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Proteomic analysis of effects of spironolactone in Heart Failure with Preserved Ejection Fraction

Ali Javaheri, MD, PhD[1,*], **Ahmed Diab, MD**[1,*], **Lei Zhao, MD, PhD**[2], **Chenao Qian, MS**[3], **Jordana B. Cohen, MD, MSCE**[3], **Payman Zamani, MD, MTR**[3], **Anupam Kumar, MD**[3], **Zhaoqing Wang, MS**[2], **Christina Ebert, PhD**[2], **Joseph Maranville, PhD**[2], **Erika Kvikstad, PhD**[2], **Michael Basso, MS**[2], **Vanessa van Empel, MD**[4], **A. Mark Richards, MD, PhD**[5,6], **Rob Doughty, MD, PhD**[6], **Ernst Rietzschell, MD, PhD**[7], **Karl Kammerhoff, BS, MBA**[2], **Joseph Gogain, PhD**[8], **Peter Schafer, PhD**[2], **Dietmar A. Seiffert, MD**[2], **David A. Gordon, PhD**[2], **Francisco Ramirez-Valle, MD, PhD**[2], **Douglas L. Mann, MD**[1], **Thomas P. Cappola, MD**[3], **Julio A. Chirinos, MD, PhD**[3]

[1]Washington University School of Medicine, St. Louis, MO.

[2]Bristol Myers Squibb Company, Lawrenceville, NJ

[3]Perelman School of Medicine. University of Pennsylvania School of Medicine/Hospital of the University of Pennsylvania. Philadelphia, PA.

[4]Department of Cardiology, Maastricht University Medical Center, Maastricht, The Netherlands

[5]Cardiovascular Research Institute, National University of Singapore, Singapore.

[6]Christchurch Heart Institute, University of Otago, Christchurch, New Zealand.

[7]Department of Cardiovascular Diseases, Ghent University Hospital, Ghent, Belgium

[8]SomaLogic, Inc., Boulder, CO, USA.

Corresponding author: Julio A. Chirinos, MD, PhD, South Tower, Rm. 11-138, Perelman Center for Advanced Medicine, 3400 Civic Center Blvd. Philadelphia, PA. 19104, Tel: 215-573-6606; Fax: 215-746-7415, julio.chirinos@pennmedicine.upenn.edu, Twitter: @JulioChirinosMd.

[*]These authors contributed equally.

Disclosures:

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Abstract

Background: The TOPCAT trial suggested clinical benefits of spironolactone treatment among patients with Heart Failure with Preserved Ejection Fraction (HFpEF) enrolled in the Americas. However, a comprehensive assessment of biologic pathways impacted by spironolactone therapy in HFpEF has not been performed.

Methods: We conducted aptamer-based proteomic analysis utilizing 5,284 modified aptamers to 4,928 unique proteins on plasma samples from TOPCAT participants from the Americas (n=164 subjects with paired samples at baseline and 1 year) to identify proteins and pathways impacted by spironolactone therapy in HFpEF. Mean percentage change from baseline was calculated for each protein. Additionally, we conducted pathway analysis of proteins altered by spironolactone.

Results: Spironolactone therapy was associated with proteome-wide significant changes in 7 proteins. Amongst these, caspase recruitment domain-containing protein 18 (CARD18), polycystin 2 (PKD2), and pregnancy-specific glycoprotein 2 (PSG2) were upregulated, whereas hepatic growth factor (HGF), phospholipid-transfer protein (PLTP), insulin growth factor 2 receptor (IGF2R), and switch associated protein 70 (SWP70) were downregulated. CARD18, a caspase-1 inhibitor, was the most up-regulated protein by spironolactone (−0.5% with placebo versus +66.5% with spironolactone, $p<0.0001$). The top canonical pathways that were significantly associated with spironolactone were apelin signaling, stellate cell activation, glycoprotein 6 signaling, atherosclerosis signaling, liver x receptor activation, and farnesoid x receptor activation. Amongst the top pathways, collagens were a consistent theme that increased in patients receiving placebo but decreased in patients randomized to spironolactone.

Conclusions: Proteomic analysis in the TOPCAT trial revealed proteins and pathways altered by spironolactone, including the caspase inhibitor CARD18 and multiple pathways that involved collagens. In addition to effects on fibrosis, our studies suggest potential anti-apoptotic effects of spironolactone in HFpEF, a hypothesis that merits further exploration.

Keywords

heart failure; spironolactone; CARD18; HFpEF

Introduction

Heart failure with preserved ejection fraction (HFpEF) is an important cause of cardiovascular morbidity and mortality [1,2], and there is an urgent need for the development of pharmacological interventions that can improve clinical outcomes in HFpEF patients. The utilization of spironolactone in HFpEF is based on the results of TOPCAT (Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist Trial), which suggested beneficial effects among patients enrolled in the Americas. Spironolactone is a mineralocorticoid receptor inhibitor that improves diastolic dysfunction in HFpEF [3,4], reduces fibrosis [5–7], and reduces blood pressure [8–12], although the effects on blood pressure do not explain the effects in HFpEF[13].

Broad proteomic scans of plasma, such as aptamer-based assays, have been successfully utilized to identify novel biomarkers involved in HF clinical outcomes and to provide a more

detailed biological understanding of HF phenotypes [14–17]. In addition, proteomic strategies can be utilized to broadly investigate potential mechanisms of drug action, which may contribute to the discovery of downstream pathways or molecules for therapeutic targeting. In the present study, we performed proteomic analysis of samples from the American subset of the TOPCAT Biorepository to further explore and understand pathways altered by spironolactone therapy.

Methods

Study population

Plasma protein quantification was performed from paired baseline and one-year follow-up samples available from the TOPCAT trial [18], which have been previously described. The raw data and analytical methods of this paper are not publicly available for purposes of reproducing the results or replicating the procedures. This data might be available subject to the establishment of appropriate data-sharing agreements and regulatory approvals. The parent TOPCAT trial data are available through the US National Institutes of Health BioLINCC website. From 2006–2012, TOPCAT randomized patients with HFpEF, defined as symptomatic HF with an EF greater than 45%, to either spironolactone or placebo. From HFpEF patients, a subset (n=218, or 6.3%) had available frozen plasma samples for *de novo* protein quantification along with clinical data, which was obtained through the National Institutes of Health BioLINCC repository. Patients from the American subset with paired samples at baseline and one-year post-randomization (n=164) were included in the present analysis. All study participants provided written informed consent, and all participating institutions received approval from local Institutional Review Boards.

Plasma Protein Quantification

All plasma samples were analyzed using the SomaScan® assay version 4, which is a multiplexed, modified aptamer-based binding assay. The SomaScan® assay utilizes Slow-Off-rate Modified Aptamer (SOMAmer) reagents, which are chemically modified nucleotides, to bind and quantify target proteins in relative fluorescent units directly proportional to the amount of target protein in the sample. Assay details have been previously described [19]. This assay includes 5,284 modified aptamer reagents to 4,928 unique protein targets. A Detailed description of the assay methodology is provided in the supplementary material.

Statistical analysis

Participant characteristics were summarized using mean (SD) for continuous variables with symmetric distribution and median (interquartile range) for continuous variables with a skewed distribution. Categorical variables are expressed as counts (percentages). Analysis of variance (ANOVA) was used to compare normally-distributed continuous variables, whereas the Kruskal-Wallis test was used for non-normally distributed variables, and the chi-square or Fisher’s exact test, as appropriate, were used for categorical data.

The percentage change from baseline was computed for each protein and each participant, and these changes were compared between the 2 treatment arms. We implemented

alpha correction for multiple comparisons based on the number of principal components underlying the variability of all measured proteins, as previously described [20,21]. There were 111 principal components corresponding to a nominal p value threshold of 0.00045. This method avoids alpha error overcorrection that may occur with the Bonferroni method due to the intrinsic correlation structure between proteins. Differences between the treatment arms were considered statistically significant if the multiplicity corrected p-value for the comparison was <0.05. All probability values presented are 2-tailed. Analyses were performed using the MATLAB statistics and machine learning toolbox (Matlab 2016b, the Mathworks; Natwick, MA).

Pathway analyses

Between-arm differences in plasma proteins between the baseline and 1-year samples were utilized to perform pathway analyses, using Ingenuity® Pathway Analysis (IPA) software (Qiagen; Hilden, Germany; www.qiagen.com/ingenuity) [22–24]. Proteins were identified according to their unique protein Identifier (UniProt ID) annotation. The totality of proteins included in the SomaScan® assay was used as the reference set and both direct and indirect experimentally confirmed relationships from all species were included. The 'Core analysis' module in IPA was used to perform pathway analysis on the differentially expressed proteins. This analysis identifies specific canonical pathways in which the changes induced by spironolactone are significantly overrepresented.

Results

Study population

The baseline characteristics of patients with available paired samples for analysis vs those who were not represented in the biorepository are shown in Table 1. Compared to the overall TOPCAT population, our subset (164 patients) was older (median [IQR]=73.5 [66,80] vs 69 [61,76]; p<0.0001), more frequently male (57% vs 48%; p=0.02), exhibited a higher BMI (median [IQR] = 32.7 [28.1,36.8] vs 30.8 [27.1,35.7] kg/m^2; p=0.01), and was more likely to report a history of smoking (60% vs 40%; p<0.0001). Our subset also exhibited a higher prevalence of diabetes (45% vs 31%; p=0.0004) and atrial fibrillation (53% vs 34%; p<0.0001), as well as higher rates of percutaneous coronary interventions (25% vs 14%; p<0.0001) and coronary artery bypass grafts (27% vs 12%; p<0.0001). Beta-blocker (85% vs 77%; p=0.009) and statin (76% vs 51%, p<0.0001) use was more prevalent, whereas ACEI/ARB (76% vs 84%, p=0.003) use was less prevalent. Baseline characteristics of patients in our subset randomized to spironolactone vs placebo are shown in Table 2. These groups were similar with respect to most clinical characteristics, although more patients in the spironolactone group required insulin therapy at baseline (13.1% vs 26.2% in the placebo vs spironolactone group, respectively, p=0.0478).

Changes in the plasma proteome associated with spironolactone therapy.

We identified significant changes in 7 proteins between the placebo and spironolactone groups, shown in Figure 1. Baseline levels of these 7 proteins did not differ and are presented in Table S1. Spironolactone induced upregulation of caspase recruitment domain-containing protein 18 (CARD18: −0.5 vs +66.5 % change in placebo and spironolactone

groups, respectively; corrected p<0.0001), polycystin 2 (PKD2; −8 vs +20 %, corrected p=0.001), and pregnancy specific glycoprotein 2 (PSG2; −11.4 vs +13.8 %, corrected p=0.02), as well as downregulation of hepatic growth factor (HGF; +6.6 vs −5 %, corrected p=0.009), phospholipid transfer protein (PLTP; +5.6 vs −4.7 %, corrected p=0.01), switch associated protein 70 (SWP70; +4.5 vs −4.7 %, corrected p=0.04), and insulin growth factor 2 receptor (IGF2R; +0.3 vs −8.2 %, corrected p=0.03). Genetic validation of aptamer specificity for these proteins is provided in the supplement. The CARD18 aptamer specificity is demonstrated by Western blotting (Supplementary Figure 1).

Additionally, because spironolactone is known to affect renal function, we performed a secondary analysis after adjustment for changes in cystatin C and found no changes in the levels of the seven significant proteins compared to our primary analysis (Table S2).

Pathways associated with spironolactone therapy.

Pathway analysis revealed 6 pathways that were differentially expressed between the spironolactone arm and the control arm. These significant canonical pathways were apelin liver signaling (p=0.00006), stellate cell activation (p=0.0003), Glycoprotein 6 (GP6) signaling (p=0.005), atherosclerosis signaling (p=0.008), LXR/RXR activation (p=0.008), and FXR/RXR activation (p=0.02) (Figure 2A). For the proteins in each significant pathway, heatmaps (Figures 2B–G) highlight individual protein changes in spironolactone vs placebo arms over the follow-up period. Notably, the top 4 canonical pathways were enriched for multiple collagens that increased in the placebo group but decreased with spironolactone.

Discussion

We report for the first time a comprehensive proteomic analysis of the effect of spironolactone therapy in the TOPCAT trial. We identified previously unknown proteins and pathways altered by spironolactone in HFpEF, including proteome-wide significant changes in CARD18, PKD2, PSG2, HGF, PLTP, IGF2R, and SWP70. These changes, along with corresponding pathway analyses, indicate the effects of spironolactone on caspase signaling, fibrosis, growth factors, and lipoprotein biology (Figure 3).

In our analysis, the most abundantly, significantly upregulated protein in the spironolactone group was CARD18, also known as iceberg. CARD18 is a small caspase recruitment domain-containing decoy molecule induced by pro-inflammatory stimuli, which inhibits caspase-1 oligomerization and activation and subsequent generation of IL-1β [25–27]. Caspase-1 is a pro-apoptotic molecule implicated in cardiomyopathy via its role in Ang II-induced cardiomyocyte hypertrophy and up-regulation of IL-1β [28]. Caspase-1 triggers the activation of the NOD-like receptor family pyrin domain containing 3 (NLRP-3) inflammasome, causing pyroptotic cell death of cardiomyocytes, a key process in HF [29]. Furthermore, spironolactone reduces levels of caspase-1 and IL-1β in a murine diabetic model [30]. Similarly, multiple recent reports have indicated a cardioprotective role of other caspase recruitment domain-containing proteins. For example, the apoptosis repressor with caspase recruitment domain (ARC), is an anti-apoptotic protein that reduces myocardial cell death in response to biomechanical and ischemic stress [31–34]. Strikingly, low-dose spironolactone could decrease infarct size and apoptosis in the reperfused myocardium

of rats by preventing the degradation of ARC [35]. In the context of the present work, larger studies should determine whether changes in CARD18 are causally involved in the therapeutic response to spironolactone or represent a biomarker of its efficacy, rather than representing a less relevant downstream effect of the drug.

Spironolactone randomized therapy, compared with placebo, was also associated with significant increases in the protein PKD2. *PKD2*, or polycystin 2, is commonly known as the one of two most commonly mutated genes in autosomal dominant polycystic kidney disease (ADPKD) [36]. In a randomized trial of patients with ADPKD, spironolactone therapy reduced blood pressure without affecting markers of endothelial dysfunction [37]. In the myocardium, PKD2 regulates cardiac diastolic function via its interaction with Ryanodine Receptor 2, which is involved in calcium handling [38]. PKD2 loss-of-function mutations impair diastolic function and predispose to HF [39]. Similarly, in PKD−/− mice, the release of natriuretic peptides (e.g., BNP) is significantly reduced compared to controls in response to β adrenergic stress [40]. However, it remains unclear whether effects on PKD2 are involved in the therapeutic response to spironolactone in HFpEF. Further mechanistic studies are needed to explore whether positive clinical effects of spironolactone in HFpEF might be, at least in part, mediated by PKD2.

A third novel finding of our study is that PLTP, a protein important for transferring phospholipids and free cholesterol from triglyceride-rich lipoproteins into HDL, was significantly downregulated in the spironolactone group. Prior studies support a potential mechanistic role for PLTP in cardiac dysfunction. High PLTP is a strong positive predictor of coronary artery disease (CAD) [41], a very common cause of HF. However, a direct mechanistic role for PLTP on HF is also possible. High PLTP activity is positively associated with LV dysfunction independent of PLTP effect on CAD [42,43]. Moreover, PLTP is linked to increased insulin resistance [44–47] and the development of diabetes [48] which can also contribute to ventricular dysfunction. Finally, PLTP has also been associated with inflammation. PLTP deficient mice fed a high-fat diet had reduced IL-6 compared to controls [49], while IL-6 dependent-induction of TNFα required PLTP [50]. Moreover, PLTP deficient mice exhibited reduced ability of LDL to induce monocyte chemotactic activity and improved the anti-inflammatory activity of HDL [51]. PLTP also impairs the reverse cholesterol transport process, as increased systemic PLTP activity decreased cholesterol efflux and from macrophages [52]. Given the possible roles for PLTP beyond atherosclerosis and CAD, our finding that spironolactone therapy is associated with a reduction in PLTP protein levels also merits further exploration. In particular, determining whether spironolactone might significantly alter PLTP activity in addition to PLTP protein levels will be a critical step forward.

Another protein downregulated by spironolactone and previously implicated in HF survival is HGF, or hepatocyte growth factor. In two cohorts of patients, circulating HGF was positively associated with mortality in patients with stable congestive HF [53] and advanced HF [54]. Since several murine studies indicate protective roles for HGF in the acute setting [55,56], HGF might be an important counter-regulatory of the cardiac stress response [57]. Given that the absolute percentage changes we observed in HGF were small, it is certainly

possible that the changes are reflective of underlying regulatory effects that are only weakly represented by changes in HGF levels.

The last two proteome-wide significant proteins downregulated by spironolactone were IGF2R and SWP70. IGF2R has been implicated in cardiomyocyte hypertrophy, fibrosis, and myocardial remodeling [58–61]. Serum IGF2R levels were increased in patients with end-stage heart failure compared to controls [62]. These findings, combined with our present data, lead to the hypothesis that spironolactone-induced reductions in IGF2R may be causally involved in its cardioprotective effects in HFpEF. Finally, SWP-70 is a guanine nucleotide exchange factor [63], with no known roles in cardiovascular biology.

Spironolactone is known to affect renal function, changes in which impact the circulating proteome. To investigate whether changes in renal function could be mediating the effect of spironolactone on circulating proteins, we adjusted for cystatin C, which is a valid surrogate for glomerular filtration rate [64,65]. In these adjusted analyses, the same 7 proteins were significantly associated with randomization to spironolactone, again with CARD18 exhibiting the most clinically and statistically significant change. These data suggest that spironolactone affected circulating CARD18 independent of effects on glomerular filtration.

We utilized pathway analysis to identify six significant pathways altered by spironolactone. One pathway is the apelin signaling pathway. Apelin is an endogenous ligand to the APJ receptor, found in myocardial tissue. Apelin has both a potent inotropic and an arterial vasodilator effect and is highly expressed in the left ventricular tissue of HF patients [66]. In a randomized trial, acute administration of apelin in HF patients produced peripheral and coronary vasodilation and improved cardiac output [67]. Furthermore, some studies have suggested a role for apelin in attenuating post-infarction remodeling [68,69], possibly by an antioxidant mechanism [70,71]. Despite these intriguing observations, our findings only establish a correlation between spironolactone and apelin signaling, without implying that changes in this pathway mediate any of the potential benefits of the drug in HFpEF. The value of apelin as a therapeutic target remains unclear and is the focus of various ongoing studies.

Two additional pathways associated with spironolactone were the liver X receptor (LXR) and the farnesoid X receptor (FXR) signaling pathways. LXR is a receptor expressed in the heart, which is activated after myocardial infarction and associated with protection against myocardial ischemia-reperfusion injury [72,73], as well as protection against pathological myocardial fibrosis and hypertrophy [74,75]. On the other hand, FXR, a regulator of apoptosis in cardiomyocytes [76], contributes to myocardial ischemia-reperfusion injury, and its knockout reduces apoptosis, fibrosis, and post-infarction remodeling in mice [77]. Evaluation of the individual components of these pathways revealed that multiple collagens changed in each pathway. Fibrillar collagen chains such as COL1A1, COL2A1, and COL3A1 were consistently downregulated with spironolactone in agreement with reports about a potential anti-fibrotic role for spironolactone [78–80]. Interestingly, spironolactone also decreases serum markers of collagen synthesis in patients with HFrEF and HFpEF [81,82].

Recently, Ferriera et al. analyzed protein biomarkers in baseline and 9-month samples of patients from the “Heart ‘Omics’ in Aging” HOMAGE trial [83]. This trial investigated the effect of spironolactone on cardiovascular function and markers of fibrosis in patients with risk factors for HF (e.g., CAD, hypertension, diabetes).[7] Consistent with our findings, Ferriera et al. reported reduced circulating markers of fibrosis and extracellular matrix metabolism in patients treated with spironolactone. In addition, while they reported several changes in markers of inflammation and insulin signaling, they did not observe any effect of spironolactone on apoptosis or apelin signaling. Differences between the present study and the work of Ferriera et al. may be related to different study populations and divergent proteomic strategies. Ferriera et al. investigated plasma from patients who had risk of developing heart failure in contrast to our population with established HFpEF. Moreover, they utilized a targeted 164 protein Olink Proseek-multiplex® cardiovascular and inflammation assay; in comparison, our aptamer-based proteomic strategy included 4,928 protein targets. The strength of the present approach is that it lends itself to a less biased discovery approach, including unexpected targets such as CARD18, but may increase the risk of type II error due to correct the alpha value for a larger number of comparisons

Our study should be interpreted in the context of its strengths and limitations. Strengths of our study include the relatively unbiased approach to interrogating plasma proteomics, which included ~5000 proteins, the randomized, double-blinded nature of the parent trial, and the highly systematic prospective data collection. Our study also has limitations. Proteins in the SomaScan® platform have been selected based on their previous identification and the ad hoc development of aptamer-based detection for incorporation into the SomaScan®. Given the large number of proteins interrogated, we applied correction for alpha error to minimize false-positive findings, which inevitably leads to a loss in power relative to analyses that utilize nominal statistical significance. In addition, since we are examining circulating factors, we cannot be certain of the tissue of origin of these factors in this clinical context. Whether the observed changes are a direct effect downstream of mineralocorticoid receptor antagonism or due to secondary alterations in the HFpEF phenotype cannot be ascertained by our analysis. It should be noted that plasma levels do not necessarily reflect in vivo compartmentalized activity; this is particularly true for neural activation pathways in which metabolomics or plasma levels of specific neurotransmitters may be more informative than proteomics. Finally, our study was based on a subset of TOPCAT participants with available plasma samples, rather than the TOPCAT population at large. The subsample included in this study exhibited some clinical differences compared to the subsample not included, which limits the generalizability of the findings. This also resulted in a smaller sample size, which does not provide sufficient statistical power to correlate the proteomics changes induced by spironolactone therapy with the risk of subsequent events. As such, our study is unable to establish whether any of the reported changes are actually involved in potential therapeutic or other clinically relevant effects of the drug in HFpEF.

In summary, we present a proteome-wide analysis of the effects of randomized spironolactone therapy in HFpEF and identify various plasma proteins that are impacted by this drug. Spironolactone altered proteins (CARD18, PKD2, PSG2, HGF, PLTP, IGF2R, SWP70) and pathways (apelin liver signaling, stellate cell activation, Glycoprotein 6 (GP6)

signaling, atherosclerosis signal, LXR-RXR activation, FXR-RXR activation) are involved in myocardial apoptosis, fibrosis, and remodeling. Whether the effects of spironolactone on these proteins and pathways are mechanistic drivers of its clinical efficacy or side effects, versus representing epiphenomena unrelated to these effects, will need to be examined through additional studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Non-standard abbreviations and acronyms:

HFpEF	Heart failure with preserved ejection fraction
TOPCAT	Treatment of Preserved Cardiac Function Heart Failure with an Aldosterone Antagonist Trial
CARD18	Caspase Associated Recruitment Domain 18
PKD2	Polycystin-2/Polycystic Kidney Disease 2
PSG2	Pregnancy Specific Beta-1-Glycoprotein 2
HGF	Hepatocyte Growth Factor
PLTP	Phospholipid Transfer Protein
IGF2R	Insulin-like Growth Factor 2 Receptor
SWP70	Switch-Associated Protein 70

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Clinical Perspective

What is new?

- Spironolactone increases levels of the anti-apoptotic protein (CARD18).
- Spironolactone alters multiple pathways including apelin liver signaling, stellate cell activation, Glycoprotein 6 signaling, atherosclerosis signaling, LXR/RXR activation, and FXR/RXR activation).
- Proteomics can be utilized to yield unexpected targets or markers of drugs in randomized controlled clinical trials.

What are the clinical implications?

- Spironolactone appears to exert broad effects in HFpEF, including alterations in anti-apoptotic pathways and pathways regulating collagens.
- Whether these effects are direct consequences of mineralocorticoid antagonism that mediate the effects of spironolactone, or markers of drug efficacy, should be explored in larger studies.

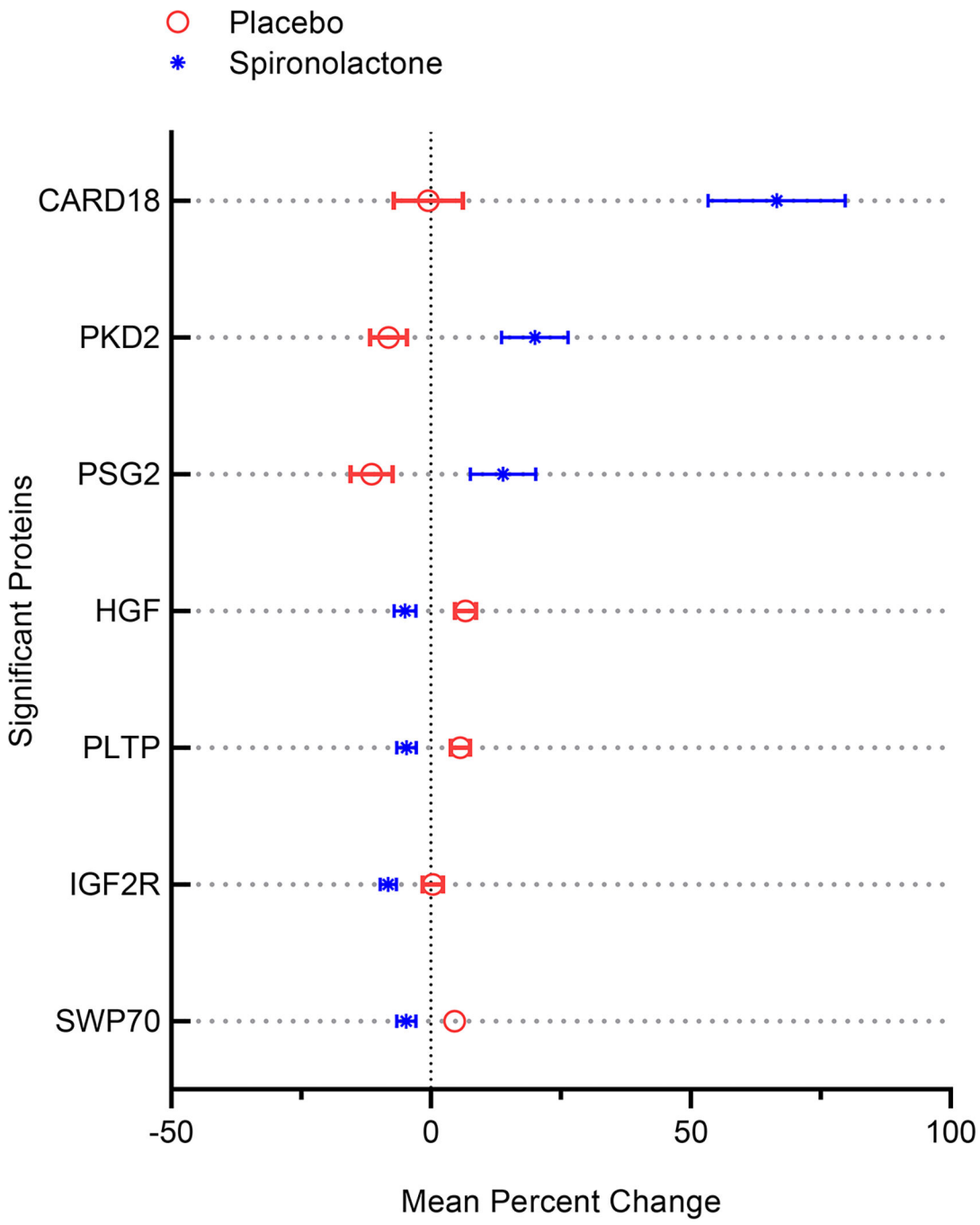

Figure 1. Mean percent change between placebo vs spironolactone group for proteins that demonstrated multiplicity corrected p-value <0.05.

Data shown include mean percentage change +/− SEM in the placebo arm (red) vs spironolactone arm (blue). CARD18: Caspase Associated Recruitment Domain 18. PKD2: Polycystin-2/Polycystic Kidney Disease 2. PSG2: Pregnancy Specific Beta-1-Glycoprotein 2. HGF: Hepatocyte Growth Factor. PLTP: Phospholipid Transfer Protein. IGF2R: Insulin-like Growth Factor 2 Receptor. SWP70: Switch-Associated Protein 70.

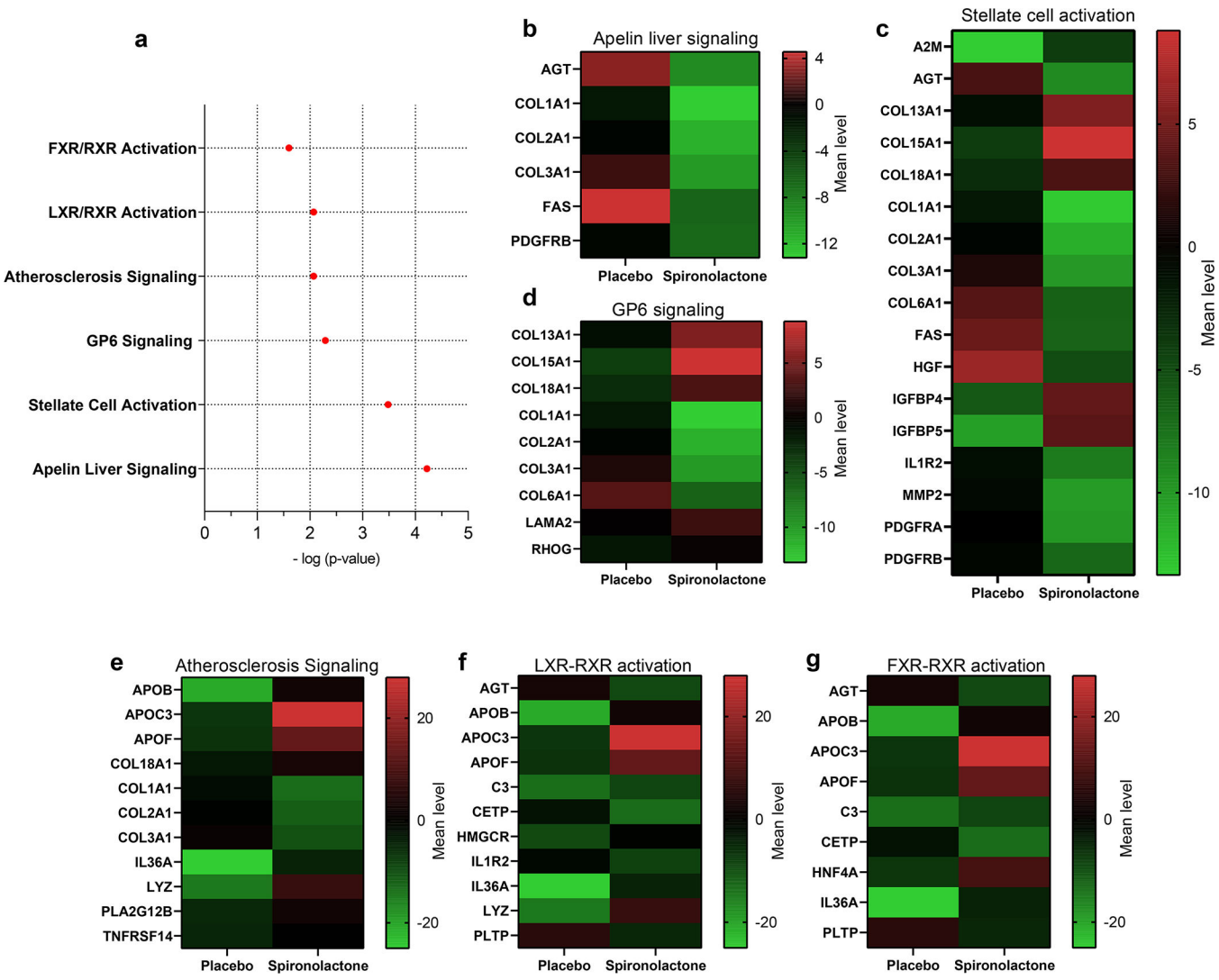

Figure 2. Pathway analysis stratified by arm.

A) Pathways that demonstrated interaction p-value < 0.05 with randomization to spironolactone arm. B-G) Heatmaps of the mean percent change for proteins involved in the pathways identified in (A) including B) apelin liver signaling pathway, C) stellate cell activation, (D) GP6 signaling, E) atherosclerosis signaling, F) liver X Receptor-Retinoid X Receptor (LXR/RXR), and G) farnesoid X Receptor-Retinoid X Receptor (FXR/RXR). A2M: alpha 2 microglobulin, AGT: Angiotensin, ApoB: Apolipoprotein B, ApoC3: Apolipoprotein C3, ApoF: Apolipoprotein F, C3: Complement protein 3, CETP: Cholesterol ester transfer protein, COL: Collagen, FAS: Fas death receptor, GP6 Signaling: Glycoprotein 6 signaling, HGF: Hepatocyte growth factor, HMGCR: Hydroxy-3-Methylglutaryl-CoA Reductase, HNF4A: Hepatocyte Nuclear Factor 4 Alpha, IGFBP: Insulin-like growth factors binding protein, IL1R2: interleukin 1 receptor type 2, IL36A: Interleukin 36 alpha, LAMA2: Laminin subunit alpha-2, LYZ: Lysozyme, MMP2: Matrix metalloproteinase 2, PDGFRA: Platelet derived growth factor receptor alpha, PDGFRB: Platelet derived growth factor receptor beta, PLA2G12B: Phospholipase A2 Group 12 B, PLTP: Phospholipid transfer

protein, RHOG: Ras Homolog Family Member G, TNRFSF14: TNF Receptor Superfamily Member 14.

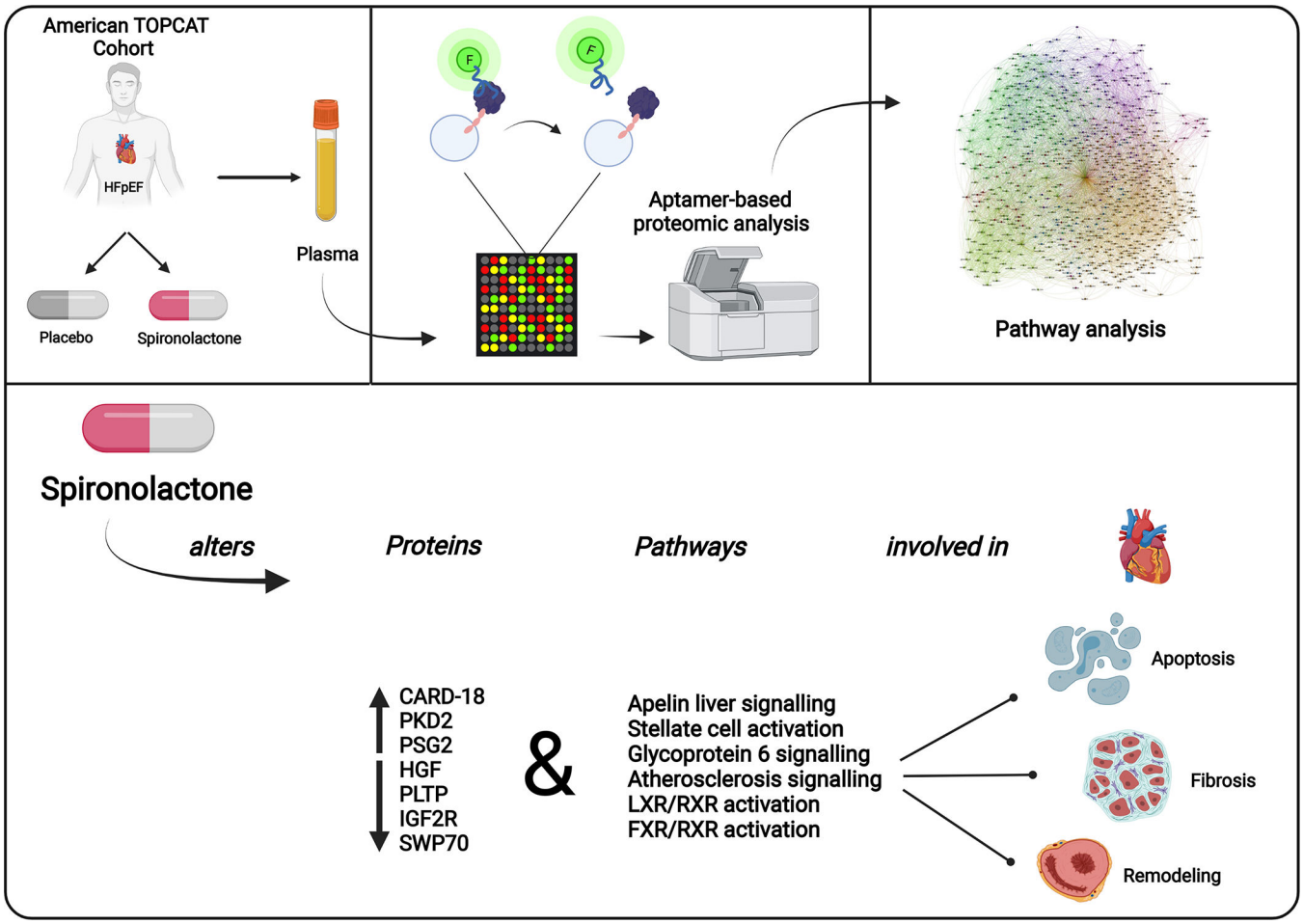

Figure 3. Plasma proteins altered by spironolactone in TOPCAT:

In HFpEF patients, spironolactone alters proteins and pathways involved in myocardial apoptosis, fibrosis, and remodeling. CARD18: Caspase Associated Recruitment Domain 18. FXR/RXR: Farnesoid X Receptor-Retinoid X Receptor. LXR/RXR: Liver X Receptor-Retinoid X Receptor. PKD2: Polycystin-2/Polycystic Kidney Disease 2. PSG2: Pregnancy Specific Beta-1-Glycoprotein 2. HGF: Hepatocyte Growth Factor. PLTP: Phospholipid Transfer Protein. IGF2R: Insulin-like Growth Factor 2 Receptor. SWP70: Switch-Associated Protein 70.

Table 1.

General Characteristics of American TOPCAT trial participants included vs. not included in this analysis.

	TOPCAT America Participants not included in this analysis. (n=1601) Median (IQR), Mean (SD), or n (%)	TOPCAT America Participants included in this analysis. (n=164) Median (IQR), Mean (SD), or n (%)	P value
Age	72 (64,79)	73.5 (66,80)	0.12
Male sex	789 (49%)	94 (57%)	0.05
Body Mass Index (BMI)	32.9 (27.9,38.6)	32.7 (28.1,36.8)	0.42
Race			0.04
White	1241 (77%)	141 (85%)	
Black	283 (17%)	19 (11%)	
Asian	77 (5%)	4 (2%)	
LVEF	58 (52,64)	60 (53.5,65)	0.35
Smoking History	802 (54%)	96 (61%)	0.09
Myocardial infarction	321 (20%)	38 (23%)	0.34
Stroke	147 (9%)	11 (6%)	0.28
Coronary artery bypass graft (CABG)	291 (18%)	45 (27%)	0.004
Percutaneous coronary intervention (PCI)	303 (19%)	41 (25%)	0.06
Chronic obstructive pulmonary disease (COPD)	274 (17%)	17 (10%)	0.02
Hypertension (HTN)	1432 (90%)	155 (95%)	0.04
Atrial fibrillation (AF)	655 (41%)	87 (53%)	0.002
Diabetes Mellitus (DM)	714 (45%)	74 (45%)	0.9
Glomerular filtration rate (GFR)	60.6 (48.8,77)	63.1 (51.7,75.6)	0.43
Hematocrit (HCT)	38.5 (35.4,41.9)	39 (35.9,41.7)	0.65
B-natriuretic peptide (BNP)	396 (188,794)	489 (190,1068)	0.13
Systolic blood pressure (SBP)	130 (118,139)	124 (118,136)	0.01
Diastolic blood pressure (DBP)	70 (62,80)	70 (61,76.5)	0.01
Insulin	347 (22%)	32 (20%)	0.52
Beta blocker (BBs)	1246 (78%)	141 (86%)	0.01
Calcium channel blocker (CCBs)	612 (38%)	69 (42%)	0.33
Angiotensinogen converting enzyme inhibitor/Angiotensin receptor blocker (ACE/ ARBs)	1269 (80%)	125 (76%)	0.35
Aspirin	927 (58%)	100 (61%)	0.45
Statins	1022 (64%)	126 (77%)	0.0009

Table 2.

Baseline Characteristics of included participants randomized to placebo or spironolactone.

	Placebo (80) Mean (SD) or n (%)	Spironolactone (84) Mean (SD) or n (%)	P value
Age	72.4(9.5)	71.6(9.4)	0.57
Male sex	47(56%)	47(59%)	0.75
Body Mass Index (BMI)	32.3(6.7)	32.6(6.8)	0.71
Race			0.43
White	71(85%)	70(88%)	
Black	10(12%)	9(11%)	
Other	3(4%)	1(1%)	
Smoking History	48(59%)	48(62%)	0.74
Myocardial infarction	22(26%)	16(20%)	0.36
Stroke	3(4%)	8(10%)	0.12
Coronary artery bypass graft (CABG)	24(29%)	21(26%)	0.86
Percutaneous coronary intervention (PCI)	20(24%)	21(26%)	0.72
Chronic obstructive pulmonary disease (COPD)	10(12%)	7(9%)	0.61
Hypertension (HTN)	80(95%)	75(94%)	0.74
Atrial fibrillation (AF)	47(56%)	40(50%)	0.53
Diabetes Mellitus (DM)	35(42%)	39(49%)	0.43
Glomerular filtration rate (GFR)	66.4(19)	62.3(19)	0.16
Hematocrit (HCT)	39(4.3)	38.6(4.3)	0.55
B-natriuretic peptide (BNP)	482.7(659.3)	498.9(665.1)	0.85
Systolic blood pressure (SBP)	124.3(14.2)	124.8(14.2)	0.82
Diastolic blood pressure (DBP)	69.3(10.8)	68.8(10.8)	0.77
Insulin	11(13%)	21(26%)	0.04
Beta blocker (BBs)	74(88%)	67(84%)	0.5
Calcium channel blocker (CCBs)	33(39%)	36(45%)	0.52
Angiotensinogen converting enzyme inhibitor/Angiotensin receptor blocker (ACE/ ARBs)	66(79%)	59(74%)	0.58
Aspirin	51(61%)	49(61%)	1
Statins	67(80%)	59(74%)	0.45