Circulation: Heart Failure

ORIGINAL ARTICLE

Proteomics Identify Clinical Phenotypes and Predict Functional Outcomes in Heart Failure With Preserved Ejection Fraction: Insights From VITALITY-HFpEF

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BACKGROUND: Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous syndrome that may emerge from overlapping systemic processes associated with comorbidities. We assessed whether unique clusters of circulating proteins are associated with specific clinical characteristics and functional status at baseline and follow-up in a well-phenotyped cohort of patients with HFpEF.

METHODS: We evaluated 368 proteins associated with cardiovascular disease and inflammation in prerandomization blood samples from 763 VITALITY-HFpEF (Vericiguat to Improve Physical Functioning in Daily Living Activities of Patients With HFpEF) participants who had a left ventricular ejection fraction ≥45% and a heart failure decompensation event within 6 months. Proteins were clustered, and their associations with clinical characteristics, baseline, and 24-week functional outcomes (Kansas City Cardiomyopathy Questionnaire Physical Limitation Score, 6-minute walk distance [6MWD], and Fried frailty phenotype) were estimated with linear regression. Elastic net regression was used to derive a proteomic summary composite to predict changes in 24-week functional outcomes.

RESULTS: Four unique protein clusters were identified, containing 24, 66, 197, and 81 proteins. At baseline, 2 protein clusters with the hub proteins caspase-3 and Dickkopf-related protein 1 were associated with increased frailty, whereas the cluster with tumor necrosis factor receptor 1 as a hub protein was associated with lower Kansas City Cardiomyopathy Questionnaire Physical Limitation Score and shorter 6MWD. By contrast, the cluster with protein C as a hub protein was associated with less frailty and longer a 6MWD. The 24-week increase in 6MWD was negatively correlated with the protein cluster with caspase-3; the protein C cluster was correlated with less frailty at 24 weeks. The baseline proteomic summary composite predicted observed changes in Kansas City Cardiomyopathy Questionnaire Physical Limitation Score and 6MWD at 24 weeks (r=0.42 and 0.30; $P<0.001$ for both).

CONCLUSIONS: Proteomics differentiate specific baseline functional traits associated with HFpEF and may facilitate phenotyping in a heterogeneous disease. These proteins also provide insights into the diverse pathophysiology of HFpEF and which patients may improve functional status during follow-up.

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Key Words: frailty ■ functional status ■ heart failure ■ proteomics ■ stroke volume

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WHAT IS NEW?

- We identified unique protein clusters, including a novel cluster led by protein C from baseline proteomic measures, in well-phenotyped clinical trial participants with heart failure with preserved ejection fraction and recent heart failure decompensation.
- The cluster is characterized by anti-inflammatory, anti-apoptotic, and anticoagulant functions.
- Higher cluster expression was associated with better baseline walking distance and an improvement in frailty status at 24 weeks.

WHAT ARE THE CLINICAL IMPLICATIONS?

- Despite some recent successes, the treatment of heart failure with preserved ejection fraction remains challenging.
- Given the diverse mechanisms contributing to this syndrome, our findings support the need for precision phenotyping of this disorder and suggest that circulating proteomics may refine baseline phenotype characterization and also identify patient cohorts most likely to show longitudinal improvement in functional outcomes.

Nonstandard Abbreviations and Acronyms

6MWD	6-minute walk distance
apoM	apolipoprotein M
BMI	body mass index
DKK1	Dickkopf-related protein 1
FFP	Fried frailty phenotype
HFpEF	heart failure with preserved ejection fraction
JAM-A	junctional adhesion molecule A
KCCQ-PLS	Kansas City Cardiomyopathy Questionnaire Physical Limitation Score
LOD	limit of assay detection
NT-proBNP	N-terminal pro-B-type natriuretic peptide
TNFR1	tumor necrosis factor receptor 1A
VITALITY-HFpEF	Vericiguat to Improve Physical Functioning in Daily Living Activities of Patients With HFpEF
WCNA	weighted coexpression network analysis

Heart failure with preserved ejection fraction (HFpEF) is a heterogeneous disease presumed to be related to multiple unique and often overlapping systemic processes (ie, inflammation, vascular calcification, tissue remodeling, and endothelial dysfunction) with associated medical comorbidities (ie, diabetes, obesity, atrial fibrillation, frailty, chronic kidney disease).[1] Clinically, HFpEF accounts for at least 50% of all heart failure (HF) admissions, making it an important therapeutic target.[2] For purposes of evaluating therapeutic interventions, HFpEF has often been treated as a homogeneous condition; with few exceptions, this approach has largely resulted in neutral trials of pharmacological interventions.[3–8] Further phenotyping based on clinical signs and symptoms, biomarkers, exercise testing, invasive hemodynamics, and echocardiographic criteria has minimally improved clinical trial designs to date.[1,9] Given the large unmet need for effective HFpEF interventions as well as the substantial cost and effort to test novel therapies, improvements in reproducible precision phenotyping have been suggested as one strategy to address this issue.[10]

Protein biomarkers, specifically natriuretic peptides, are routinely used to screen patients for inclusion in HFpEF clinical trials but lack precision for the diagnosis. The advent of high-throughput technologies to measure many diverse circulating proteins, that is, proteomics, offers an opportunity to objectively characterize and better differentiate seemingly heterogeneous HFpEF patient phenotypes.[11,12]

To address proteomics for phenotyping, our study examined 2 objectives in this carefully characterized advanced HFpEF population: first, to assess the associations between a spectrum of circulating proteins and baseline clinical and functional characteristics; and second, to evaluate the predictive capability of baseline protein levels for forecasting changes in functional parameters over a 24-week period.

METHODS

The design and primary results of the VITALITY-HFpEF study (Vericiguat to Improve Physical Functioning in Daily Living Activities of Patients With HFpEF; [NCT03547583]) have been previously reported.[3,13] In summary, this was a phase IIb, multicenter, double-blind, placebo-controlled trial of HFpEF participants randomized to receive vericiguat, an oral soluble guanylate cyclase stimulator titrated up to 10 or 15 mg daily, versus placebo over 24 weeks. The primary objective was to measure improvement in the patient-reported Kansas City Cardiomyopathy Questionnaire Physical Limitation Score (KCCQ-PLS), and the secondary objective was to measure change in exercise capacity from time of randomization to 24 weeks using the 6-minute walk distance (6MWD). Change in self-reported frailty from baseline to 24 weeks was a post hoc exploratory end point. Institutional review board or ethics committee approval was obtained at each study site; all patients provided written informed consent. The data that support the findings of this study are available from the senior author upon reasonable request. For this secondary analysis of a clinical trial, we used the Strengthening the Reporting of Observational studies in Epidemiology cohort checklist when writing our report and uploaded the Strengthening the Reporting of Observational studies in Epidemiology checklist as a supplemental file of the article.

Proteomic Substudy Participants

All 789 randomized participants were eligible for inclusion in this targeted discovery proteomics substudy. Proteomics for this analysis were measured from blood collected at the baseline visit (prerandomization). Participants enrolled in VITALITY-HFpEF were ≥45 years of age with a history of chronic HFpEF (left ventricular ejection fraction ≥45%) and had New York Heart Association class II or III symptoms, elevated natriuretic peptide levels, and an acute HF decompensation event within 6 months (hospitalization or need for outpatient intravenous diuretics). Additional inclusion and exclusion criteria for VITALITY-HFpEF are reported elsewhere.[3]

Baseline and Follow-Up Study Functional Assessments

For the purposes of this proteomics analysis, we utilized the prerandomization and 24-week KCCQ-PLS, the 6MWD in meters, and the Fried frailty phenotype (FFP) assessments. The KCCQ-PLS is a participant report of functional capacity for activities of daily living in patients with HF and is reported on a scale score of 0 to 100, with a higher score representing greater function. The FFP was assessed on a 5-point scale based on a patient's response to questions about weight loss, exhaustion, physical activity, and for this study, subjective assessment of grip strength and 5-m gait speed (Table S1).[14,15] A score of 3 to 5 is considered consistent with a frail phenotype, 1 to 2 is prefrail, and 0 is nonfrail.[15]

Proteomic Measurements

For the targeted discovery proteomic analysis, serum samples drawn at the baseline study visit were collected and stored at −80 °C. Samples were sent to Olink (Watertown, MA) for analysis with 4 Olink Target-96 panels, including the cardiometabolic, cardiovascular II and III, and inflammation panels with 368 unique proteins measured using the proximity extension assay technology.[16] Values are not reported as concentrations but as normalized protein eXpression values expressed in log base 2.[17] These panels were chosen in part based on prior work showing unique differentiation of proteins between patients with heart failure with reduced ejection fraction versus HFpEF as well as a knowledge-based curation of potential mechanisms that could influence HFpEF pathophysiology.[11,18] Reproducibility and validation information about the proteins are reported by Olink.[19] A list of proteins by panel included in the analysis can be found at the Olink website.[20] Quality control measures for the Olink Target-96 panels are available on the Olink website.[21]

Statistical Analysis

Categorical variables are reported as frequencies (percentages) and continuous variables as medians (25th, 75th percentiles) for patient characteristics at baseline (randomization). The frequency of nonmissing data for each variable and end point of interest was reported, and missing data were not imputed. Protein levels below the limit of assay detection (LOD) were imputed halfway between 0 and the LOD.

Individual Protein Analysis

The relationships of the individual proteins as well as clusters of proteins with a priori selected features including age, sex, body mass index (BMI), KCCQ-PLS, 6MWD, and FFP at baseline were evaluated. For the individual protein associations, we estimated the magnitude of effect size (β coefficient) of each of the features on each protein using a series of univariable linear regressions. The FFP assessment (nonfrail, prefrail, and frail) was considered an ordinal independent variable with the regression coefficient (β) measuring the relative change in the protein level as frailty status gets worse. Age, BMI, KCCQ-PLS, and 6MWD were considered as continuous variables, and β represents the change in the protein per 1 SD (1 SD, age 9.4 years, BMI 6.0 kg/m^2, KCCQ-PLS 24.4, and 6MWD 110 m) increase in the respective variable. For sex at birth, β represents the relative mean increase in the protein level in females compared with males. Statistical tests were adjusted for multiple comparisons using the Benjamini-Hochberg method at an overall false discovery rate of 5%. Given an absence of standardization of normalized protein eXpression units between different proteins, we ranked individual proteins' association with clinical characteristics based on *P* values after correction for false discovery rate.

Protein Clustering

We applied the method of weighted coexpression network analysis (WCNA). The WCNA is an unsupervised data-driven approach that defines clusters of proteins based on their correlation to each other.[22] Once the proteins are grouped into clusters, a representative summary composite of each cluster is determined using the module eigengene (first principal component). Eigengene values were standardized. Furthermore, for each cluster, we estimated a quantitative measure of cluster/module membership as the correlation of the cluster eigengene and the individual protein expression profile. The eigengene summary values were subsequently applied in linear regressions, as described above, to determine the association of the clusters with the baseline phenotypes and 24-week changes in functional measures. A correlation heat map was used to present correlations of the clusters with the phenotypes and functional variables at baseline. Similarly, heat maps were used to present adjusted associations of the clusters with functional measures at baseline and changes at 24 weeks. These associations were adjusted for age, sex, BMI, atrial fibrillation, NT-proBNP (N-terminal pro-B-type natriuretic peptide), creatinine, and New York Heart Association class. A pathway overrepresentation analysis was performed on each of the 4 identified clusters to distinguish the predominant pathways to which they belong. For this analysis, we used the following 3 online databases: (1) gene ontology (GO); (2) Kyoto Encyclopedia of Genes and Genome; and (3) Reactome Pathway Database. The pathway overrepresentation analysis applies the standard accumulative hypergeometric statistical test to identify pathways where input biomarkers show significant presence, automatically clustering resultant terms to reduce redundancy. The pathway analysis was stratified by the 4 WCNA-identified clusters. The proteins in each cluster were the input biomarkers for the analysis that was performed in Metascape online application.[23]

Prediction Modeling for Longitudinal Functional Change

To identify the optimal proteins that jointly predict relative change in the functional outcomes, we used an elastic net regression ("glmnet" R package) for variable selection and to regularize (shrinkage) the coefficients. Elastic net regression focuses on the selection of individual protein associations with

the outcomes and is efficacious in scenarios such as this study where the number of potential predictors (proteins) exceeds the number of outcomes. For tuning the model, we applied a leave-one-out cross-validation criterion to select the optimal amount of shrinkage in the penalized regression.

For each functional outcome, we determined a summary score for a participant using the linear predictor, a linear combination of the set of selected proteins with their mixing coefficients. Predictive performance was evaluated using an internally cross-validated C-index. To evaluate predictive performance, we plotted the cross-validated predicted values versus observed change over 24 weeks and calculated a Pearson correlation coefficient.

All analyses were conducted agnostic to the assigned study treatment (placebo, 10 or 15 mg vericiguat), as there was an absence of study treatment effect with these functional outcomes at 24 weeks.[3,15] SAS, version 9.4 (SAS Institute Inc, Cary, NC), and R, version 4.0.2, were used to perform the statistical analysis, and a 2-sided *P* value of <0.05 was considered statistically significant.

One author (W. Alemayehu) had full access to all the data in the study and takes responsibility for its integrity and the data analysis.

RESULTS

Patient Characteristics

Of 789 participants randomized in the main study, 763 had baseline blood samples for measurement that passed quality assurance metrics on all 4 panels and were included in this proteomic analysis (Table 1). These are older adults (median age of 73.0 years), nearly evenly divided by sex (48.4% female), of predominantly White race (86.8%), and the majority were overweight or obese. Approximately two-fifth had New York Heart Association class III symptoms; their median NT-proBNP was 1394 pg/mL, and left ventricular ejection fraction was 55%. The KCCQ-PLS, 6MWD, and FFP assessments were available at baseline in 739 (97%), 699 (92%), and 732 patients (96%), respectively. The median KCCQ-PLS was 62.5, the median 6MWD was 300 m, and approximately one-third of participants were classified as frail, all consistent with moderate impairment of functional capacity at baseline.

Table 1. Baseline Characteristics of Participants Enrolled in VITALITY-HFpEF With Proteomic Measurements at Randomization

	Total (n=763)
Age, y, median (25th, 75th)	73.0 (66.0, 80.0)
Female, n (%)	369 (48.4%)
Race, n (%)	
White	662 (86.8%)
Black or African American	20 (2.6%)
Asian	62 (8.1%)
American Indian or Alaska Native	11 (1.4%)
Not reported	2 (0.3%)
Multiple	6 (0.8%)
Body mass index, kg/m^2, median (25th, 75th)	30.4 (26.2, 34.6)
Systolic BP, mm Hg, median (25th, 75th)	128.3 (120.0, 138.3)
Diastolic BP, mm Hg, median (25th, 75th)	73.3 (66.3, 80.0)
Atrial fibrillation: central ECG assessment, n (%)	
Atrial fibrillation	263 (34.5%)
Sinus rhythm	500 (65.5%)
Creatinine, mg/dL, median (25th, 75th)	1.1 (0.9, 1.4)
NT-proBNP, pg/mL, median (25th, 75th)	1394.2 (617.0, 2966.3)
NYHA class, n (%)	
II	445 (58.3%)
III	318 (41.7%)
LVEF (%), median (25th, 75th)	55.0 (50.0, 61.0)
KCCQ-PLS, median (25th, 75th)	62.5 (41.7, 79.2) n=739
6MWD, m, median (25th, 75th)	300.0 (219.0, 372.0) n=699
Fried frailty phenotype, n (%)	n=732
Not frail	195 (26.6%)
Prefrail	278 (38.0%)
Frail	259 (35.4%)

6MWD indicates 6-minute walk distance; BP, blood pressure; KCCQ-PLS, Kansas City Cardiomyopathy Questionnaire Physical Limitation Score; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; and VITALITY-HFpEF, Vericiguat to Improve Physical Functioning in Daily Living Activities of Patients With Heart Failure With Preserved Ejection Fraction.

Proteomic Measures

Individual protein associations (β coefficients) are shown for the prespecified, prerandomization clinical characteristics including sex, age, and BMI (Table S2). All 368 unique proteins were analyzed, in which proteins below the LOD were assigned with half of the LOD value. There were 21 proteins having >50% of values below the LOD. After adjustment for false discovery, 101 proteins were associated with sex, 134 associated with age, and 79 associated with BMI. We then evaluated individual protein associations with the prerandomization functional measures of the KCCQ-PLS, 6MWD, and FFP (Table S3). After adjustment for false discovery, there were 167 proteins significantly associated with the KCCQ-PLS, 161 with 6MWD, and 139 with frailty as assessed using the FFP.

Protein Clustering and Pathway Analysis

Clustering by WCNA identified 4 unique clusters that are presented in Table 2. The number of proteins in each cluster is shown with the central hub protein and other top contributing proteins, ordered by cluster membership. Next, we evaluated the association of each cluster with the 3 prerandomization clinical characteristics of sex, age, and BMI and the prerandomization measures of

Table 2. Summary of the Clusters Identified by the Weighted Correlation Network Analysis

Cluster	No. of proteins in cluster	Central hub* (MM)	Other top contributors*
1	24	CASP3 (0.93)	SIRT2, STAMBP, AXIN1, STK4, NEMO, DECR1, ITGB1BP2, SRC, JAM_A
2	66	DKK1 (0.89)	ANGPT1, HB_EGF, SORT1, TNFSF14, PDGFSUBUNITB, CD84, PDGFSUBUNITA, LAPTGF_BETA_1 TGF_ALPHA
3	197	TNFR1 (0.88)	LTBR, TNF_R2, EPHB4, IL_18BP, TRAIL_R2, PLC, TNFRSF14, TNFRSF11A, U_PAR, TFF3, CD40, CST3, IL_15RA, CD93, TNFRSF10A, TNFRSF9, PGF, IL_10RB, IGFBP_7
4	81	PROC (0.76)	SERPINA5, F7, F11, FETUB, APOM, LDLRECEPTOR, CNDP1, IGFBP3, CFHR5

Full names for other top contributor proteins can be found in reference 20. CASP3 indicates caspase-3; DKK1, Dickkopf-related protein 1; MM, module membership; PROC, protein C; and TNFR1, tumor necrosis factor receptor 1A.

*For each cluster, we estimated a quantitative measure of cluster/MM as the correlation of the cluster eigengene (first principal component) and the protein expression profile.

functional status, KCCQ-PLS, 6MWD, and FFP. A heat map representation of the strength of association of each of the 4 clusters with participant baseline and functional characteristics is shown in Figure 1. Clusters represented by the central hub proteins caspase-3, DKK1 (Dickkopf-related protein 1), and TNFR1 (tumor necrosis factor receptor 1A) are positively associated with progressive frailty. The cluster represented by TNFR1 is also positively associated with older age and inversely associated with KCCQ-PLS and 6MWD. In contrast to the directionality of the associations of clusters 1 to 3, the cluster represented by protein C is associated with female sex, younger age, higher BMI, greater 6MWD, and less frailty. After multivariable adjustment, the trends for each cluster's associations with baseline functional measures remained similar, with the TNFR1 hub cluster remaining significantly associated with all functional measures, the DKK1 cluster was associated with greater frailty, and the protein C hub cluster was associated with a greater 6MWD and with less frailty (Figure S1).

To explore potential mechanisms that may explain these associations, we performed a pathway analysis. Overrepresented pathways on each of the 4 clusters are shown in Figure 2. Clusters 3 and 4, which have the strongest associations with the baseline clinical features and measures of physical function, are predominantly represented by nonspecific pathways of cytokine-cytokine interaction, reflecting the predominance of inflammatory/anti-inflammatory mechanisms represented by these clusters. Additional pathways shown in Figure 2 reflect the multifunctional roles of these circulating proteins. Evaluation of top cluster membership proteins in clusters 3 and 4 is shown in Figure S2A and S2B, and the directionality of the individual top membership proteins is consistent with the overall directionality of the cluster associations with the baseline clinical features.

The KCCQ-PLS, 6MWD, and FFP assessments were available at 24-week follow-up in 630 (83%), 627 (82%), and 622 patients (82%), respectively. The KCCQ-PLS baseline and 24-week scores were a median 62.5 (41.7,

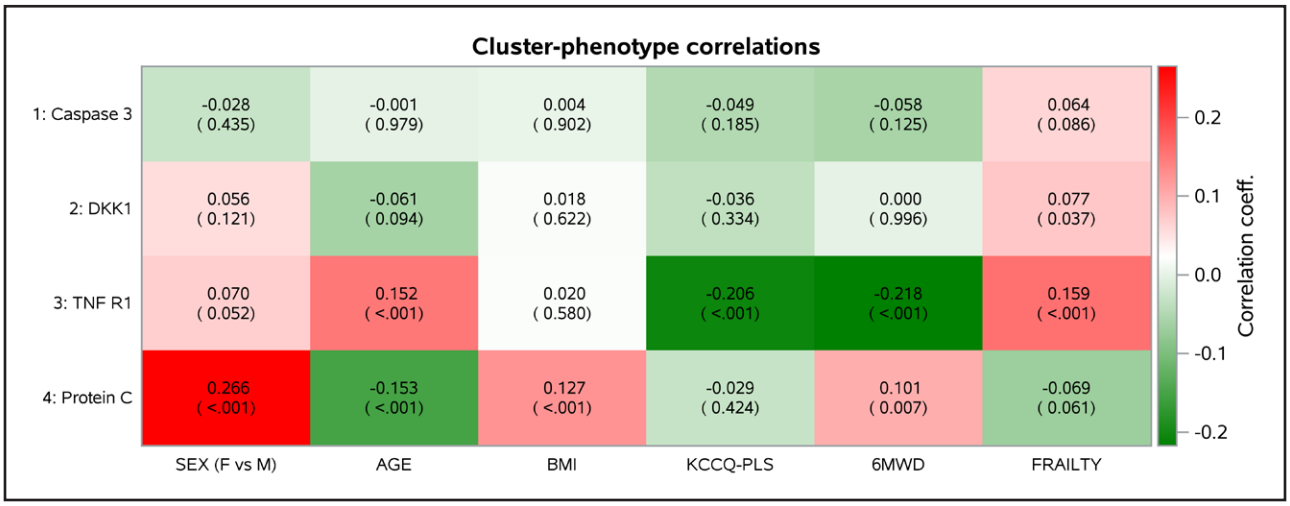

Figure 1. Association of weighted coexpression network analysis–identified protein clusters with prerandomization clinical and functional characteristics of participants in the VITALITY-HFpEF study.
Clusters 1 to 4 are shown as rows identified by their hub proteins. The number in each cell is an unadjusted (Spearman for female and frailty or Pearson for the rest) correlation coefficient (*P* value). Red color represents a positive correlation, and green color represents a negative correlation. 6WMD indicates 6-minute walk distance; BMI, body mass index; DKK1, Dickkopf-related protein 1; KCCQ-PLS, Kansas City Cardiomyopathy Questionnaire Physical Limitation Score; TNFR1, tumor necrosis factor receptor 1; and VITALITY-HFpEF, Vericiguat to Improve Physical Functioning in Daily Living Activities of Patients With Heart Failure With Preserved Ejection Fraction.

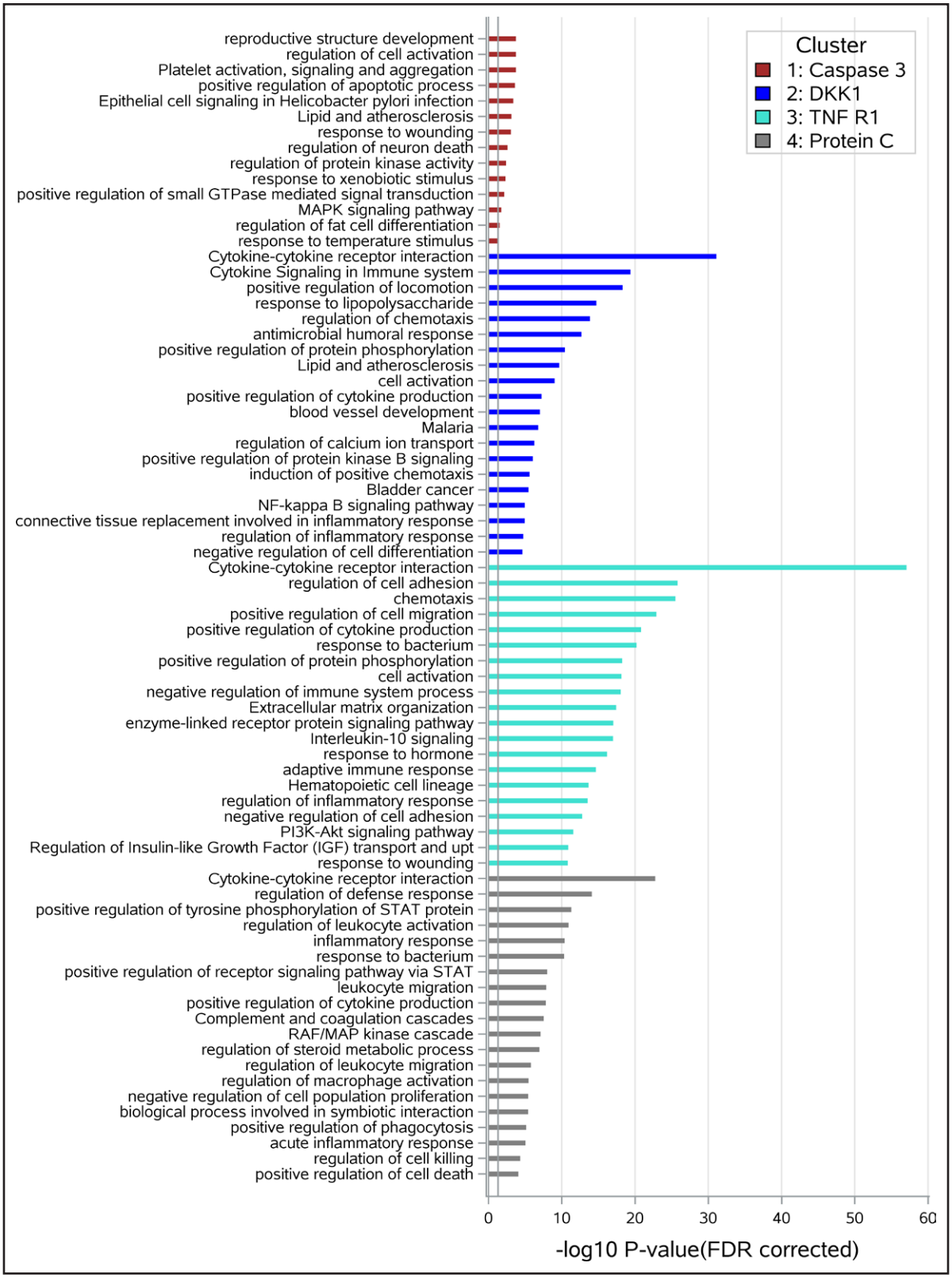

Figure 2. Overrepresented pathways for the 4 weighted coexpression network analysis–identified protein clusters; top pathways selected based on false discovery rate (FDR)-corrected *P* value (x axis).
Up to 20 significant pathways per cluster are shown (vertical reference line just to the right of 0 represents the FDR-corrected *P*=0.05). Caspase-3 protein cluster pathways are red, DKK1 (Dickkopf-related protein 1) protein cluster pathways are blue, TNFR1 (tumor necrosis factor receptor 1) protein cluster pathways are turquoise, and protein C cluster pathways are gray. MAP indicates mitogen-activated protein; MAPK, mitogen-activated protein kinase; RAF, rapidly accelerated fibrosarcoma; and STAT, signal transducer and activator of transcription.

79.2) and 75.0 (50, 90), and 6MWD at baseline and 24 weeks were a median 300 (219, 372) m and 320 (240, 400) m across the randomized arms. By 24 weeks, 46.5% of patients had no change in frailty, 46.5% had become less frail, and 7.1% had become frailer. Adjusted associations of the 4 clusters with 24-week change in functional status are shown in Figure 3. The caspase-3 cluster was inversely associated with an increased 6MWD over 24 weeks, whereas the protein C cluster was positively associated with a change to a less frail state over 24 weeks. The unadjusted associations of the clusters with 24-week functional outcomes are shown in Figure S3. Volcano plots illustrate the adjusted associations of individual proteins from within the caspase-3 and protein C clusters with 24-week change in 6MWD and change in frailty, respectively (Figure S4A and S4B). JAM-A (junctional adhesion molecule A) shows the strongest association with a decrease in 6MWD, and apoM (apolipoprotein M) shows the highest odds ratio for an improvement in frailty status.

Proteomics Composite Metrics and Functional Outcomes

Specific proteins and their mixing coefficients calculated by elastic net regression for each functional outcome of interest are shown in Table S4. A baseline proteomics composite differentiated longitudinal functional outcomes for individual participants with a moderate correlation between the predicted and observed 24-week change in KCCQ-PLS and 6MWD (r=0.42 and r=0.30, $P<0.001$ for both; Figures S5 and S6). Participants who were classified as less frail (n=273), defined as a decrease of 1 or more in the frailty category, at 24-week follow-up had a lower median frailty-specific proteomic summary composite compared with participants who either had no change or became more frail (n=321; Figure S7).

DISCUSSION

We identified 3 primary findings in this targeted discovery proteomics approach to patients with symptomatic HFpEF focusing on circulating proteins associated with inflammation, cardiovascular, and cardiometabolic disorders. First, we characterized 4 unique clusters in the VITALITY-HFpEF study population that are associated with baseline clinical and functional characteristics. Second, these protein clusters were also associated with the longitudinal functional changes that occur in patients with HFpEF during study follow-up. Third, using the same proteins, we developed a proteomic summary composite that could predict 24-week functional longitudinal change. To arrive at these findings, we used different statistical methodologies: clustering examined correlations between protein expression levels to derive mechanistic insights between HFpEF and clinical phenotypes. Thereafter, a specialized form of regression modeling for high-dimensional data was used identifying single or multiple proteins most strongly associated with longitudinal change in physical function measures.

Protein Clustering and Insights Into Mechanisms Associated With HFpEF

The 4 protein clusters represent distinct biological pathways. The cluster represented by caspase-3 is involved in the execution phase of apoptosis.[24] Specific to HF, caspase-3 activation was found in the myocytes of patients with end-stage cardiomyopathies at the time

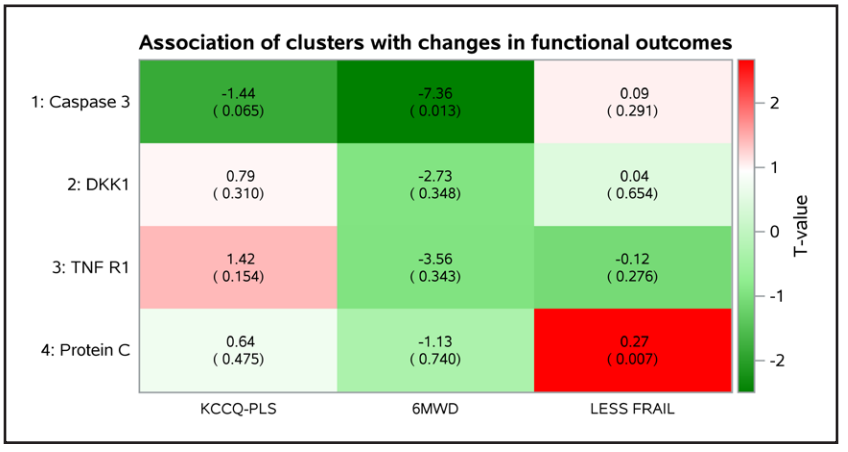

Figure 3. Association of weighted coexpression network analysis–identified protein clusters with changes in functional outcomes.
Clusters 1 to 4 are shown as rows identified by the cluster number and its hub protein. The number in each cell is the beta regression coefficient (*P* value). Red color represents a positive association, and green color represents a negative association. Associations adjusted for age, sex at birth, BMI, creatinine, New York Heart Association class, and NT-proBNP. 6WMD indicates 6-minute walk distance; BMI, body mass index; DKK1, Dickkopf-related protein 1; KCCQ-PLS, Kansas City Cardiomyopathy Questionnaire Physical Limitation Score; NT-proBNP, N-terminal pro-B-type natriuretic peptide; and TNFR1, tumor necrosis factor receptor 1.

of transplant but not in healthy donor hearts, emphasizing the role of apoptosis in end-stage heart failure with reduced ejection fraction.[25] However, in HFpEF, the role of caspase-3 within the myocardium is less certain, as apoptotic pathways were not overexpressed in the myocytes of patients with HFpEF undergoing myocardial biopsy.[26] Caspase-3 was also shown to be a major HFpEF network protein in BIOSTAT-CHF (Biology Study to Tailored Treatment in Chronic Heart Failure) but not in other HFpEF cohorts.[11,18,27,28]

The cluster with the hub protein DKK1 was associated with frailty. DKK1 is expressed in many tissues, including higher expression in adipose tissue, but is minimally expressed within myocytes.[29] Potentially relevant to HFpEF pathophysiology are its associations with vascular fibrosis and calcification when upregulated in experimental animal models.[30] In a proteomic analysis in patients by the DIAST-HF (Diastolic Dysfunction and Diastolic Heart Failure) investigators, DKK1 was found to be associated with HFpEF but not heart failure with reduced ejection fraction, and was also noted to be a hub protein in their network analysis.[28] The association of DKK1 with fibrosis and calcification could imply that this and correlated proteins are relevant to HFpEF pathophysiology.

The cluster represented by the hub protein TNFR1 is the largest cluster consisting of 197 proteins. It was positively associated with progressive frailty, older age, and lower baseline 6MWD and KCCQ-PLS scores. Tumor necrosis factor alpha is a potent inflammatory cytokine produced by macrophages.[31] TNFR1 is prognostic in HF and has been suggested as a substitute for measuring tumor necrosis factor alpha, as it is more stable and has a higher dynamic range for measurement.[32] The inclusion of tumor necrosis factor receptors as hub proteins or major components of network analyses is seen across multiple HFpEF cohorts.[11,18,28]

The fourth cluster with the hub protein, protein C, is associated with anticoagulant, anti-inflammatory, anti-apoptotic, and endothelial barrier protection functions.[33] Unlike the other 3 protein clusters, this protein cluster appears to have protective associations in HFpEF. It is positively associated with BMI and female sex (characteristics that can be associated with better outcomes in HFpEF),[34,35] younger age, higher 6MWD, and less frailty. It is also positively associated with longitudinal improvement in frailty status. Identification of this cluster is a unique finding of this analysis of VITALITY-HFpEF. Other top proteins in this cluster that provide mechanistic insights include protein C inhibitor (SERAPINA5), coagulation factor 7 (F7), and coagulation factor X1 (F11), consistent with this cluster's role in the coagulation system. Important additional top eigengene value selected proteins include apoM that has been shown to be inversely associated with mortality in multiple HFpEF cohorts and is thought to work predominantly through its anti-inflammatory effects, supporting the anti-inflammatory role of this cluster.[36]

Functional Outcomes Assessment by Proteomic Composite

We demonstrate that baseline proteomics can identify participants at risk for progression of frailty, predict self-reported changes in quality of life and physical function (KCCQ-PLS), and change in objective measures of physical function (6MWD). It is notable that the protein selection for longitudinal functional outcomes is different from the WCNA selection of top proteins. In part, this reflects differences in the statistical methodologies and objectives. WCNA was applied for cross-sectional mechanistic analysis to reduce the dimensionality of the individual protein data and identify clusters of proteins that show highly correlated expression patterns. Those clusters were later tested for their associations with participant traits such as functional status. In contrast, elastic net regression, a form of supervised machine learning, associates individual proteins with functional outcomes with the aim of identifying the best set of predictor proteins. However, the 2 statistical methodologies can identify similar signals. The protein most strongly associated with 24-week change in 6MWD from within the cluster analysis was JAM-A, and it was also the only protein selected by elastic net regression to predict 6MWD longitudinal change. JAM-A is expressed on the surface of many cell types and maintains a barrier function for the endothelium. However, soluble JAM-A has a causal role in atherosclerosis and acute coronary syndromes through platelet activation and potentially monocyte conversion to foam cells with resulting inflammation.[37,38]

Limitations

Notwithstanding the absence of a comorbidity-matched control population without HF, the substantial size of our study population (≈2–3× larger than the next 3 largest HFpEF cohorts) as well as their recent acute hospitalization provides additional potential mechanistic insights into HFpEF pathophysiology as compared with other more stable cohorts evaluating circulating proteins. This study and others that analyze circulating proteins assume that measurement of soluble proteins adequately reflects HFpEF pathophysiology. When evaluating high-dimensional circulating proteomic data, a larger study sample size and greater breadth and heterogeneity of measured proteins could refine the clusters and improve the strength of the associations. Furthermore, our cross-sectional proteomic mechanistic clustering findings deserve validation in external cohorts. With respect to functional longitudinal outcomes, our post hoc analysis and proteomic findings and their relationship to clinical characteristics and functional measures over time are hypothesis generating and require external validation. We also acknowledge our patient-reported frailty may have limitations.

Conclusions

Our findings from a well-characterized clinical trial population with HFpEF confirm that circulatory inflammatory and apoptosis-inducing proteins are associated with clinical features of frailty and poor functional status by both qualitative and quantitative measures. Importantly, we also identify a counterbalancing cluster of proteins that are associated with a mitigating role in HFpEF morbidity and decline in function over time. Validation and investigation with an expanded portfolio of proteins may provide a phenotyping strategy for patients with HFpEF along with clinical features to better characterize baseline pathophysiology for treatment selection and prediction of functional outcomes.

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Supplemental Material

STROBE Checklist
Tables S1–S4
Figures S1–S7

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