

**Biological Pathways and Gene Networks Link Inflammation and Vascular Remodeling
to Both Heart Failure with Preserved and Reduced Ejection Fraction
in Women across Ethnicities**

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Abstract

Introduction: Heart failure (HF) is understudied among women; especially, genomic evidence implicating shared or unique mechanisms of HF with respect to reduced or preserved ejection fraction (HFrEF, HFpEF) is lacking across ethnic populations of women. Prior genome-wide association studies (GWAS) have identified approximately 30 suggestive genetic variants for HF, although none have been specifically linked to HFrEF or HFpEF.

Objectives: We aimed to define, replicate, and annotate genetic variants to HFrEF, HFpEF, or both, as well as to investigate potential biological mechanisms underlying HFrEF and HFpEF among African American (AA) and European American (EA) women in three well-characterized, high-quality prospective cohorts, the Women's Health Initiative (WHI) study, the Jackson Heart Study (JHS), and the Framingham Heart Study (FHS).

Methods: GWAS analysis on HFrEF and HFpEF were first performed among 7,982 AA and 4,133 EA in the WHI, followed by pathway analysis employing two independent methodological platforms (GSA-SNP and Mergeomics) curating KEGG, Reactome, and

BioCarta pathway databases. GWAS signals and biological pathways identified using the WHI were replicated in the JHS and FHS. For all replicated pathways, we performed cross-phenotype and cross-ethnicity validation analyses to examine shared pathways between HFrEF and HFpEF, and phenotype-specific pathways, across ethnicities. We further prioritized key driver genes for HF according to specific pathways identified.

Results: We validated one previously reported genetic locus and identified six new ones, among which one locus was allocated to HFrEF and five to HFpEF. Additionally, we defined five biological pathways shared between HFrEF and HFpEF and discovered six HFpEF-specific pathways. These pathways overlapped in two main domains for molecular signaling: 1) inflammation and 2) vascular remodeling (including angiogenesis and vascular patterning), involving key driver genes from collagen and HLA gene families.

Conclusions: Our network analysis of three large prospective cohorts of women in the United States defined several novel loci for HF and its subtypes. In particular, several key driver genes reinforce the mechanistic role of inflammation and vascular remodeling in the development of HF, especially HFpEF. Given that therapeutic strategies developed for left ventricular dysfunction have had limited success for HFpEF, several new targets and pathways identified and validated in this study should be further assessed in risk stratification as well as the design of potential new HF interventions.

1 **Introduction**

2 According to the American Heart Association (AHA), approximately 6.5 million U.S.
3 adults have heart failure (HF) in 2018¹, representing a major cause of morbidity and mortality
4 in the United States. Heart failure is phenotypically and genetically heterogeneous, and much
5 remains unknown about the etiology for subtypes of HF, including HF with preserved
6 ejection fraction (HFpEF) and HF with reduced ejection fraction (HFrEF). Of note as many
7 as 40-71% of patients have HFpEF for which limited clinical treatment options are available
8 with little to no impact on outcomes². Moreover, HF is understudied in women, who
9 experience a higher mortality than men¹. African American (AA) women have the highest
10 HF incidence, followed by Hispanic Americans (HA) and European Americans (EA), and
11 morbidity and mortality are twice as high for AA women relative to EA³⁻⁶. Observational
12 studies have shown that while patients with HFpEF and HFrEF often display similar clinical
13 symptoms, they often have markedly different risk factors, underlying pathophysiological
14 processes, and responses to clinical therapies⁷. Many therapies with unequivocal benefit in
15 HFrEF have failed to show efficacy for HFpEF^{8,9}. Thus, there is a urgent need to further
16 research biological pathways and gene networks for HFpEF and HFrEF that could provide
17 mechanistic insight into the disease processes and identify potential targets for novel
18 treatment modalities, particularly for women, who are disproportionately affected by HF¹.

19 Recent candidate gene studies and genome-wide association studies (GWAS) have
20 identified several genetic loci (such as *ADRB1*, *USP3*, *ITPK1*, and *BAG3* genes^{10,11}
21 associated with HF risk. However, these studies are primarily focused on genes associated
22 with inherited HFrEF and are limited by reproducibility, effect size, and lack of ethnic
23 diversity^{10,11}. Although women are at higher risk of HF, no studies to date have
24 examined/observed sex-specific genetic variants. Moreover, few studies have directly
25 investigated the genetic mechanisms underlying the two HF subtypes, HFpEF and

26 HFrEF^{12,13}; and no studies were conducted in an ethnically diverse population of women. As
27 genes tend to behave conjointly on HF processes, analyzing a cluster of genes with related
28 biological functions, using an integrative pathway and network approach^{14,15}, improves the
29 statistical power to identify genetic variants of biological importance. To enhance the
30 understanding of biological mechanisms underlying different HF phenotypes (HFpEF versus
31 HFrEF) as well as genomic and ethnic diversity in women with HF, we therefore investigated
32 genetic risk factors and biological pathways predisposing to HF and its subtypes among AA
33 and EA women in the Women's Health Initiative (WHI) study. We replicated our findings
34 using AA women from the JHS, EA from FHS, and HA women from the WHI.

35

36 **Methods**

37 Study Population

38 *Discovery Population*

39 The WHI study enrolled 161,808 postmenopausal women aged between 50 and 79
40 years old from 1993 to 1998. The original WHI study has two major components: a partial
41 factorial randomized clinical trial (CT) including 68,132 participants and an observational
42 study (OS) of 93,676 participants. The detailed study design has been reported elsewhere¹⁶.
43 Briefly, medical records from enrollment through September 2014 for 44,174 WHI
44 participants, including all women randomized to the hormone trial component (n = 27,347)
45 and all AA participants (n = 11,880) and HA participants (n = 4,947) from the CT and the
46 OS, were sent to the University of North Carolina (UNC) for HF adjudications.

47 Of the participants enrolled in the WHI-OS, 8,515 self-identified AA women had
48 consented to and were eligible for the WHI-SNP Health Association Resource (SHARe), and
49 of the participants enrolled in the WHI hormone trial, 4,909 EA women were included in the
50 WHI-Genomics and Randomized Trials Network (GARNET). After quality control, the

51 standard GWAS and pathway analyses were conducted among 8,298 AA and 4,257 EA
52 participants of the WHI. Considering the lack of replication cohort of HA participants in the
53 WHI-SHARe, we treated the HA participants as a replication sample in the pathway analysis.

54

55 *Population for validation and replication*

56 The current study included three populations as validation and replication: AA in the
57 Jackson Heart Study (JHS), EA in the Framingham Heart Study (FHS), and HA in the WHI-
58 SHARe. Considering the WHI only enrolled postmenopausal women, we replicated the
59 proposed analyses among women in the JHS and FHS. The main FHS enrolled three
60 generations: the original generation (started in 1948), offspring generation (started in 1971),
61 and generation three (started in 2005). Because of the poor measurement of left ventricular
62 ejection fraction (LVEF) among the original generation and the relatively young age of
63 generation three (baseline age < 40 years old), we only included the offspring generation in
64 the analysis. In total, we conducted the proposed analyses among 1,871 AA women in the
65 JHS, 1,764 EA women in the FHS, and 3,461 HA women in the WHI-SHARe.

66

67 Definition of Heart Failure

68 In the WHI, HF adjudication was based on the Atherosclerosis Risk in Communities
69 (ARIC) classification guidelines¹⁷, in which HF was defined as having acute decompensated
70 HF (ADHF) and chronic stable HF¹⁸. Participants with adjudicated HF were further classified
71 as HFrEF or HFpEF according to their LVEF. For patients with ADHF, those with LVEF <
72 45% were considered as HFrEF and those with LVEF \geq 45% were considered as HFpEF. For
73 patients with chronic stable HF, baseline LVEF or lowest estimated LVEF on medical
74 records were used to classify HF subtypes. Similar criteria were applied to replication

75 cohorts, the JHS and FHS. Participants without LVEF were excluded from the analysis
76 (n=440).

77

78 Genotype Data

79 Genome-wide genotyping of the WHI-SHARE and JHS participants were performed
80 using the Affymetrix 6.0 array (Affymetrix, Inc, Santa Clara, CA), and WHI-GARNET and
81 FHS participants were genotyped using Illumina HumanOmni1-Quad SNP platform
82 (Illumina, Inc, San Diego, CA). As the gene chips used for genotyping are designed to
83 capture common genetic variants, genetic variants with frequency $\geq 5\%$ were genotyped.
84 Reference panels from the 1000 Genomes (1000G) Project Consortium (Version 3, March
85 2012 release), which provide near complete coverage of common genetic variation with
86 minor allele frequency $\geq 0.5\%$, were used for genotype imputation.

87

88 Statistical Analysis

89 *Genome-wide association analyses*

90 We performed standard GWAS analysis for HF_rEF and HF_pEF for AA and EA
91 women, using multivariable logistic regressions. The regression models were implemented
92 using allelic dosage at each SNP (single-nucleotide polymorphism) as the independent
93 variable, with covariate adjustment for age, age², and first four principal components (PCs)
94 for global ancestry in all three cohorts. We also adjusted for region in two WHI cohorts, and
95 randomized hormone treatment group and baseline hysterectomy status in the WHI-
96 GARNET study. Since the associations between germline genetic variants and HF are not
97 confounded by demographic and lifestyle factors, no other confounders were adjusted in the
98 GWAS analysis. The general form of the GWAS model is specified as follows:

99

$$\text{logit Pr}[Y|G, V] = \alpha_0 + \alpha_g G + \alpha_v V,$$

100 where Y denotes HF subtype, G denotes SNPs, and V denotes adjusted covariates. Common
101 genetic variants reaching the suggestive significance (5×10^{-6}) were identified as potential
102 GWAS hits. Suggestive SNPs were validated in the JHS and FHS using nominal *P* value <
103 0.05, followed by cross-ethnicity meta-analyses combining AA, EA, and HA women from
104 WHI, JHS, and FHS using METAL¹⁹ (FDR-adjusted *q* value < 0.05).

105

106 *Pathway analysis*

107 We obtained knowledge-driven metabolic and signaling pathways from three
108 databases: the Kyoto Encyclopedia of Genes and Genomes (KEGG)²⁰, Reactome²¹, and
109 BioCarta²². SNPs showing potential associations with HF subtypes (*P* value < 0.05 in GWAS)
110 were mapped to relevant genes based on their chromosome locations or functions, and further
111 mapped to biological pathways. Each pathway was tested for enrichment of genetic signals
112 for HFrEF and HFpEF by ethnic groups. To avoid potential biases due to a particular method,
113 we applied two different well-established methods based on known biological pathways: 1)
114 GSA-SNP¹⁴, and 2) Mergeomics¹⁵. Pathways were defined as significant if they met the
115 following criteria: 1) identified by both methods from the WHI study with a FDR-adjusted *q*
116 value < 0.2; and 2) validated by GSA-SNP or Mergeomics with a significant *P* value after
117 Bonferroni correction in JHS (as replication of WHI-AA) and FHS (as replication of WHI-
118 EA). We then performed cross-phenotype and cross-ethnicity analyses in WHI to examine
119 shared pathways between HFrEF and HFpEF, as well as phenotype-specific pathways, across
120 ethnicities (AA, EA, and HA).

121

122 *Key Driver Analysis for Identification of Key Regulatory Genes for HF-related Pathways*

123 As hundreds of genes are involved in the biological pathways, we seek to further
124 prioritize key driver (KD) genes, defined as genes that played a central role in the disease

125 progress and once perturbed, should have major impact on many other genes. We integrated
126 all genes involved in significant pathways with seven Bayesian networks and one protein-
127 protein interaction network using KD analysis methods^{15,23,24}. We designed a normalized
128 rank score (NRS) to summarize the consistency and strength of identified KD genes across
129 multiple networks²⁵, where $NRS = \frac{C_{KD}}{N} \times \sum_{i=1}^{C_{KD}} R_{KD_i}$; C_{KD} is the count of networks from
130 which a KD was identified; C_{KD} is then normalized by total number of networks N to
131 represent the consistency of a KD across all networks tested (Bayesian networks from seven
132 tissues, including adipose, blood, brain, islet, liver, kidney, and muscle, and one protein-
133 protein interaction). The KD strength is represented by the summation of normalized
134 statistical rank in each network i (R_{KD_i}) across all networks from which the KD is identified;
135 $R_{KD_i} = \frac{Rank_{KD_i}}{N_{KD_i}}$, which was calculated by dividing the rank of a KD based on the P values of
136 the Fisher exact test in descending order ($Rank_{KD_i}$) by the total number of KDs identified
137 from a network i (N_{KD_i}). KDs with high NRS were those with high network enrichment for
138 pathways and high consistency across tested networks.

139

140 **Results**

141 Among WHI participants, 860 (10.4%) AA, 601 (14.1%) EA, and 165 (4.7%) HA
142 were initially identified as having HF. After excluding those without LVEF measurement
143 (316 WHI-AA and 124 WHI-EA), we performed primary analyses among 7,982 AA and
144 4,133 EA women in the WHI, and replication analyses among 1,853 AA women in the JHS,
145 1,755 EA women in the FHS, and 3,461 HA women in the WHI. The descriptive statistics on
146 demographic and lifestyle factors of each study population are shown in **Table 1**. Compared
147 to WHI-EA, WHI-AA women were younger in age and less physically active, had higher
148 BMI and lower intakes of alcohol and total calories, and with a higher proportion of
149 cardiovascular disease and diabetes.

150

151 Identification of Significant Genetic Loci Using Standard GWAS Analysis

152 In the validation analysis of previously reported 30 loci for HF from the GWAS
153 catalog²⁶, we validated one locus and further allocated it to HFpEF in the WHI, JHS, and
154 FHS populations with FDR-adjusted q value < 0.05. The validated SNP rs4420638 is located
155 on chromosome 19 and close to *APOE* and *APOC* genes. Detailed information regarding the
156 validated locus can be found in **Supplemental Table 1**.

157 The standard GWAS results for HF_rEF and HF_pEF within WHI-AA (n=7,982) and
158 WHI-EA (n=4,133) are shown in the Manhattan plots (**Supplementary Figure 1**). Among
159 AA, this discovery analysis revealed one significant ($P < 5 \times 10^{-8}$, rs35900865) and 57
160 suggestive ($P < 5 \times 10^{-6}$) SNPs related to HF_rEF, and three significant ($P < 5 \times 10^{-8}$, rs7834398,
161 rs78668964, and rs12203350) and 94 suggestive ($P < 5 \times 10^{-6}$) SNPs related to HF_pEF.
162 Among EA, we failed to identify significant SNPs, but found 50 suggestive ($P < 5 \times 10^{-6}$)
163 SNPs related to HF_rEF and 47 SNPs for HF_pEF.

164 In the replication analysis for AA women among JHS (n=1,853) participants, eight
165 SNPs from four loci (lead SNPs: rs12067046, rs114553497, rs10229703, and rs149663839)
166 out of 94 SNPs for HF_pEF reached the threshold of $P < 0.05$. In the replication analysis for
167 EA women among FHS (n=1,755) participants, one SNP (rs12719020) reached the $P < 0.05$
168 threshold among the 50 suggestive SNPs for HF_rEF; and 19 SNPs (concentrated on
169 chromosome 16, lead SNP: rs12599260) among the suggestive 47 SNPs for HF_pEF reached
170 the threshold of $P < 0.05$. The effect of all lead SNPs on HF was in the same direction in the
171 discovery population and the ethnicity-specific replication population. In the cross-ethnicity
172 meta-analysis combining AA, EA, and HA women from the WHI, JHS and FHS, all loci
173 passed ethnicity-specific validation and were further validated with FDR-adjust q value of <

174 0.05 (**Table 2**). More information regarding the newly discovered loci can be found in
175 **Supplemental Table 1**.

176

177 Identification of Biological Pathways Using Integrative Pathway Analysis

178 We initially identified 21 pathways for HFrEF (nine for EA and 12 for AA) and 42
179 pathways for HFpEF (31 for EA and 17 for AA) among WHI participants, of which 11
180 pathways were validated for HFrEF and 15 pathways for HFpEF, among the JHS and FHS
181 women. The results of cross-phenotype and cross-ethnicity analysis were presented in **Table**
182 **3 and Supplemental Tables 2 and 3**. Based on the functions of the pathways, we identified
183 two main overarching domains with some cell signaling and metabolism common to both: 1)
184 angiogenesis and vascular patterning and 2) inflammation. Five pathways, emerging from
185 angiogenesis and vascular patterning, were shared between HFrEF and HFpEF across AA,
186 EA, and HA women, namely, extracellular matrix (ECM)-receptor interaction, cell adhesion
187 molecules (CAMs), axon guidance, netrin-1 signaling, and developmental biology (**Figure**
188 **1**). The five shared pathways were highly interconnected as demonstrated by a shared
189 common set of 256 genes among them (**Figure 2**).

190 In addition, we found six pathways specifically enriched for HFpEF across AA and
191 EA, namely, adherens junction, endocytosis, phosphatidylinositol signal system, vascular
192 smooth muscle contraction, and heparan sulfate/heparin (HS)-glycosaminoglycan (GAG)
193 biosynthesis and degradation; all of which corresponded to the domain of angiogenesis and
194 vascular patterning (**Figures 1 and 3**). Of the aforementioned six HFpEF-specific pathways,
195 the first three pathways were further replicated in the WHI-HA participants.

196

197 Identification of Key Drivers for HFpEF and HFrEF

198 In the KD analysis to identify potential genes that played a central role in the
199 significant pathways for HF, we used eight different regulatory or interaction networks that
200 capture gene-gene or protein-protein interactions among the pathways. The top 10 KD genes
201 for the five shared pathways (developmental biology, axon guidance, netrin-1 signaling,
202 ECM-receptor interaction, and CAMs) between HFrEF and HFpEF across two ethnicities are
203 *COL1A1*, *COL1A2*, *COL3A1*, *COL4A2*, *COL5A1*, and *COL6A3* from ECM and axon
204 guidance pathways, and *HLA-DQA1*, *HLA-DQB1*, *HLA-DRB1*, and *HLA-DMB* from CAMs
205 pathway (**Figure 2 and Supplemental Figure 2**). For the six pathways specific for HFpEF,
206 the top 10 KD genes are *MYH11*, *MYLK* and *PRKACB* from vascular smooth muscle
207 contraction, *PRKCG*, *PIK3R1* from phosphatidylinositol signal system, *HGS*, *EGF*, and
208 *SH3KBP1* from endocytosis, and *CTNNB1* and *RAC1* from adherens junction (**Figure 3 and**
209 **Supplemental Figure 2**). Variants in the identified top KD genes collectively account for 15-
210 19% and 15-16% variations of HFrEF and HFpEF among women in the WHI.

211

212 **Discussion**

213 In this GWAS analysis of 7,982 AA and 4,133 EA women from the WHI, we
214 validated one previously reported genetic locus and allocated it to HFpEF, and additionally
215 discovered one HFrEF and five HFpEF novel genetic loci of potential importance. Also, five
216 biological pathways appeared to be shared for both HFrEF and HFpEF across AA, EA, and
217 HA women, and six pathways were specific for HFpEF across AA and EA women. Our
218 results suggested the presence of core mechanisms across HF subtypes (HFrEF and HFpEF),
219 such as vascular remodeling and inflammation alone with some common overlapping
220 mechanisms of cell signaling and metabolism. It is important to note the paucity of
221 cardiomyocyte-specific gene variants, including those for nuclear envelope proteins,

222 sarcomere proteins, cytoskeletal, and calcium regulatory proteins, given their extensive
223 involvement in familial dilated cardiomyopathies. Our data highlight the genetic and/or
224 biological significance of the vascular remodeling and inflammation, rather than that of the
225 cardiomyocyte in the acquisition of HFpEF and HFrEF.

226 Given the increased diversity of gene involvement, the genetic architecture underlying
227 HFrEF and HFpEF remains challenging to delineate. We validated one previously reported
228 locus close to *APOE* and *APOC*, and further allocated it to HFpEF. Genes in the
229 apolipoprotein family (*APOE*, *APOC1*, *APOC2*, etc.) encode lipid transport proteins that
230 regulate cholesterol metabolism and are associated with obesity and cardiovascular
231 disease^{27,28}. Of note, we were not able to validate other suggestive HF loci reported from
232 previous European-based cohorts, which may be due to effect modifications by sex and/or
233 ethnicity.

234 In addition, we discovered one HFrEF and five HFpEF loci from intergenic regions.
235 Variant rs12719020, associated with HFrEF, is located upstream (< 20 Kb) to *COBL*, a gene
236 related to vasculitis and type 1 diabetes²⁹. For variants associated with HFpEF, rs12067046 is
237 located 500 Kb downstream of *PLXNA2*, which is related to the development of blood
238 vessel³⁰ and inflammatory-induced immune disorders³¹; the linkage disequilibrium (LD)
239 block around rs12599260 is upstream (5 Kb) to *HEATR3*, which regulates inflammatory
240 immune response³²; rs149663839 is located upstream (50 Kb) to *CAT*, a key antioxidant
241 enzyme, which is hypothesized to play a role in the development of many chronic or late-
242 onset diseases such as HF³³; *ACTA1* (60 Kb to the LD block around rs114553497) and
243 *CALDI* (5 Kb to rs10229703) are fundamental genes for skeletal/smooth muscle contraction
244 and had been linked to pulmonary hypertension in animal studies^{34,35} (**Table 2 and**
245 **Supplemental Table 1**).

246 Genetic pathway and network analysis, as novel approaches to integrate genetic
247 signals that complements current GWAS analysis, have been yielded new insight into the
248 biology of coronary heart disease³⁶, type 2 diabetes²⁵, obesity³⁷, and LV function³⁸. Our
249 pathway-based analysis revealed five consistent pathways between HFrEF and HFpEF across
250 the two ethnicities (**Table 3**). All the five pathways were linked to angiogenesis and vascular
251 patterning, among which three pathways, ECM-receptor interaction, CAMs, and Netrin-1
252 signaling were also linked to inflammation (**Figure 1**). From the five pathways shared by
253 both HFrEF and HFpEF, three pathways, axon guidance, ECM-receptor interaction, and
254 CAMs, had been implicated previously in thromboembolic cardiovascular disease, type 2
255 diabetes, and LV function^{25,38}.

256 There appears to be a substantial overlap in the major areas of inflammation and
257 angiogenesis among shared pathways between HFrEF and HFpEF. The ECM is an intricate
258 network composed of multidomain macromolecules organized to support mechanical and
259 structural properties of cells and tissue but also to control behavioral characteristics of cells,
260 including proliferation, adhesion, migration, polarity and differentiation^{39,40}. Major
261 components include collagens, proteoglycans, elastin, and cell-binding glycoproteins, each
262 with distinct physical and biochemical properties. ECM molecules connect to the cells
263 through integrins, syndecans, and other receptors which provides signaling input in addition
264 to mechanical support⁴¹. This ECM-receptor interaction contributes to angiogenesis and
265 vascular patterning in multiple ways, including the organization and maintenance of gradients
266 for angiogenic factors like vascular endothelial growth factor (VEGF)-A^{42,43}. Endothelial
267 ECM receptors like intergrins play a critical role in adhesion and migration via control of
268 cytoskeletal dynamics while at the same time directing cell-cell interactions through
269 pathways like Notch signaling in order to coordinate sprouting and tube organization in early
270 capillary networks⁴⁴. ECM-CAM interactions also have the ability to influence inflammatory

271 state of both vascular and immune cells through focal adhesion complexes comprised of
272 integrins, protein kinases such as focal adhesion kinase (FAK), Src and many other kinases,
273 adaptor proteins such as Shc, signaling intermediates such as phosphoinositide 3-kinase
274 (PI3K), Rho and Rac GTPases, and actin binding cytoskeletal proteins⁴⁵. Further, cardiac
275 ECM (primarily collagen I) may also play a critical role in providing a platform for
276 cardiomyocytes to maintain structure and function, and any change in ECM properties
277 following an insult has potential to drive the progression toward HF, including myocardial
278 fibrosis and altered ECM protein orientation^{46,47}. Details regarding the main functions of
279 other identified pathways can be found in **Supplemental Table 4**.

280 Taken together, the above identified pathways highlight the previously known but
281 likely underappreciated importance of angiogenesis and vascular patterning as well as
282 inflammation on HF that appear to link HFrEF and HFpEF to other cardio-metabolic health
283 outcomes via multiple mechanisms. The fact that these pathways were consistently identified
284 across multiple ethnicities further highlights a convergent or central role in the joint
285 mechanisms between interrelated cardiac diseases.

286 We additionally identified six pathways specifically for HFpEF across ethnicities. All
287 the six pathways were linked to angiogenesis and vascular patterning, from which three
288 pathways (phosphatidylinositol signal system, HS-GAG biosynthesis and degradation) were
289 additionally linked to inflammation (**Figure 1**). None of the six HFpEF pathways have
290 previously been implicated in pathway-based studies, making these findings novel.

291 Specifically, the phosphatidylinositol signaling system is critically linked to
292 inflammation and vascular remodeling, and in particular angiogenesis and vascular
293 patterning. Activation of the PI3K pathway can occur in response to a variety of extracellular
294 (e.g., ECM, CAM) as well as growth factor (e.g., fibroblast growth factor, VEGF-A)
295 signaling and can regulate broad spectrum of molecular functions which involve

296 proliferation, adhesion, migration, invasion, metabolism and cell survival^{48,49}. Activation of
297 the PI3K pathway involves recruitment via Src-homology 2 (SH2) to phosphotyrosine
298 residues on the intracellular portion of membrane receptors, followed by phosphorylation of
299 phosphatidylinositol-4,5-bisphosphate (PIP2) to generate the second messenger molecule
300 PIP3. The Akt family of serine/threonine kinases has been shown to be the primary
301 downstream mediator of the effects of PI3K. Through the phosphorylation of I κ B kinase
302 (IKK) and activation of nuclear factor κ B (NF- κ B) transcriptional activity, Akt leads to
303 upregulation of inflammatory and prosurvival genes⁵⁰⁻⁵³. Akt can also activate mTOR,
304 resulting in stabilization of hypoxia inducible factor-1 (HIF-1) and consequent expression of
305 VEGF-A in order to promote angiogenesis⁵⁴⁻⁵⁶. PI3K signaling may also play a role in
306 regulating cardiomyocyte size, survival, and inflammation during cardiac hypertrophy and
307 HF, in part via calcium signaling^{57,58}. Details regarding the main functions of other identified
308 pathways can be found in **Supplemental Table 4**. Detailed knowledge of these relationships
309 at the molecular level will allow researcher to understand the distinct mechanisms underlying
310 HFpEF and enable the development of effective therapeutic strategies.

311 In addition, we observed two pathways with highly significant *P* values: Rac1
312 pathway for HF_rEF within EA and HFpEF within AA, and signaling by bone morphogenetic
313 proteins (BMP) pathway for HFpEF among AA (**Supplemental Tables 2, 3, and 4**). Rac1 is
314 a GTPase protein, a member of the Rac subfamily of the Rho family of GTPases, and plays a
315 critical role in inflammation and vascular remodeling. Activated Rac1 can promote NF- κ B
316 signaling and reactive oxygen species (ROS) production via nicotinamide adenine
317 dinucleotide phosphate (NADPH) oxidase, both known activators of the NACHT, LRR, and
318 PYD domains-containing protein 3 (NLRP3) inflammasome protein complex that promotes
319 expression of the critical inflammatory cytokine, interleukin-1 β (IL-1 β)⁵⁹⁻⁶¹. A number of
320 studies have found that activation of Rac1 is associated with atrial fibrillation⁶²,

321 atherosclerotic calcification⁶³, cardiac hypertrophy⁶⁴, and HF⁶⁵, suggesting Rac1 may have
322 strong potential as a new therapeutic target⁶⁶. BMPs belong to transforming growth factor
323 beta (TGF β) superfamily, which is one of the most potent profibrogenic cytokine systems
324 governing cardiac fibrosis⁶⁷. BMP signaling is also increasingly recognized for its influence
325 on endocrine-like functions in postnatal cardiovascular and metabolic homeostasis⁶⁸. Some
326 BMP molecules, such as BMP9 and BMP10, had been found to reduce pulmonary arterial
327 hypertension, cardiac fibrosis, and myocardial infarction, thereby providing potentially
328 benefits for HF patients^{68,69}.

329 The KD gene analysis prioritized KD genes of coronary heart disease⁷⁰, type 2
330 diabetes²⁵, and obesity⁷¹, but has not been performed for HF. In our KD gene analysis based
331 on shared pathways between HFrEF and HFpEF, we found that the KD genes belong to the
332 collagen gene family, shared between axon guidance and ECM-receptor interaction, and
333 HLA genes from CAMs pathway. HLA gene family members are components of the major
334 histocompatibility complex (MHC) and play a central role in the immune system with
335 established allelic contributions to type 1 diabetes susceptibility⁷², and a host of inflammatory
336 disorders, including rheumatoid arthritis⁷³, Sjögren's⁷⁴, ulcerative colitis⁷⁵, and systemic
337 lupus erythematosus⁷⁶. Collagen gene family, as previously described, encodes proteins to
338 regulate vascular patterning and maintain the structure and function of cardiomyocytes.
339 These KD genes further highlight the effect of angiogenesis, vascular patterning and
340 inflammation in HF. Importantly, genes *COL1A1* and *COL3A1* were also found to be the KD
341 genes for thromboembolic cardiovascular disease and type 2 diabetes²⁵; thus, showing
342 potential shared biological mechanisms underlying these interrelated diseases as well as the
343 pleiotropic effects of these KD genes. *COL4A2* is a critical component of the basement
344 membrane, and loss of function leads to disordered capillary networks during
345 angiogenesis^{77,78}. The C-terminal portion of *COL4A2* is a potent inhibitor of angiogenesis,

346 prevents proliferation and migration of endothelial cells and induces apoptosis⁷⁹. Moreover,
347 variants of *COL4A2* are implicated in vascular cell survival, atherosclerotic plaque stability
348 and risk of myocardial infarction, as well as hemorrhagic stroke^{80,81}.

349 The KD genes for HFpEF were mainly from the vascular smooth muscle contraction,
350 phosphatidylinositol signal system and endocytosis pathways, which further highlight the
351 functional roles of inflammation and systemic vascular remodeling in the pathogenesis of
352 HFpEF. Two examples of genes intricately linked to vascular wall mechanics rather than
353 cardiomyocyte mechanics include *MYH11* and *MYLK*. *MYH11* encodes one of the smooth
354 muscle cell myosin heavy chains, and variants are associated with familial thoracic aneurysm
355 syndrome^{82,83}. *MYLK* encodes a myosin light chain kinase that is implicated in inflammatory
356 responses, apoptosis, and vascular permeability. Variants of *MYLK* are associated with
357 arterial and aortic aneurysmal disease^{84,85}.

358 Several strengths and limitations need to be considered when interpreting these
359 findings. First, this study represents the first attempt to systematically examine and integrate
360 genetic variants for HF phenotypic subtypes using pathway and network approaches with
361 special emphasis on revealing mechanistic similarities and differences between HFpEF and
362 HFpEF with higher statistical efficiency. The second strength is the large, previously
363 validated and high-quality phenotyping of women of different ethnic backgrounds, which
364 allows the detection of HF mechanisms shared across ethnicities. Thirdly, two additional
365 high-quality cohorts, JHS and FHS, served as replication populations supporting the
366 robustness of our findings. One major limitation is that our results were based upon germline
367 mutations. Therefore, it is unclear whether mutations in the identified genomic regions and
368 pathways would impact downstream expression levels in particular tissues of interest, and
369 whether the identified genes and pathways are up-/down-regulated before and after HF
370 events. This highlights the critical need for future studies that will quantify the downstream

371 gene expression changes by comparing population with and without HF. Moreover, it will be
372 important to validate these results in men in order to examine the effect of sex on HF and to
373 replicate our finding using suitable animal models of HF in order to further validate these
374 newly discovered pathways.

375

376 **Conclusion**

377 This study validated previously identified locus and defined novel loci for HF and its
378 subtypes, implicating specific molecular pathways, some shared and others unique, that
379 contribute to HF and its subtypes. We highlight the significant mechanistic role of the
380 inflammation and vascular remodeling (angiogenesis and vessel patterning) in the genetic
381 signals associated with HFpEF and HFrEF, supporting the concept that HF is largely a
382 disease of the systemic vasculature. Finally, this work defines several leading and novel
383 targets and pathways for risk stratification and design of potential new HF interventions.

384

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413

414 **Disclaimer**

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Tables and Figures

Table 1. Baseline Characteristics of African and European American Women in Study Populations ^a

	Discovery Populations		Replication Populations ^b		
	WHI-AA (N=7,982)	WHI-EA (N=4,133)	JHS-AA (N=1,853)	FHS-EA (N=1,755)	WHI-HA (N=3,461)
No. of participants with HF, n (%) ^c	544 (6.8)	477 (11.5)	166 (9.0)	108 (6.2)	100 (2.9)
HFpEF, n (%)	345 (63.4)	304 (63.7)	140 (84.3)	103 (95.4)	67 (67.0)
HFrfEF, n (%)	199 (36.6)	173 (36.3)	26 (15.7)	5 (4.6)	33 (33.0)
Years of follow-up, year (SD)	10.0 (5.2)	8.9 (5.3)	6.4 (2.3)	38.9 (4.8)	10.4 (5.3)
Age, year (SD)	61.5 (7.0)	65.6 (6.9)	55.5 (12.7)	34.8 (9.8)	60.2 (6.7)
Region, n(%) ^d					
Northeast	1410 (17.7)	1039 (25.1)	--	--	436 (12.6)
South	3902 (48.9)	879 (21.3)	--	--	1423 (41.1)
Midwest	1854 (23.2)	1185 (28.7)	--	--	126 (3.6)
West	816 (10.2)	1030 (24.9)	--	--	1476 (42.6)
Current smoking, n (%)	901 (11.3)	449 (10.9)	202 (10.9)	723 (41.2)	233 (6.7)
Cardiovascular disease, n (%) ^e	1355 (17)	647 (15.7)	191 (10.3)	0 (0)	356 (10.3)
Diabetes, n (%)	1054 (13.2)	285 (6.9)	436 (23.5)	8 (0.5)	278 (8)
BMI, kg/m² (SD)	31 (6.5)	29.6 (6.1)	33.3 (7.8)	24 (4.4)	28.9 (5.8)
Physical Activity, MET-h/week (SD) ^d	9.7 (12.7)	10.2 (12.8)	--	--	10.8 (13.8)
Alcohol drinking, serving/week (SD)	1.1 (3.9)	2.3 (5.1)	1.5 (6.2)	3.5 (5.8)	1.3 (3.9)
Total energy intake, kcal/day (SD)	1614.1 (759.5)	1683.3 (663.1)	1826.2 (751.8)	1757.0 (579.6)	1656.7 (777.4)

Abbreviations: AA (African American), ADHF (acute decompensated heart failure), BMI (body mass index), EA (European American), FHS (Framingham Heart Study), HA (Hispanic American), HF (heart failure), HFpEF (heart failure with preserved ejection fraction), HFrfEF (heart failure with reduced ejection fraction), JHS (Jackson Heart Study), LVEF (left ventricular ejection fraction), SD (standard deviation), WHI (Women's Health Initiative Study).

^a Continuous variables were presented as mean (SD).

^b 62.0% in the JHS and 52.5% in the FHS participants were women.

^c HF was defined as having ADHF or chronic stable HF, and further classified as HFrEF (LVEF < 45%) or HFpEF (LVEF ≥ 45%). For WHI patients with ADHF, LVEF closest to the diagnosis date of ADHF was used, and for patients with chronic stable HF, baseline LVEF or lowest estimated LVEF on medical records were used to classify HF subtypes. In the JHS, considering over 50% of ADHF patients were missing LVEF, baseline LVEF was used to determine HFrEF and HFpEF for those without coronary heart disease before HF, and for chronic stable HF patients, baseline LVEF was used. In the FHS, HF was defined as incident or prevalent congestive HF. Since LVEF was not measured at baseline, LVEF closest to the diagnosis date of HF was used to define HFrEF and HFpEF. Participants without LVEF measurement were excluded in the analysis.

^d Results in the replication populations were not presented for variables which were not applicable (region) or measured in different scales (physical activity) .

^e Cardiovascular disease was defined as self-reported coronary heart disease, heart failure, stroke, and peripheral artery disease at baseline.

Table 2. Newly Discovered Loci for Heart Failure among Women across Ethnicities in the Women’s Health Initiative Study, Jackson Heart Study and Framingham Heart Study.

Lead SNP	Position (hg19)	Candidate Gene ^a	AI 1/ AI 2	Discovery ^b		Validation ^c		Cross-ethnicity Meta-analysis ^d	
				β (SE)	P value	β (SE)	P value	Direction	FDR-q value
European American - HFrEF									
rs12719020	7:51066547	Near <i>COBL</i>	A/C	1.08 (0.2)	3.1×10 ⁻⁶	2.59 (0.9)	0.02	+++?+ ^e	8.8×10 ⁻⁴
European American - HFpEF									
rs12599260	16:50093238	Near <i>HEATR3</i>	A/G	-0.42 (0.1)	4.9×10 ⁻⁶	-0.43 (0.2)	5.5×10 ⁻³	+----	0.01
African American - HFpEF									
rs12067046	1:208913216	Near <i>PLXNA2</i>	A/G	0.47 (0.1)	4.2×10 ⁻⁶	0.44 (0.2)	6.6×10 ⁻³	++---	7.2×10 ⁻⁴
rs114553497	1:229507916	Near <i>CCSAP, ACTA1</i>	T/C	0.92 (0.2)	5.2×10 ⁻⁷	0.67 (0.3)	0.04	+?+?	1.6×10 ⁻⁴
rs10229703	7:134661230	Near <i>CALD1, AGBL3</i>	A/G	0.49 (0.1)	3.6×10 ⁻⁶	0.41 (0.2)	0.02	+++--	7.6×10 ⁻³
rs149663839	11:34408645	Near <i>ABTB2, CAT</i>	A/G	2.61 (0.9)	1.4×10 ⁻⁶	1.69 (0.8)	5.1×10 ⁻³	+??+?	7.1×10 ⁻⁶

Abbreviations: AI (allele), FDR (false discovery rate), HFpEF (heart failure with preserved ejection fraction), HFrEF (heart failure with reduced ejection fraction), SE (standard error), SNP (single-nucleotide polymorphism).

^a Closest genes within ± 300 kb of the lead SNPs.

^b The discovery populations were African Americans (N=7,982) and European Americans (N=4,133) within the Women’s Health Initiative study.

^c The validation populations were African Americans women (N=1,853) in the Jackson Heart Study and European American women (N=1,755) in the Framingham Heart Study.

^d The cross-ethnicity meta-analysis was performed among African Americans, European Americans, and Hispanic Americans in the Women’s health Initiative study, and the Jackson Heart Study and Framing Heart Study.

^e The following symbols denote the directions of the ethnicity-specific GWAS results: + denotes a positive association between the genetic variant and HF; - denotes a negative association between the genetic variant and HF; and ? denotes the tested variant was not available in the population.

Table 3. Biological Pathways Enriched for HFrEF and HFpEF among African and European American Women across Ethnicities ^a.

Pathway	HFrEF			HFpEF		
	EA	AA	HA	EA	AA	HA
Angiogenesis and Vascular Patterning						
Extracellular matrix-receptor interaction	X	X	X	X	X	X
Cell adhesion molecules	X	X	X	X	X	X
Axon guidance	X	X	X	X	X	X
Netrin-1 signaling	X	X	X	X	X	X
Developmental biology	X	X	X	X	X	X
Adherens junction		X	X	X	X	X
Endocytosis		X	X	X	X	X
Phosphatidylinositol signal system				X	X	X
Vascular smooth muscle contraction	X		X	X	X	
HS-GAG biosynthesis		X		X	X	
HS-GAG degradation				X	X	
Mucin type O-glycan biosynthesis		X	X	X		X
Pre-Notch expression and processing				X		X
Signaling by BMP					X	
Cell-cell junction organization			X		X	
Intrinsic pathway	X		X			X
Inflammation						
Extracellular matrix-receptor interaction	X	X	X	X	X	X
Cell adhesion molecules	X	X	X	X	X	X
Netrin-1 signaling	X	X	X	X	X	X
Phosphatidylinositol signal system				X	X	X
HS-GAG biosynthesis		X		X	X	
HS-GAG degradation				X	X	
Rac1 pathway	X				X	
NRAGE signals death through JNK		X		X		
Signaling by Rho GTPases		X	X	X		X
Cell-cell junction organization			X		X	
Intrinsic pathway	X		X			X

Abbreviations: AA (African Americans), BMP (Bone morphogenetic proteins), EA (European Americans), GAG (glycosaminoglycan), HA (Hispanic American), HFpEF (heart failure with preserved ejection fraction), HFrEF (heart failure with reduced ejection fraction), HS (Heparan sulfate), JNK (JUN Kinase).

^a Biological pathways presented in the table were those identified from AA and EA women in the Women’s Health Initiative Study, and validated in the Jackson Heart Study and Framingham Heart Study. A pathway was marked with “X” when nominal *P* value from GSA-SNP or Mergeomics < 0.05 in the Women’s Health Initiative Study

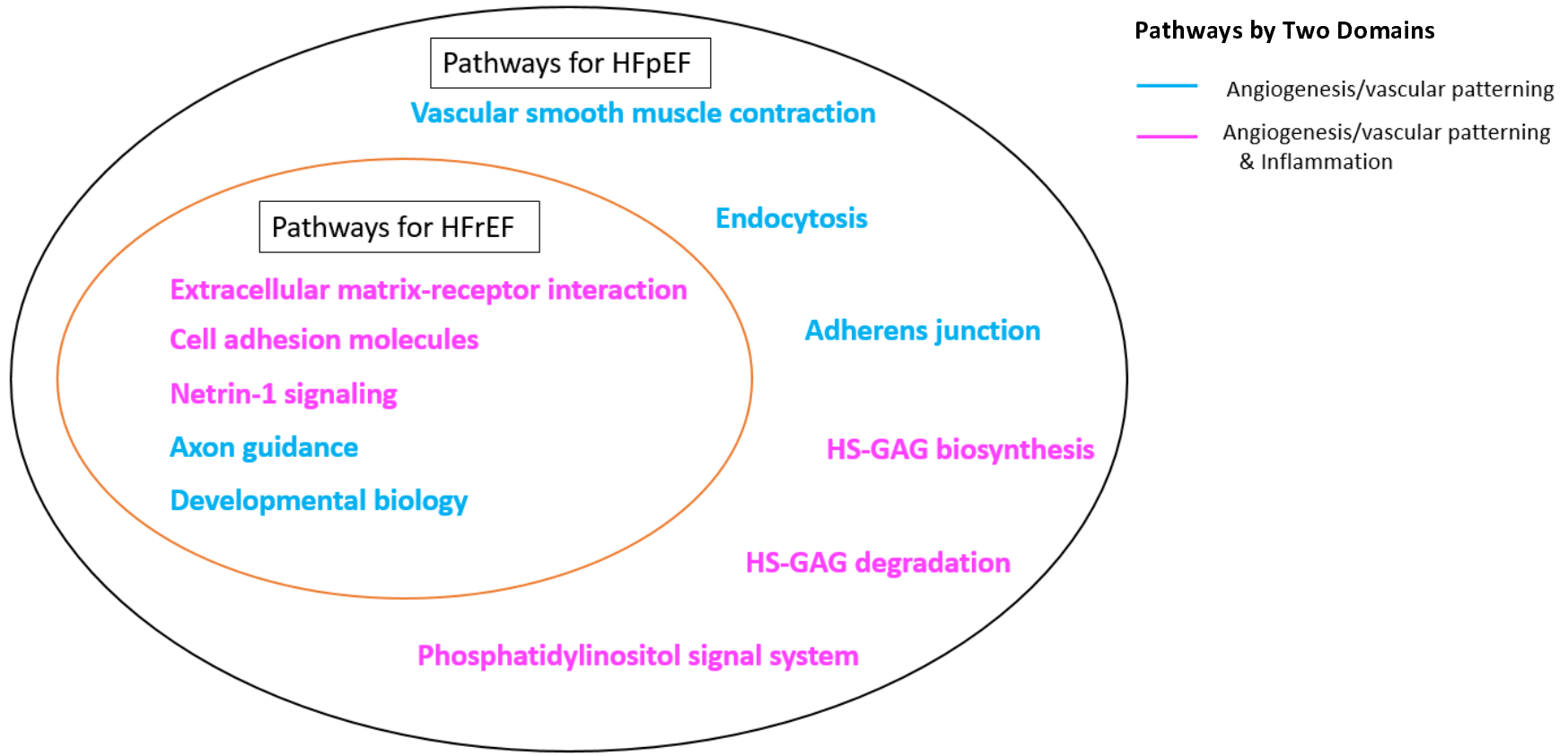


Figure 1. Venn Diagram for Biological Pathways Enriched for HFrEF and HFpEF among African and European American Women across Ethnicities

Abbreviations: GAG (glycosaminoglycan), HFpEF (heart failure with preserved ejection fraction), HFrEF (heart failure with reduced ejection fraction), HS (Heparan sulfate).

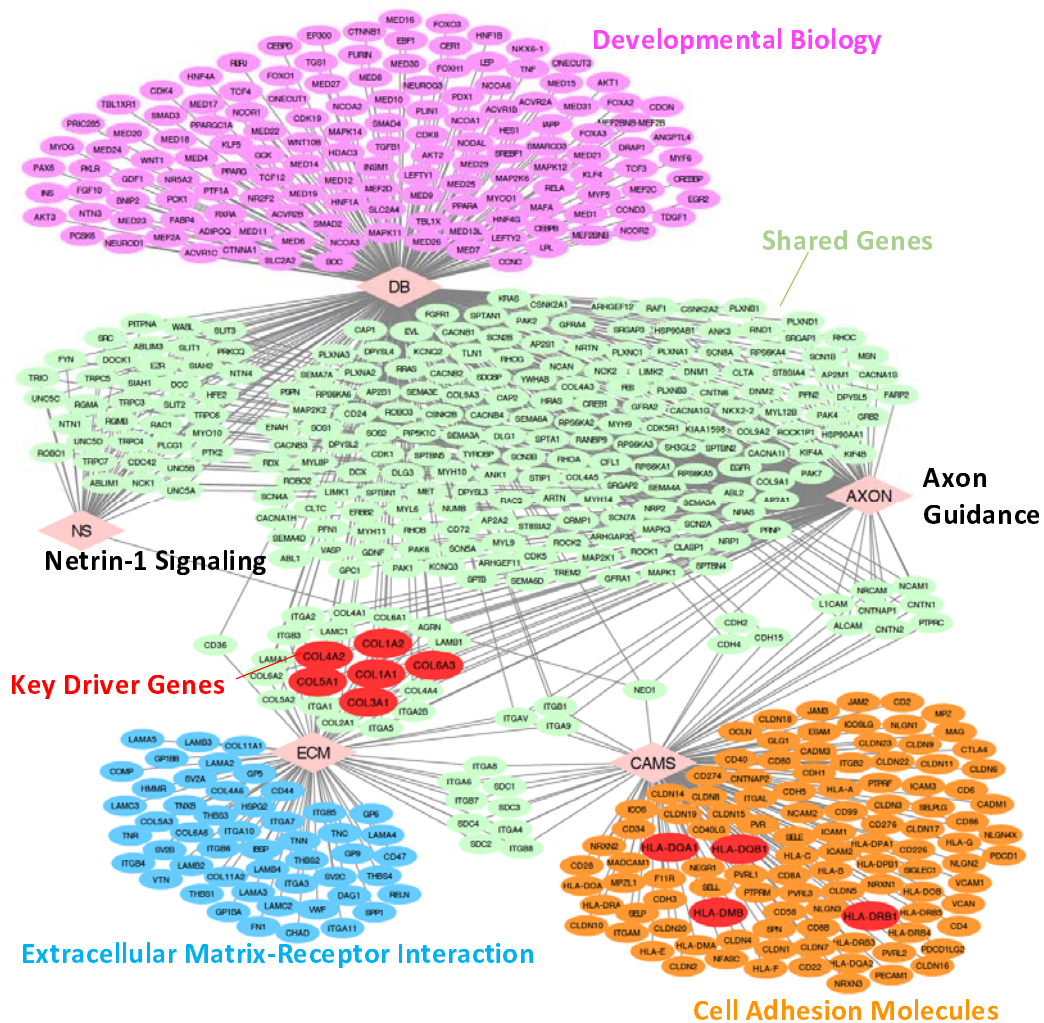


Figure 2. Network of 5 Pathways Enriched for HFReF and HFpEF with Top 10 Key Driver Genes among African and European American Women.

The diamond nodes represent pathway and the ellipse nodes represent genes, and the edge shows the interaction, that is, the association between a gene and a pathway. The color nodes are: red, top 10 key driver genes; light green, genes involved in ≥ 2 pathways; others are pathway-specific genes. The figure was created using Cytoscape⁸⁶.

Abbreviations: AXON (axon guidance), CAMS (Cell adhesion molecules), DB (developmental biology), ECM (Extracellular matrix-receptor interaction), HFpEF (heart failure with preserved ejection fraction), HFReF (heart failure with reduced ejection fraction), NS (Netrin-1 signaling).

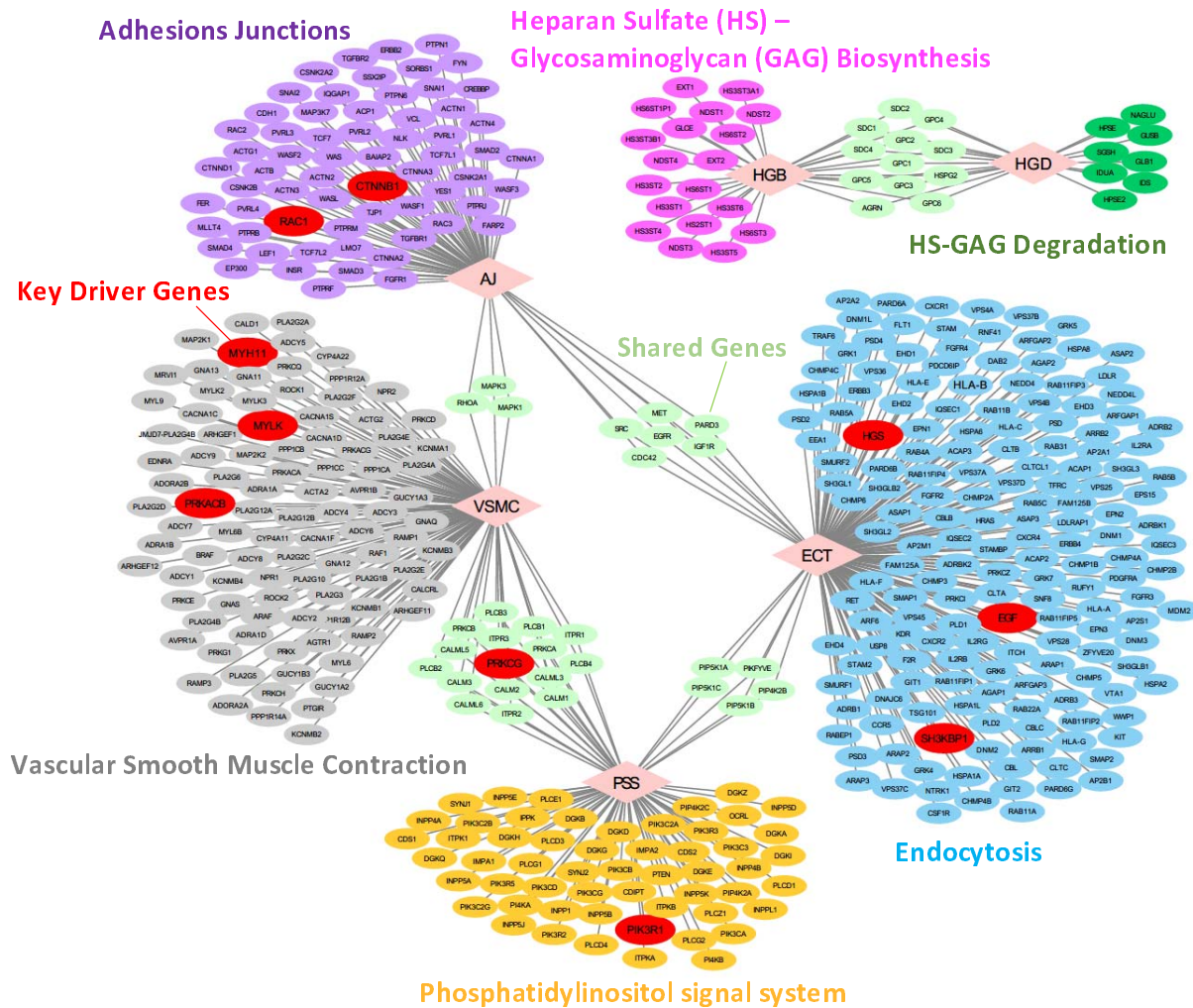


Figure 3. Network of 6 Pathways Enriched for HFpEF with Top 10 Key Driver Genes among African and European American Women.

The diamond nodes represent pathway and the ellipse nodes represent genes, and the edge shows the interaction, that is, the association between a gene and a pathway. The color nodes are: red, top 10 key driver genes; light green, genes involved in ≥ 2 pathways; others are pathway-specific genes. The figure was created using Cytoscape⁸⁶.

Abbreviations: AJ (adherens junction), ECT (endocytosis), HGB (heparan sulfate-glycosaminoglycan biosynthesis), HGD (heparan sulfate-glycosaminoglycan degradation), HFpEF (heart failure with preserved ejection fraction), PSS (phosphatidylinositol signal system), VSMC (vascular smooth muscle contraction).

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