF) is a clinical syndrome caused by structural or functional cardiac defects that result in inadequate cardiac output or elevated ventricular filling pressures [1, 2]. Global prevalence is estimated at 1%–3%, contributing substantially to cardiovascular disease (CVD) morbidity and mortality, placing a substantial burden on healthcare and societies [3, 4]. Despite therapeutic advances, outcomes remain poor, partly due to demographic and biological heterogeneity that complicates treatment decisions [5]. Morphologically, HF is classified by ejection fraction and further subdivided into ischemic and non-ischemic forms, including dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), restrictive cardiomyopathy, arrhythmogenic cardiomyopathy, and peripartum cardiomyopathy (PPCM) [6, 7]. These subtypes exhibit distinct phenotypes and prognoses [8]. Further shaped by sex, identical

transcription factors may regulate different gene sets in male and female cardiomyocytes, driving sex-specific cardiac remodeling [9].

Clinically, men often develop HF at an earlier age, experience more severe symptoms, and respond differently to treatments compared to women [10–12]. The biological pathways underlying sex-divergent remodeling, including eccentric versus concentric hypertrophy transition and differential treatment responses, remain incompletely understood [11]. Whether cardiac gene expression varies between sexes in a manner that is independent of etiology remains poorly characterized [10] owing to small sample sizes, heterogeneous cohorts, analytical limitations, and costs [13, 14]. Transcriptomic profiling has revealed extensive gene expression changes across HF subtypes with potential therapeutic targets [15], yet whether sex hormones modify genetic targets in a sex-specific manner remains unresolved [16]. Because not all sex-biased genes drive functional differences, establishing a precise link between RNA abundance and phenotypes is essential [16]. Liu et al. analyzed only six samples without sex stratification [13], leaving sex-specific transcriptomic mechanisms largely uncharacterized. A microarray-based analysis identified 1,837 sexually dimorphic genes in HF myocardium [17]; yet probe-dependent design and limited dynamic range likely underestimate the true breadth of sex-specific transcriptomic remodeling. Current therapeutic target discovery is limited by a reliance on sex-aggregated data, which masks crucial interactions between sex-biased molecular phenotypes and clinical variables [10]. Furthermore, it remains unclear whether cardiac transcriptomes can identify sex-specific subtypes with unique pathophysiological features and prognoses. Correlating these sex-biased transcriptomic patterns with clinical outcomes could ultimately improve personalized treatment and patient care.

RNA-sequencing (RNA-seq) enables unbiased, high-resolution transcriptome profiling with superior sensitivity for detecting sex-specific differences compared with the microarray platform [18]. This study characterizes sex-stratified differential gene expression across non-failing donor (NFD) and HF myocardial samples to identify molecular signatures. We hypothesized that sex-dependent HF subtypes exhibit distinct myocardial transcriptomics, and that sex-stratified analysis identifies distinct differential gene expression and regulatory patterns overlooked when sex is treated merely as a covariate.

## Methodology: Data source, sample population, and covariates

This study utilized left ventricular transcriptomic data from the Myocardial Applied Genomics Network (MAGNet) consortium, accessed via the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) dataset GSE141910 [19, 20]. The cohort comprised 366 myocardial tissue samples (200 failing hearts and 166 non-failing donor [NFD] controls). RNA sequencing was performed using poly-A–selected libraries on the Illumina HiSeq 2500 high-throughput sequencing platform [9, 13]. Raw counts and normalized $\log_2$ matrices were obtained from GEO. Clinical metadata retrieved from MAGNetique GitHub repository were integrated using unique sample identifiers [9]. Standardized protocols minimized cold ischemia and post-mortem intervals to safeguard RNA stability [21]. Samples were categorized into NFD controls (n = 166) and HF cases (n = 200), including DCM, HCM, and PPCM.

The primary predictors were normalized expression values for 20781 genes. Clinical and demographic variables included disease etiology, sex (male versus female), race (African American versus Caucasian), age at tissue collection (in years), body weight (kg), height (cm), left ventricular ejection fraction, and binary clinical conditions (hypertension, diabetes, atrial fibrillation [AF], and ventricular fibrillation [VF]). This study used publicly available de-identified data and is exempt from Institutional Review Board (IRB) approval, adhering strictly to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) reporting guidelines [22].

# Statistical analysis

Transcriptomic and clinical datasets were harmonized using unique sample identifiers. Data quality assessment included evaluation of sample-level mean expression, $\log_2$-transformed count distribution, and gene-level variance; mean-variance relationships were characterized via locally weighted regression (LOESS) to identify outliers and assess variance stabilization. Core gene overlap across NFD, DCM, and HCM was explored by Venn analysis, restricted to genes expressed above the 10th percentile. Demographic and clinical characteristics were summarized by sex as median (range) for continuous variables and frequency (percentage) for categorical variables. Associations between HF etiology and demographics were evaluated using bivariate multinomial logistic regression, with NFD as the reference. A two-sided significance threshold of $\alpha = 0.05$ was applied. Benjamini-Hochberg false discovery rate (FDR) correction applied for multiple testing across differential gene expression (DGE), analysis of covariance (ANCOVA), transcription factor (TF), and functional enrichment analysis.

Principal Component Analysis (PCA) of normalized expression data assessed global transcriptomic variation and sample clustering by sex and HF phenotype. DGE analysis was conducted using DESeq2 (raw counts) with age and race as covariates; limma (log2-transformed data) was used for secondary confirmation, with concordance between methods assessed for the top-ranked genes. Pairwise comparisons between HF subtypes were conducted using Tukey-adjusted estimated marginal means. Unsupervised hierarchical clustering of the top 500 most variable genes identified expression patterns, visualized via dendrograms and heatmaps. ANCOVA modeled gene expression as a function of etiology, adjusting for age and race within each sex cohort. Functional enrichment was assessed by Gene Ontology Biological Process (GO: BP) analysis. TF enrichment analysis was conducted on the top 500 HF-associated genes (ranked by FDR-adjusted p-values and fold change) to evaluate key upstream regulators. All analyses were conducted in R (version 4.6.1).

## Results

# Descriptive statistics

The cohort comprised 366 samples (NFD: n = 166; DCM: n = 166; HCM: n = 28; PPCM: n = 6). Median age was 55 years (range: 15–83), with 53% male and 66% Caucasian participants. Males were younger than females (54 vs. 56 years; p = 0.034) (Table 1). Individuals with HF were younger than controls (median 53

vs. 57 years; $p < 0.001$) and exhibited higher prevalence of AF (45.4% vs. 10.3%; $p < 0.001$) and VF (44.2% vs. 0.6%; $p < 0.001$), but lower prevalence of hypertension (42.0% vs. 61.8%; $p < 0.001$) (Table S1). HF was present in 54.6% (200/366) of the cohort and was more frequent in males than females (58.5% vs. 41.5%; $p = 0.021$). Males exhibited higher median body weight (85 vs. 71 kg; $p < 0.001$), height (178 vs. 163 cm; $p < 0.001$), heart weight (513 vs. 388 g; $p < 0.001$), and prevalence of AF (36.3% vs. 21.6%; $p = 0.002$) and VF (30.6% vs. 17.5%; $p = 0.004$). DCM was more frequent in males (51.5% vs. 38.4%; $p = 0.002$). Across etiologies, AF (75.0%) and VF (50.0%) were more common in HCM, while diabetes (24.1%) and hypertension (47.0%) were more prevalent in DCM (Table S2). Transcriptomic overlap was highly conserved: 15,714 genes (97.8%) were co-expressed in both sexes, and 18,196 genes (94.2%) were shared across NFD, DCM, and HCM. Etiology-specific transcripts comprised ≤ 1.1% of the total for each group (Figure S1).

Table 1
Baseline characteristics of the participants (n = 366) in the Myocardial Applied Genomics Network (MAGNet) consortium, stratified by sex

| **Variables*** | **Female** **N = 172** | **Male** **N = 194** | **P value** |
|---|---|---|---|
| **Age**, median (range),years | 56 (16, 83) | 54 (15, 81) | 0.034† |
| **Race**, n (%) | | | 0.400 ‡ |
| African American | 62 (36.0) | 62 (32.0) | |
| Caucasian | 110 (64.0) | 132 (68.0) | |
| **Weight**, median (range), kg | 71 (27, 152) | 85 (57, 267) | < 0.001† |
| **Height**, median (range), cm | 163 (55, 180) | 178 (75, 196) | < 0.001† |
| Missing** | 1 | 0 | |
| **Diabetes**, n (%) | 41 (23.8) | 39 (20.3) | 0.400 ‡ |
| Missing | 0 | 2 | |
| **Hypertension**, n (%) | 88 (51.2) | 98 (50.8) | 0.900‡ |
| Missing** | 0 | 1 | |
| **Heart weight**, median (range), gm | 388 (159, 773) | 513 (151, 923) | < 0.001† |
| Missing** | 3 | 4 | |
| **Left Ventricular Ejection Fraction**, median (range), % | 0.20 (0.05, 0.75) | 0.20 (0.05, 0.80) | 0.300† |
| Missing** | 48 | 32 | |
| **Atrial Fibrillation**, n (%) | 37 (21.6) | 69 (36.3) | 0.002† |
| Missing** | 1 | 4 | |
| **Ventricular Fibrillation**, n (%) | 30 (17.5) | 59 (30.6) | 0.004† |
| Missing** | 1 | 1 | |
| **Heart Failure**#, n (%) | | | 0.002§ |
| NFD | 89 (51.7) | 77 (39.7) | |
| DCM | 66 (38.4) | 100 (51.5) | |

| Variables* | Female<br>N = 172 | Male<br>N = 194 | P value |
|---|---|---|---|
| HCM | 11 (6.4) | 17 (8.8) | |
| PPCM | 6 (3.5) | 0 (0.0) | |
| * All continuous variables reported as median (min, max), and categorical variables as n(%) | | | |
| † Wilcoxon rank sum test | | | |
| ‡ Pearson's Chi-squared test | | | |
| § Fisher's exact test | | | |
| #NFD = Non-Failing Donor; DCM = Dilated Cardiomyopathy; HCM = Hypertrophic Cardiomyopathy; PPCM = Peripartum Cardiomyopathy | | | |
| **Missing values are presented as frequency. | | | |

## Association between HF and key demographics

Multinomial logistic regression (crude) demonstrated that each 1-year increase in age was associated with lower odds of DCM (OR 0.97; 95% CI, 0.95–0.99; p = 0.0026), HCM (OR 0.95; 95% CI, 0.92–0.98; p = 0.0025), and PPCM (OR 0.89; 95% CI, 0.84–0.95; p = 0.0001) (Table S3). Males had 77% higher odds of DCM compared with females (OR 1.77; 95% CI, 1.15–1.74; p = 0.0100). African American participants had ~ 2.5 times higher odds of DCM (OR 2.45; 95% CI, 1.55–3.90; p = 0.0001) but 90% lower odds of HCM (OR 0.10; 95% CI, 0.01–0.79; p = 0.0293) compared with Caucasian participants.

## Sex-Stratified Analysis of HF Transcriptomics

**Principal Component Analysis (PCA)**: PCA revealed sex-specific transcriptomic clustering across etiologies (Fig. 1). PC1 captured approximately 31% of the variance in both sexes. In males (PC1: 31.9%, PC2: 7.3%), NFD and DCM subtypes occupied largely non-overlapping regions along PC1. HCM demonstrated intermediate PC1 scores with overlap of both NFD and DCM ellipses. In females (PC1: 30.7%, PC2: 7.5%), NFD and DCM separated along PC1 but showed greater overlap than in males. HCM samples clustered near the origin (n = 11). PPCM samples, present only in females, colocalized within the DCM ellipse.

### Unsupervised Hierarchical Clustering

Hierarchical clustering of the top 500 most variable genes revealed two broad expression clusters that did not fully separate by etiology (Figure S2). NFD samples partially co-segregated in a left-sided cluster

characterized by high Z-scores (> 1) in the upper gene cluster and low Z-scores (<-1) in the lower cluster. DCM samples dominated the central region with an inverted Z-score pattern. HCM and PPCM samples were interspersed across both major branches without distinct isolation. The dendrogram (Figure S3) revealed all four groups interdigitating across major branches.

**Differential Gene Expression**

DESeq2 identified 13375 of 20781 genes (64%) as significantly differentially expressed between HF and control samples (FDR-adjusted $p < 0.05$), adjusting for age, sex, and race. Sex-stratified, age- and race-adjusted modeling identified 2990 significant DEGs (14.4%) in males and 2382 (11.5%) in females. In males, DCM vs. NFD yielded 4998 DEGs (2438 upregulated, 2560 downregulated). HCM vs. NFD yielded 19 DEGs (all upregulated). In females, DCM vs. NFD yielded 410 DEGs (292 upregulated, 118 downregulated). HCM vs. NFD yielded 42 DEGs (33 upregulated, 9 downregulated). Volcano plots (Fig. 2) demonstrated that fold-change magnitude was lower for HCM contrasts than for DCM contrasts in both sexes.

**ANCOVA and Shared Transcriptomic Architecture**: ANCOVA revealed 8876 significantly dysregulated genes (FDR $p < 0.05$) in males and 8795 in females, after adjustment for age and race. Intersection of these gene lists revealed that 6403 genes (~ 73%) were shared between both sexes. SMOC2 (ENSG00000112562) expression was significantly upregulated as a sex- and etiology-independent marker of pathological remodeling (Male: FDR $p = 9.82 * 10^{-55}$; Female: FDR $P = 2.20 * 10^{-38}$; Tukey-adjusted pairwise comparisons) (Fig. 3). In males, SMOC2 expression was lower in NFD compared with DCM and HCM (-1.81 to -1.84 log2 units; all $p < 0.0001$). In females, NFD expression was similarly lower compared with DCM, HCM, and PPCM (-1.81 to -1.90 log2 units; all $p < 0.0001$). No significant differences were observed between disease groups within either sex. The statistical metrics and descriptions for the top 10-ranked genes in each sex are summarized in Table S4.

**Upstream Transcription Factor (TF) Analysis**

Analysis of upstream TF of the top 500 HF-associated genes influencing HF revealed 5 significant TFs in males and 4 TFs in females (Fig. 4). STAT5A, STAT5B, and PGR were shared across sexes; SPI1 and HIF1A were male-specific, and SP1 was female-specific. In the sex-divergent regulatory network analysis (Fig. 5), females revealed SP1 as the primary hub, with target genes including ABCG2, IL10, PTN, APLNR, NT5E, APOA1, ASPN, CD83, CD163, IL2RB, LSPI, ALOX5, EDNRB, TIMP2, PTN, and CD163. In males, SPI1 anchored the network, with targets including CD163, FCER1A, CTSG, CTSK, and STAT3. HIF1A was associated with metabolic and vascular targets, including ACE, SLC2A1, and NT5E. Shared STAT5B/STAT5A and PGR targets included ESR1, CCND1, IL10, and ABCG2.

**Functional Enrichment Analysis**: Functional enrichment analysis revealed distinct sex-stratified transcriptomic profiles (Table 2). Males demonstrated a broad response across 16 biological processes, led by adaptive immune response (count = 257, FDR $p = 0.0004$) and regulation of trans-synaptic signaling (count = 236, FDR $p = 0.0032$). Female responses were restricted to 3 pathways, centered on

extracellular matrix (ECM) organization (count = 162, FDR p = 0.0316). Etiology-specific stratification (Table 3) demonstrated that DCM converged on immune regulation in both sexes: lymphocyte activation in males (FDR $p = 3.67 \times 10^{-10}$, intersection = 196) and regulation of immune response in females (FDR $p = 9.84 \times 10^{-20}$, intersection = 243). HCM revealed divergent molecular patterns: male gene was characterized by regulation of cell migration (FDR $p = 1.86 \times 10^{-12}$, intersection = 236) and cytokine production (FDR $p = 5.29 \times 10^{-11}$, intersection = 200), whereas female HCM shifted toward cell morphogenesis (FDR $p = 1.38 \times 10^{-20}$, intersection = 298) and post-translational protein modification (FDR $p = 3.38 \times 10^{-19}$, intersection = 289).

Table 2

Global Sex-Stratified Gene Ontology Enrichment Axis in Human Heart Failure. Top Gene Ontology (GO) biological processes enriched among differentially expressed genes in failing left ventricles compared to non-failing donors. Gene ratio indicates the proportion of differentially expressed genes matching the specific GO term relative to the total query size. *Benjamini-Hochberg false discovery rate (FDR) corrected p-value < 0.05.

| Gene ID | Description | Count | Gene Ratio | p.adjust* |
|---|---|---|---|---|
| **Male** | | | | |
| GO:0002250 | Adaptive immune response | 244 | 244/6237 | 0.0001 |
| GO:0009611 | Response to wounding | 240 | 240/6237 | 0.0006 |
| GO:0043062 | Extracellular structure organization | 139 | 139/6237 | 0.0017 |
| GO:0045229 | External encapsulating structure organization | 139 | 139/6237 | 0.0017 |
| GO:0030198 | Extracellular matrix organization | 138 | 138/6237 | 0.0019 |
| GO:0002252 | Immune effector process | 267 | 267/6237 | 0.0033 |
| GO:0099177 | Regulation of trans-synaptic signaling | 213 | 213/6237 | 0.0039 |
| GO:0050804 | Modulation of chemical synaptic transmission | 211 | 211/6237 | 0.0055 |
| GO:0045123 | Cellular extravasation | 42 | 42/6237 | 0.0090 |
| GO:0051056 | Regulation of small GTPase mediated signal transduction | 165 | 165/6237 | 0.0119 |
| GO:0042060 | Wound healing | 186 | 186/6237 | 0.0176 |
| GO:0009410 | response to xenobiotic stimulus | 152 | 152/6237 | 0.0181 |
| GO:0002460 | Adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains | 157 | 157/6237 | 0.03321 |
| GO:0089718 | Amino acid import across plasma membrane | 29 | 29/6237 | 0.03321 |
| GO:0061564 | Axon development | 191 | 191/6237 | 0.03321 |
| GO:0048667 | Cell morphogenesis involved in neuron differentiation | 221 | 221/6237 | 0.03321 |
| **Female** | | | | |
| GO:0030198 | Extracellular matrix organization | 139 | 139/6448 | 0.0210 |
| GO:0043062 | Extracellular structure organization | 139 | 139/6448 | 0.0210 |
| GO:0045229 | External encapsulating structure organization | 139 | 139/6448 | 0.0210 |

Table 3

Etiology-Specific and Sex-Stratified Functional Pathway Enrichment in Dilated Cardiomyopathy (DCM) and Hypertrophic Cardiomyopathy (HCM). Term size reflects the total number of annotated pathway genes; intersection size represents the number of differentially expressed genes that overlap with the specific pathway. All listed pathways satisfy a strict significance threshold of * Benjamini-Hochberg false discovery rate (FDR) adjusted p-value < 0.05.

| Subtype | Term name | Term size | Adjusted p-value* | Intersection size |
|---|---|---|---|---|
| **Male** | | | | |
| DCM | Lymphocyte activation | 811 | $3.67 \times 10^{-10}$ | 196 |
| DCM | Leukocyte activation | 975 | $3.09 \times 10^{-09}$ | 223 |
| DCM | Small GTPase-mediated signal transduction | 493 | $1.99 \times 10^{-07}$ | 126 |
| DCM | Positive regulation of immune response | 775 | $2.42 \times 10^{-07}$ | 179 |
| DCM | Cell projection assembly | 615 | $2.68 \times 10^{-07}$ | 149 |
| HCM | Regulation of cell migration | 944 | $1.86 \times 10^{-12}$ | 236 |
| HCM | Cytokine production | 786 | $5.29 \times 10^{-11}$ | 200 |
| HCM | Regulation of cytokine production | 780 | $9.22 \times 10^{-11}$ | 198 |
| HCM | Response to peptide | 993 | $1.14 \times 10^{-10}$ | 239 |
| HCM | Enzyme-linked receptor protein signaling pathway | 988 | $1.17 \times 10^{-10}$ | 238 |
| **Female** | | | | |
| DCM | Regulation of immune response | 970 | $9.84 \times 10^{-20}$ | 243 |
| DCM | Positive regulation of immune response | 775 | $5.50 \times 10^{-17}$ | 199 |

| Subtype | Term name | Term size | Adjusted p-value* | Intersection size |
|---|---|---|---|---|
| **Male** | | | | |
| DCM | Regulation of cytokine production | 780 | $1.20 \times 10^{-16}$ | 199 |
| DCM | Cytokine production | 786 | $1.33 \times 10^{-16}$ | 200 |
| DCM | Leukocyte activation | 975 | $2.33 \times 10^{-16}$ | 234 |
| HCM | Cell morphogenesis | 998 | $1.39 \times 10^{-20}$ | 298 |
| HCM | Post-translational protein modification | 977 | $3.38 \times 10^{-19}$ | 289 |
| HCM | Protein modification by small protein conjugation or removal | 927 | $1.16 \times 10^{-18}$ | 276 |
| HCM | Mitotic cell cycle | 893 | $1.25 \times 10^{-17}$ | 262 |
| HCM | Protein modification by small protein conjugation | 810 | $2.73 \times 10^{-15}$ | 239 |

# Discussion

This study demonstrates etiology-specific sexual dimorphism in the human myocardial transcriptome. Most transcripts were shared across sexes and HF subtypes, but sex-specific genes shaped distinct remodeling programs. Differential expression was greater in males with DCM, whereas females exhibited greater divergence in HCM. Functional enrichment revealed sex- and etiology-specific pathway associations: in males, DCM was enriched for lymphocyte/leukocyte activation and HCM for cell migration and cytokine signaling; in females, DCM was enriched for immune and cytokine regulation, and HCM for cell morphogenesis and post-translational protein modification. Upstream analysis identified male SPI1/HIF1A and female SP1 networks converging on a shared STAT5/PGR core. SMOC2 was consistently upregulated across all HF phenotypes irrespective of sex. Together, these findings support a sex-biased framework for HF heterogeneity that extends beyond what adjustment for sex as a covariate would capture.

This study adds to evidence that sex-specific transcriptomic regulation extends beyond what adjustment for sex as a covariate would capture.

While Boheler et al. demonstrated that many HF-responsive genes vary more by sex and age than by etiology [23], our analysis extends this observation across a larger, multi-etiology cohort (n = 366). Although 6403 genes (~ 73%) of the cardiac transcriptome are shared between sexes, the remaining differentially regulated subset reflects significant functional partitioning [24]. In DCM, ~ 12-fold greater DGE burden in males than females (4998 vs. 410 DEGs) suggests more extensive transcriptional reprogramming in the male myocardium. This pattern is consistent with population data showing a persistent male predominance in DCM [25], and reflects a complex interplay of inflammation, environmental, genetic architecture, and hormonal influences that drive known sex differences in disease progression and therapeutic response [10, 11, 26, 27]. Conversely, the reverse pattern in HCM, where females exhibited greater DGE than males (42 vs. 19 DEGs), may reflect stronger heritability of HCM (a Mendelian condition)[28–31] interacting with female-specific hormonal and epigenetic modifiers to produce greater transcriptomic divergence. As an observational, cross-sectional analysis of explanted myocardium, this study cannot distinguish whether sex-divergent transcriptomic patterns are a cause or a downstream consequence of etiology-specific disease trajectories, and the direction of that relationship should not be inferred from these data.

A key contribution of this study is the identification of a sex-divergent regulatory network architecture through upstream TF analysis. SP1 exhibited the highest connectivity in the female regulatory network, consistent with the hypothesis that female HF remodeling involves structural and matrix-stabilizing programs. SP1 is a zinc finger TF previously implicated in cardiovascular pathology through fibrosis, ECM deposition, and hypertrophy [32]. This aligns with experimental evidence that female cardiac fibroblasts exhibit differential TGF-beta1 responsiveness compared with male cells, while males show increased alpha-SMA stress fiber formation and enhanced SMAD2/3 nuclear localization, and enhanced resistance to TGF-beta receptor inhibition [33]. Clinically, these sex differences in fibroblast activation may underlie the observed female predominance in HF with preserved ejection fraction, where diastolic dysfunction and ventricular stiffness are hallmark features. SPI1 anchored the male network around myeloid-lineage targets, consistent with its established role in immune inflammation [34]. Zhang et al. previously reported SPI1 and SP1 as shared regulators in DCM [35]; our multi-etiology, sex-specific design instead resolves these as sex-divergent rather than shared, suggesting that sex-specific transcriptional architecture may be obscured in single-etiology designs. HIF1A was an additional male-specific regulator, consistent with previous reports implicating this factor in metabolic adaptation, facilitating the shift toward anaerobic glycolysis during pressure overload and oxygen deprivation [36]. These sex-divergent upstream regulatory patterns are consistent with the long-observed clinical phenomenon of greater inflammatory activation in male HF and relatively preserved matrix architecture in female HF [37]. The shared STAT5A/B and PGR targets across both sexes suggest a conserved sex-hormone-responsive transcriptional layer that persists despite divergent downstream remodeling. STAT5 has been implicated in cytokine and growth factor signaling with cardioprotective functions [38, 39], while PGR (progesterone receptor) participates in hormonally regulated cardiac adaptation [40]. The co-

localization of these shared regulators with sex-specific hubs (SP1 in females, SPI1/HIF1A in males) suggests a model in which hormonal signals provide a common regulatory scaffold that is differentially interpreted by sex-specific transcriptional machinery, yielding divergent remodeling patterns. This architecture has therapeutic implications: interventions targeting the shared STAT5/PGR axis might offer broad applicability, whereas sex-specific modulation of SP1 or SPI1 pathways could enable precision therapeutic approaches. The biological interpretation of these divergent pathways is further supported by the functional enrichment results. The male HF transcriptome's focus on immune-inflammatory and wound-healing pathways mirrors established observations that male hearts exhibit a heightened propensity toward immune-inflammation and leukocyte recruitment. In contrast, the female transcriptome's dominance in structural, matrix-driven adaptive remodeling is consistent with a "fibro-protective" or "matrix-stabilizing" response, which is consistent with recent evidence [24].

SMOC2 (secreted protein, acidic and rich in cysteine-related modular calcium-binding protein 2) appeared the most consistent transcriptomic signal across all HF phenotypes, irrespective of sex. Biologically, SMOC2 has been linked to the fibroblast-to-myofibroblast transition, a hallmark of pathological fibrosis [41, 42]. Our finding aligns with recent studies demonstrating that SMOC2 mRNA is stabilized by METTL3-mediated m6A methylation, which enhances cardiac fibroblast proliferative and differentiation capacity through the TGF-beta1/Smad3 signaling axis and modulation of autophagy [42–44]. In a prospective cohort of ischemic cardiomyopathy (n = 188), elevated plasma SMOC2 was associated with higher HF rehospitalization risk (HR = 1.02; 95% CI, 1.012–1.655), and correlated with NT-proBNP, left ventricular end-diastolic diameter, and hospital stay length, and was inversely associated with ejection fraction and fractional shortening [45]. The consistent upregulation of SMOC2 ($p = 2.2 \times 10^{-16}$) observed in this study suggests it is associated with pathological remodeling across sexes and etiologies, representing a conserved molecular axis in HF that may warrant further investigation as a potential therapeutic target.

PCA and unsupervised clustering indicate that sex and etiology define continuous remodeling gradients rather than fully separable transcriptomic classes. The sharper separation of male NFD and DCM along PC1, together with the larger DEG burden in male DCM, is compatible with broader transcriptional reprogramming in men with DCM. The greater ellipse overlap across etiologies in females suggests transcriptomic change may be distributed across multiple variance axes rather than one dominant program. The limited dendrogram separation warrants caution; bulk tissue sequencing blends diverse cardiac cell types, creating compositional shifts and inter-patient heterogeneity that can obscure etiologic boundaries despite robust underlying signals [46]. Our findings extend the microarray-era observations of Fermin et al. by showing a broader and more etiology-dependent sex-biased remodeling landscape than that earlier probe-based platforms could resolve [17]; as an exploratory analysis, we attribute this expanded variance largely to RNA-seq's superior dynamic range and gene coverage. Age- and race-adjusted ANCOVA results suggest that sex-related differences in HF are reflected in regulatory state and network coordination rather than by gene content alone. Although 73% of significant transcripts overlapped between men and women, the shared transcriptome appears to be utilized

differently across sexes, consistent with evidence that sex-biased expression often reflects reweighting of shared pathways rather than entirely distinct gene sets [37].

This study is strengthened by its comprehensive sex-stratified analytical framework utilizing a large, well-characterized MAGNet consortium cohort for high-resolution left ventricular transcriptomic profiling (Illumina HiSeq 2500). By integrating an age- and race-adjusted multi-method approach, we demonstrated a highly consistent biologically relevant signature across analyses. Importantly, this sex-stratified design addresses a critical gap in HF research by exposing divergent transcriptomic signatures typically obscured in sex-aggregated analyses. Several limitations warrant consideration: 1) lack of genetic data to distinguish familial from non-familial cases, particularly relevant for HCM, where sarcomeric gene mutations are prevalent; 2) bulk RNA-sequencing precludes cell-type-specific attribution of transcriptomic changes; 3) sample size imbalance between DCM (n = 166) and HCM (n = 28) may limit the statistical power for sex-specific conclusions; 4) residual confounding from medications, comorbidities, or disease duration could not be fully addressed; 5) due to the small sample size and sex-specific nature, PPCM was excluded from inferential modeling; 6) transmural biopsies represent end-stage explanted tissues, so temporal progression of remodeling cannot be captured; 7) findings require external validation in independent cohorts with comprehensive clinical follow-up; 8) observational, cross-sectional analysis of explanted myocardium cannot establish whether sex-divergent transcriptomic patterns precede or follow etiology-specific disease trajectories; the direction of association should not be inferred from these data.

In conclusion, sex-stratified analyses revealed largely shared but etiologically divergent myocardial remodeling programs, with male-predominant immune/inflammatory signatures, female-associated structural/ECM-related signatures, distinct candidate TF networks, and SMOC2 as a conserved HF-associated remodeling signal. These exploratory insights offer a framework for future translational studies toward sex-specific therapeutic targets in HF. Future research integrating single-cell or single-nucleus transcriptomics with prospective validation is needed to determine whether the regulatory hubs identified represent tractable therapeutic entry points and whether they are consistent across sex and etiology to translate into clinical utility. Bridging the gap between these sex-biased transcriptomic signatures and clinical practice will be essential for achieving equity-focused precision medicine in cardiovascular care.

# Abbreviations

**AF:** Atrial fibrillation.

**ANCOVA**: Analysis of covariance.

**CVD**: Cardiovascular disease.

**DCM**: Dilated cardiomyopathy

**DESeq2**: **Differential expression sequencing 2**.

**DGE**: Differential gene expression.

**ECM:** Extracellular matrix.

**FDR**: False discovery rate.

**GEO**: Gene Expression Omnibus.

**GO:BP**: Gene Ontology Biological Process.

**HCM**: Hypertrophic cardiomyopathy

**HF**: Heart failure.

**IRB**: Institutional Review Board

**MAGNet**: Myocardial applied genomics network.

**NCBI**: National Center for Biotechnology Information

**NFD**: Non-failing donor.

**PCA**:  Principal component analysis.

**PPCM**: Peripartum cardiomyopathy

**RNA**: Ribonucleic acid.

**Seq**: Sequencing.

**SMOC2:** Secreted modular calcium-binding protein 2.

**STROBE**: Standards Outlined In The Strengthening The Reporting Of Observational Studies In Epidemiology

**VF**: Ventricular fibrillation.

**VST**: Variance stabilizing transformation

# Declarations

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**Ethics approval and consent to participate:** The dataset is publicly accessible and de-identified, and therefore, exempt from Institutional Review Board (IRB) approval.

**Availability of data and materials**: The datasets supporting the conclusions of this article are de-identified, publicly available data in the GEO database and the dedicated GitHub repository associated with the Magnetique analytical framework.

**Author's contributions:** TB: Conceptualization and design, data acquisition, formal statistical analysis and interpretation of results, drafting manuscript, reviewing critically for intellectual content, and editing. MS: Critical revision and editing. HC: Critical revision and editing. All authors read and approved the final manuscript.

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# Statement on AI use

During the preparation of this manuscript, the authors used Claude (Version Sonnet 4.6) to improve the readability of the manuscript. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the publication's content.

## References

1. McDonagh TA, Metra M, Adamo M, Baumbach A, Böhm M, Burri H et al. 2021 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. European Heart Journal. Oxford University Press; 2021. pp. 3599–726. 10.1093/eurheartj/ehab368 PubMed PMID: 34447992.

2. Heidenreich PA, Bozkurt B, Aguilar D, Allen LA, Byun JJ, Colvin MM, et al. 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: Executive Summary: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation. Lippincott Williams and Wilkins; 2022. pp. E876–94. 10. 1161/CIR.0000000000001062 PubMed PMID: 35363500.
3. Savarese G, Becher PM, Lund LH, Seferovic P, Rosano GMC, Coats AJS. Global burden of heart failure: a comprehensive and updated review of epidemiology. Cardiovascular Research. Oxford University Press; 2022. pp. 3272–87. 10.1093/cvr/cvac013 . PubMed PMID: 35150240.
4. Valente V, Laborante R, Abdin A, Becher PM, Lainscak M, Polovina M, et al. The Global Epidemiology of Heart Failure: A Comprehensive and Contemporary Review. Eur J Heart Fail. 2026 Apr;7. 10.1093/ejhf/xuag121 . PubMed PMID: 41955514.
5. Heidenreich PA, Bozkurt B, Aguilar D, Allen LA, Byun JJ, Colvin MM, et al. 2022 AHA/ACC/HFSA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. Circulation. Lippincott Williams and Wilkins; 2022. pp. E895–1032. 10. 1161/CIR.0000000000001063 PubMed PMID: 35363499.
6. Stamp KD, Prasun MA, McCoy TP, Lisa R. Utility of the New York heart association functional classification compared to other measures: A systematic review. Health Sciences Review. Elsevier Ltd; 2025. 10.1016/j.hsr.2025.100241
7. Bozkurt B, Colvin M, Cook J, Cooper LT, Deswal A, Fonarow GC, et al. Current Diagnostic and Treatment Strategies for Specific Dilated Cardiomyopathies: A Scientific Statement from the American Heart Association. Circulation. Lippincott Williams and Wilkins; 2016. pp. e579–646. 10. 1161/CIR.0000000000000455 PubMed PMID: 27832612.
8. Kittleson MM, Ye SQ, Irizarry RA, Minhas KM, Edness G, Conte JV, et al. Identification of a gene expression profile that differentiates between ischemic and nonischemic cardiomyopathy. Circulation. 2004;110(22):3444–51. .11 PubMed PMID: 15557369.
9. Britto-Borges T, Ludt A, Boileau E, Gjerga E, Marini F, Dieterich C. Magnetique: an interactive web application to explore transcriptome signatures of heart failure. J Transl Med. 2022;20(1). 10.1186/s12967-022-03694-z . PubMed PMID: 36345035.
10. Vaura F, Palmu J, Aittokallio J, Kauko A, Niiranen T. Genetic, Molecular, and Cellular Determinants of Sex-Specific Cardiovascular Traits. Circ Res. 2022;130(4):611–31. 10.1161/CIRCRESAHA.121.319891 . PubMed PMID: 35175841.
11. Solomon SD, McMurray JJV, Anand IS, Ge J, Lam CSP, Maggioni AP, et al. Angiotensin–Neprilysin Inhibition in Heart Failure with Preserved Ejection Fraction. N Engl J Med. 2019;381(17):1609–20. 10.1056/nejmoa1908655 . PubMed PMID: 31475794.
12. McMurray JJV, Jackson AM, Lam CSP, Redfield MM, Anand IS, Ge J, et al. Effects of Sacubitril-Valsartan Versus Valsartan in Women Compared With Men With Heart Failure and Preserved

Ejection Fraction Insights From PARAGON-HF. Circulation. 2020;141(5):338–51. 10.1161. /CIRCULATIONAHA.119.044491 PubMed PMID: 31736337.

13. Liu Y, Morley M, Brandimarto J, Hannenhalli S, Hu Y, Ashley EA, et al. RNA-Seq identifies novel myocardial gene expression signatures of heart failure. Genomics. 2015;105(2):83–9. 12.002 PubMed PMID: 25528681.
14. Lanzer JD, Ramirez Flores RO, Liñares Blanco J, Steier M, Rangrez AY, Frey N, et al. A cross-study transcriptional patient map of heart failure defines conserved multicellular coordination in cardiac remodeling. Nat Commun. 2025;16(1). 10.1038/s41467-025-62219-6 . PubMed PMID: 41173856.
15. Lteif C, Huang Y, Guerra LA, Gawronski BE, Duarte JD. Using Omics to Identify Novel Therapeutic Targets in Heart Failure. Circulation: Genomic and Precision Medicine. Lippincott Williams and Wilkins; 2024. pp. 277–93. 10.1161/CIRCGEN.123.004398 . PubMed PMID: 38766848.
16. Deegan DF, Nigam P, Engel N. Sexual Dimorphism of the Heart: Genetics, Epigenetics, and Development. Frontiers in Cardiovascular Medicine. Front Media SA. 2021. 10.3389/fcvm.2021.668252.
17. Fermin DR, Barac A, Lee S, Polster SP, Hannenhalli S, Bergemann TL, et al. Sex and age dimorphism of myocardial gene expression in nonischemic human heart failure. Circ Cardiovasc Genet. 2008;1(2):117–25. 10.1161/CIRCGENETICS.108.802652 . PubMed PMID: 20031553.
18. Sá ACC, Sadee W, Johnson JA. Whole Transcriptome Profiling: An RNA-Seq Primer and Implications for Pharmacogenomics Research. Clin Transl Sci. 2018;11(2):153–61. 10.1111/cts.12511 . PubMed PMID: 28945944.
19. Tan WLW, Anene-Nzelu CG, Wong E, Lee CJM, Tan HS, Tang SJ, et al. Epigenomes of Human Hearts Reveal New Genetic Variants Relevant for Cardiac Disease and Phenotype. Circ Res. 2020;127(6):761–77. 10.1161/CIRCRESAHA.120.317254 . PubMed PMID: 32529949.
20. Flam E, Jang C, Murashige D, Yang Y, Morley MP, Jung S, et al. Integrated landscape of cardiac metabolism in end-stage human nonischemic dilated cardiomyopathy. Nat Cardiovasc Res. 2022;1(9):817–29. 10.1038/s44161-022-00117-6.
21. White K, Yang P, Li L, Farshori A, Medina AE, Zielke HR. Effect of Postmortem Interval and Years in Storage on RNA Quality of Tissue at a Repository of the NIH NeuroBioBank. Biopreserv Biobank. 2018;16(2):148–57. 10.1089/bio.2017.0099 . PubMed PMID: 29498539.
22. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. J Clin Epidemiol. 2008;61(4):344–9. PubMed PMID: 18313558.
23. Boheler KR, Volkova M, Morrell C, Garg R, Zhu Y, Margulies K et al. Sex-and age-dependent human transcriptome variability: Implications for chronic heart failure. PNAS [Internet]. 2003;100(5):2754–9. Available from: www.pnas.orgcgidoi10.1073pnas.0436564100.
24. Sweet ME, Cocciolo A, Slavov D, Jones KL, Sweet JR, Graw SL, et al. Transcriptome analysis of human heart failure reveals dysregulated cell adhesion in dilated cardiomyopathy and activated

immune pathways in ischemic heart failure. BMC Genomics. 2018;19(1). 10.1186/s12864-018-5213-9 . PubMed PMID: 30419824.

25. Bergan N, Prachee I, Curran L, Mcgurk KA, Lu C, De Marvao A, et al. Systematic Review, Meta-Analysis, and Population Study to Determine the Biologic Sex Ratio in Dilated Cardiomyopathy. Circulation. 2025;151(7):442–59. 10.1161/CIRCULATIONAHA.124.070872 . PubMed PMID: 39895490.
26. Luo Y, Safabakhsh S, Palumbo A, Fiset C, Shen C, Parker J, et al. Sex-Based Mechanisms of Cardiac Development and Function: Applications for Induced-Pluripotent Stem Cell Derived-Cardiomyocytes. International Journal of Molecular Sciences. Multidisciplinary Digital Publishing Institute (MDPI); 2024. 10.3390/ijms25115964 . PubMed PMID: 38892161.
27. Deegan DF, Nigam P, Engel N. Sexual Dimorphism of the Heart: Genetics, Epigenetics, and Development. Frontiers in Cardiovascular Medicine. Front Media SA. 2021. 10.3389/fcvm.2021.668252.
28. Hespe S, Waddell A, Asatryan B, Owens E, Thaxton C, Adduru ML, et al. Genes Associated With Hypertrophic Cardiomyopathy: A Reappraisal by the ClinGen Hereditary Cardiovascular Disease Gene Curation Expert Panel. J Am Coll Cardiol. 2025;85(7):727–40. 10.1016/j.jacc.2024.12.010 . PubMed PMID: 39971408.
29. Hershberger RE, Cowan J, Jordan E, Kinnamon DD. The Complex and Diverse Genetic Architecture of Dilated Cardiomyopathy. Circ Res. 2021;128(10):1514–32. 10.1161/CIRCRESAHA.121. .318157 PubMed PMID: 33983834.
30. Puckelwartz MJ, Pesce LL, Dellefave-Castillo LM, Wheeler MT, Pottinger TD, Robinson AC, et al. Genomic context differs between human dilated cardiomyopathy and hypertrophic cardiomyopathy. J Am Heart Assoc. 2021;10(7). 10.1161/JAHA.120.019944 . PubMed PMID: 33764162.
31. Stroeks SLVM, Merlo M, Mora-Ayestaran N, Jason M, Tayal U, Wang P, et al. Sex Differences in Prognosis of Patients With Genetic Dilated Cardiomyopathy. Circ Heart Fail. 2025;18(11):e012592. 10.1161/. CIRCHEARTFAILURE.124.012592 PubMed PMID: 40988625.
32. Ding J, Fayyaz AI, Ding Y, Liang D, Luo M. Role of Specificity Protein 1 (SP1) in Cardiovascular Diseases: Pathological Mechanisms and Therapeutic Potentials. Biomolecules. Multidisciplinary Digital Publishing Institute (MDPI); 2024. 10.3390/biom14070807 . PubMed PMID: 39062521.
33. Faust MN, Nguyen AK, Gorashi RM, Félix Vélez NE, Loud MC, Aguado BA. Transforming Growth Factor β1 Modulates Sex Differences in Cardiac Myofibroblast Activation on Hydrogel Biomaterials [Internet]. 2026. Available from: http://biorxiv.org/lookup/doi/10.64898/2026.01.16.699818 doi:10.64898/2026.01.16.699818.
34. Liu Z, Huang S. Upregulation of SPI1 during myocardial infarction aggravates cardiac tissue injury and disease progression through activation of the TLR4/NFκB axis. Am J Transl Res. 2022;14(4):2709–27.
35. Zhang M, Wang X, Chen W, Liu W, Xin J, Yang D, et al. Integrated bioinformatics analysis for identifying key genes and pathways in female and male patients with dilated cardiomyopathy. Sci

Rep. 2023;13(1). 10.1038/s41598-023-36117-0 . PubMed PMID: 37268658.

36. Rahman MA, Rahaman KA, Harrath AH, Al-Zharani M, Jalouli M. Hypoxia-inducible factor-1α in cardiovascular disease, mechanistic insights, pathophysiological roles, and therapeutic targeting strategies. Biochemical Pharmacology. Elsevier Inc.; 2026. 10.1016/j.bcp.2026.117753.
37. Regitz-Zagrosek V, Kararigas G. Mechanistic Pathways of Sex Differences in Cardiovas-cular Disease. Physiol Rev. 2017;97:1–37. 10.1152/physrev.00021.2015.-Major.
38. Jin G, Wang L, Ma J. Inhibiting STAT5 significantly attenuated Ang II-induced cardiac dysfunction and inflammation. Eur J Pharmacol. 2022;915. 10.1016/j.ejphar.2021.174689 . PubMed PMID: 34919891.
39. Heusch G, Musiolik J, Kottenberg E, Peters J, Jakob H, Thielmann M. STAT5 activation and cardioprotection by remote ischemic preconditioning in humans. Circ Res. 2012;110(1):111–5. 10.1161/CIRCRESAHA.111.259556 . PubMed PMID: 22116817.
40. Sim CB, Phipson B, Ziemann M, Rafehi H, Mills RJ, Watt KI, et al. Sex-Specific Control of Human Heart Maturation by the Progesterone Receptor. Circulation. 2021;143(16):1614–28. 10.1161/CIRCULATIONAHA.120.051921 . PubMed PMID: 33682422.
41. Yan W, Li Y, Wang G, Huang Y, Xie P. Clinical application and immune infiltration landscape of stemness-related genes in heart failure. ESC Heart Fail. 2025;12(1):250–70. 10.1002/ehf2. 15055 PubMed PMID: 39275894.
42. Ren Y, Wu Y, He W, Tian Y, Zhao X. SMOC2 plays a role in heart failure via regulating TGF-β1/Smad3 pathway-mediated autophagy. Open Med (Poland). 2023;18(1). 10.1515/med-2023-0752.
43. Meng L, Chen H, min, Zhang Jsheng, Wu Yrong, Xu Yzhou. Matricellular proteins: From cardiac homeostasis to immune regulation. Biomedicine and Pharmacotherapy. Elsevier Masson s.r.l.; 2024. 10.1016/j.biopha.2024.117463 PubMed PMID: 39305814.
44. Yun X, Ling Y, Gaoyuan L, Saiyang X, Mengyao L, Nan Z, et al. SMOC2 accelerates myocardial fibrosis following myocardial infarction by promoting lipid peroxidation through inhibition of the LKB1/AMPKα/FOXO3 pathway. J Adv Res. 2026. 10.1016/j.jare.2026.03.022.
45. Chen X, Zhong X, Luo D, Lei Y, Huang R. Plasma SMOC2 Predicts Prognosis in Patients with Heart Failure: A Prospective Cohort. Int J Gen Med. 2024;17:1651–64. 10.2147/ijgm.s445457.
46. Koenig AL, Shchukina I, Amrute J, Andhey PS, Zaitsev K, Lai L, et al. Single-cell transcriptomics reveals cell-type-specific diversification in human heart failure. Nat Cardiovasc Res. 2022;1(3):263–80. 10.1038/s44161-022-00028-6 . PubMed PMID: 35959412.

# Figures

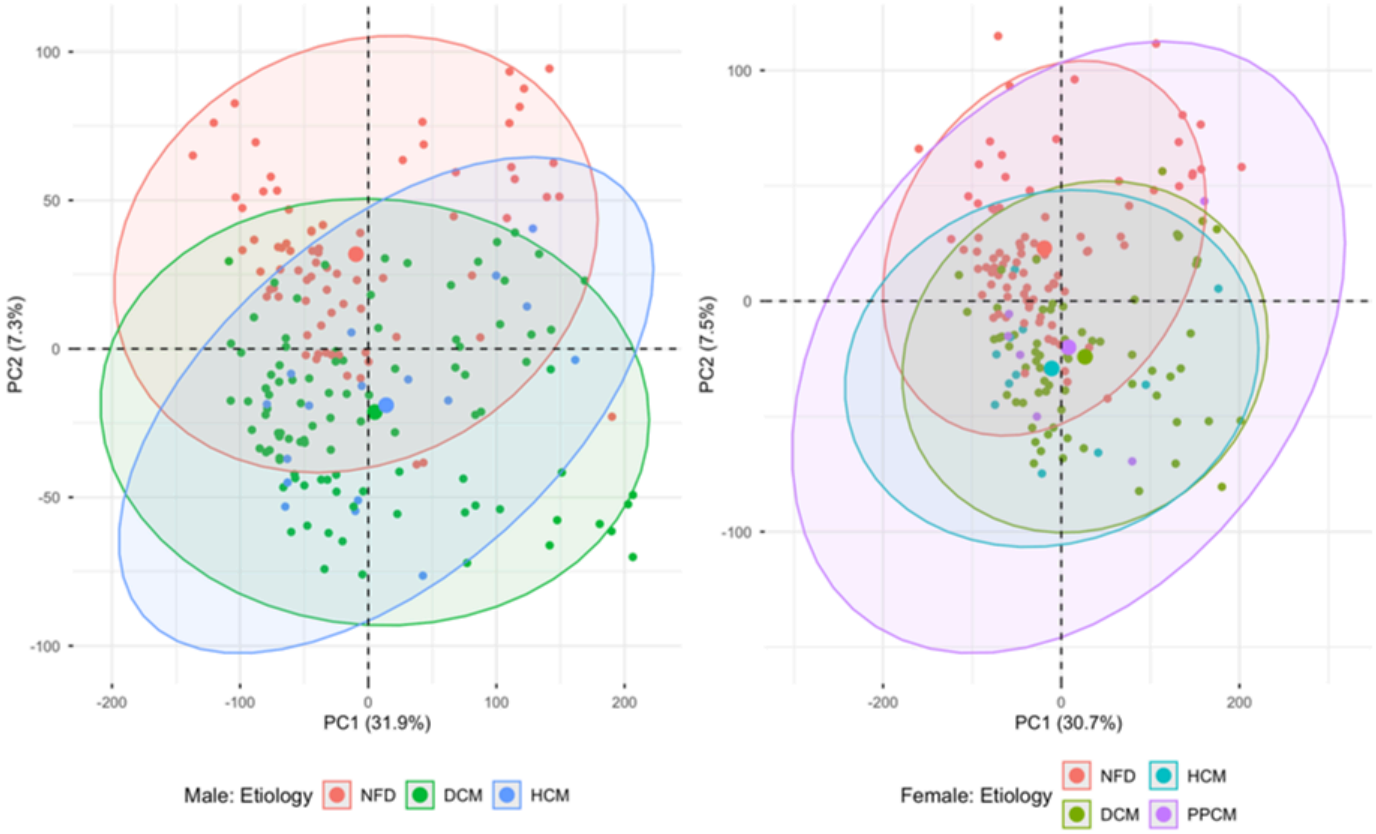



**Figure 1**

Sex-stratified Principal component analysis of transcriptomic profiles. Partial separation among etiology groups in males and females. Ellipses indicate group dispersion, with PC1 and PC2 explaining the variance shown for each subset.

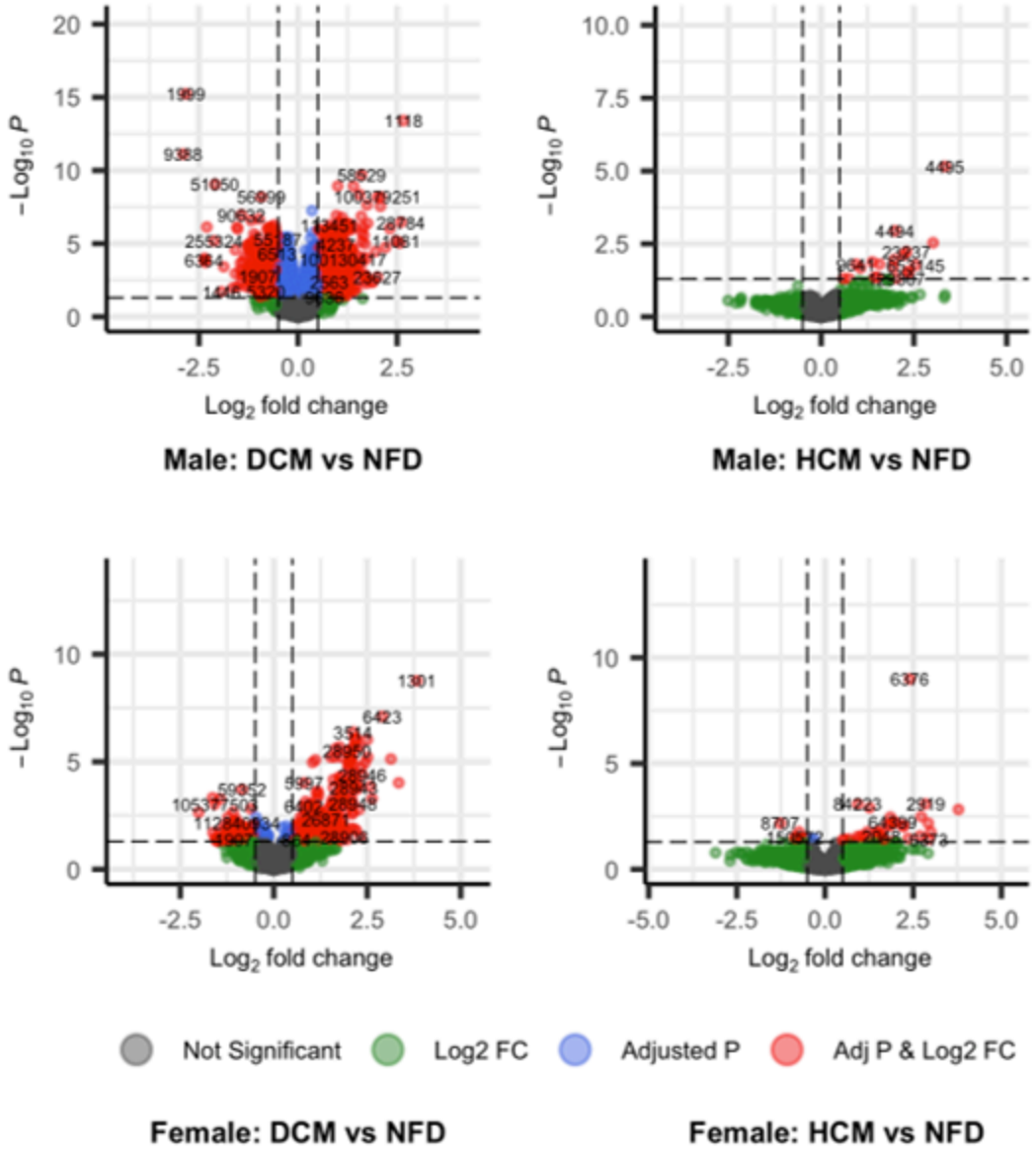



**Figure 2**

**Sex-stratified volcano plots of differential gene expression.** These volcano plots illustrate transcriptomic shifts in DCM and HCM, compared to NFD, derived from raw integer counts using DESeq2 negative binomial modeling. Significant genes were identified using an absolute log2 fold change threshold of 0.5 and a Benjamini-Hochberg-adjusted p-value < 0.05. The male DCM cohort exhibits a greater number of both upregulated and downregulated genes than the female. The HCM phenotype demonstrates sexual dimorphism, with females showing a broader set of significantly upregulated and downregulated transcripts compared to males, suggesting a more constrained molecular divergence state in men with HCM.

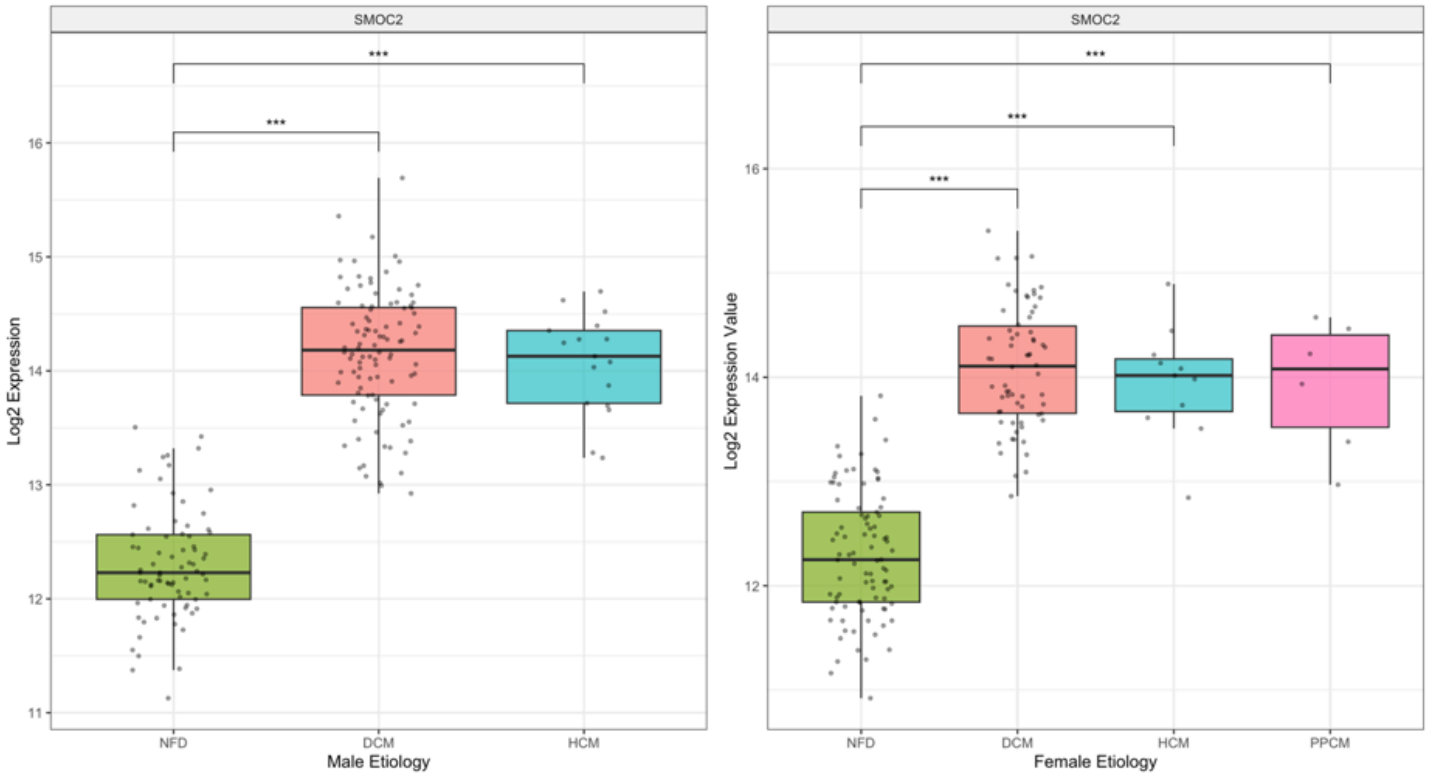



**Figure 3**

**Sex-stratified differential expression of ENSG00000112562 (SMOC2) across heart failure etiologies**. These boxplots illustrate transformed expression levels for the top differentially expressed gene identified in male (left) and female (right) cohorts. Comparisons include dilated cardiomyopathy (DCM), hypertrophic cardiomyopathy (HCM), and peripartum cardiomyopathy (PPCM; female-only) relative to non-failing donors (NFD). Despite sex-specific cohort differences, ENSG00000112562 (SMOC2) emerged as the top-ranked gene in both sexes, with profound statistical significance ($p = 2.2 \times 10^{-16}$), from Tukey-adjusted post-hoc pairwise comparisons following ANCOVA, while models adjusted for age and race. In males, SMOC2 expression was significantly lower in NFD compared with DCM and HCM (-1.81 to -1.84 log2 units, all $p < 0.0001$ ). In females, NFD expression was similarly lower compared with DCM, HCM, and PPCM ( -1.81 to -1.90 log2 units, all $p < 0.0001$). Notably, no significant differences were observed between disease groups within either sex (e.g., Male DCM vs. HCM, $p = 0.98$; Female DCM vs. PPCM, $p > 0.99$ ).

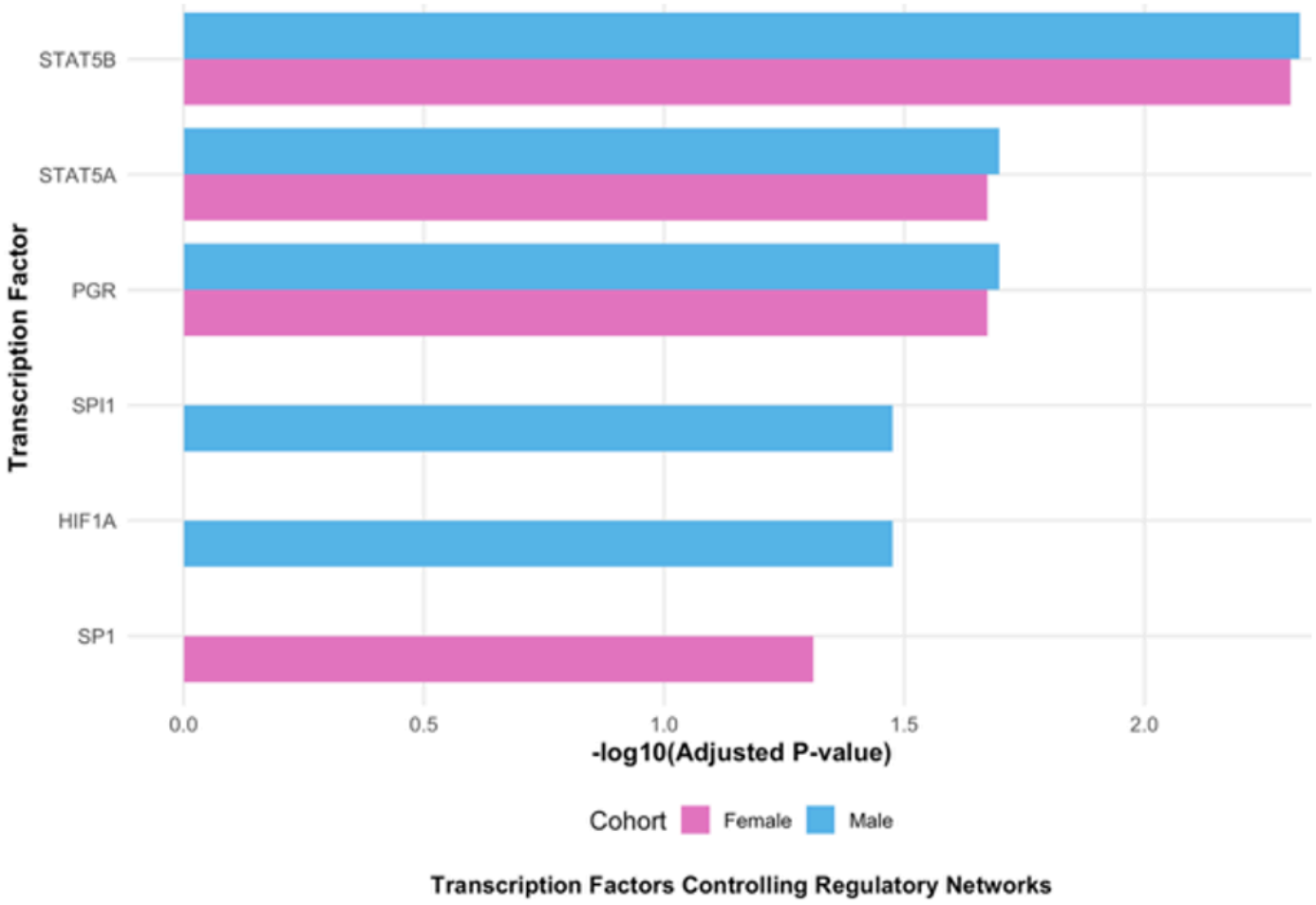



**Figure 4**

**Sex-stratified transcription factor analysis.** Bar plot of significant upstream transcription factors (TFs) identified from the top 500 HF-driving genes in male (blue) and female (pink) cohorts, ranked by $-\log_{10}$(FDR-adjusted p-value). STAT5B, STAT5A, and PGR were shared across sexes; SPI1 and HIF1A were male-specific; SP1 was female-specific.

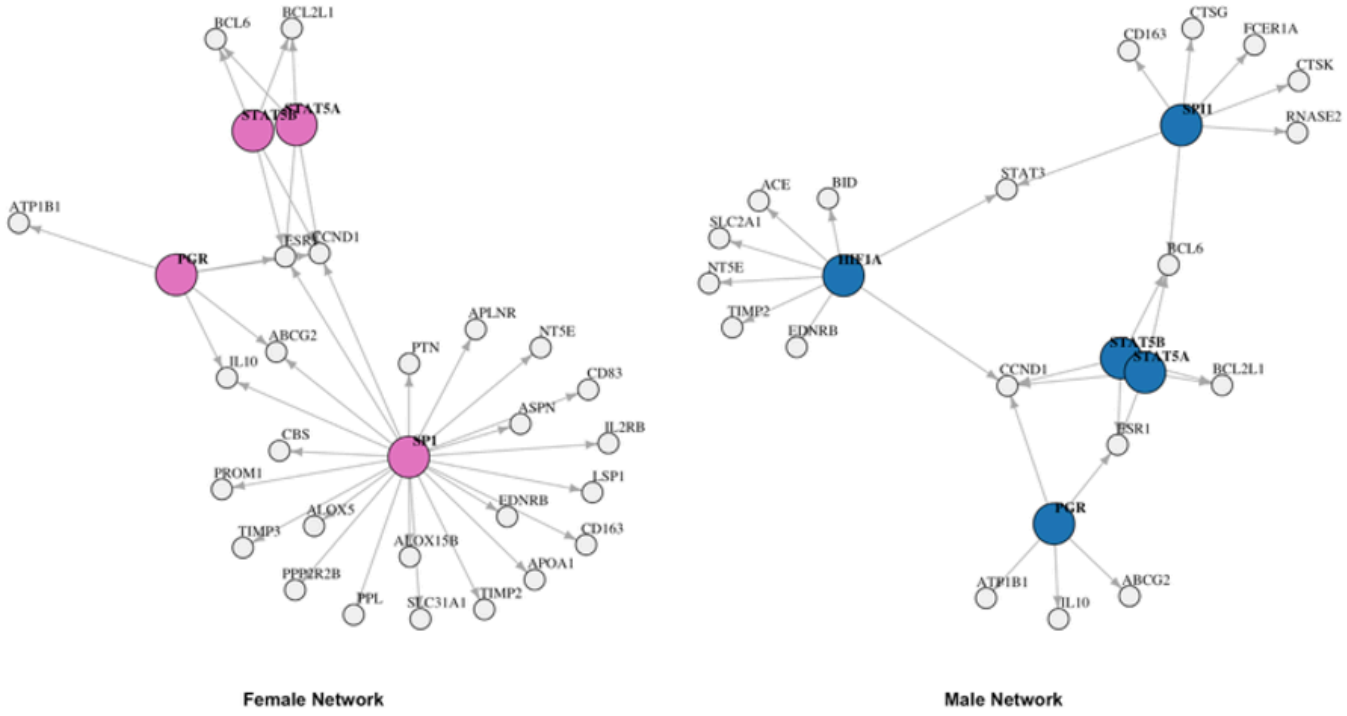



**Figure 5**

Sex-stratified regulatory networks depicting transcription factor (TF)-target gene interactions in female (left) and male (right) cohorts. Node size reflects connectivity. SP1 serves as the dominant hub in the female network, regulating a broad set of ECM, lipid, and inflammatory target genes. SPI1 anchors the male network, targeting myeloid immune effector genes. Shared STAT5B/STAT5A and PGR regulatory modules across both networks indicate a conserved sex-hormone-responsive transcriptional layer underlying sex-divergent HF remodeling.

## Supplementary Files

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- SupplementaryDocuments.docx