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Plasma proteomic biomarkers of physical frailty in heart failure: a propensity score matched discovery-based pilot study

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Abstract

Background Physical frailty is highly prevalent in heart failure (HF), but we lack an understanding of the underlying pathophysiology. Proteomic evaluation of plasma samples may elucidate potential mechanisms and biomarkers of physical frailty in HF.

Objectives We aimed to identify plasma proteomic biomarkers that are differentially expressed between physically frail and non-physically frail adults with HF.

Methods This was a secondary analysis of a subset of data and plasma samples from a study of frailty among patients with New York Heart Association (NYHA) Functional Classification I-IV HF. Physical frailty was measured using the Frailty Phenotype Criteria. Propensity score matching was used to match pairs of physically frail ($n=20$) vs. non-physically frail ($n=20$) patients on clinical characteristics. Plasma samples were processed using a sensitive liquid chromatography mass spectrometry platform, utilizing a multiplexed tandem mass tag-labeled quantitative proteomics approach. Differentially expressed proteins were quantified individually using paired t tests with associated log fold change of 0.3 and Fisher's combined p values.

Results The sample ($n=40$) was 62.8 ± 16.9 years old, 58% female, and 55% NYHA Class III/IV. Proteomic analysis revealed 7 proteins differentially expressed using full differential criteria: matrix metalloproteinase-14 was downregulated in frailty, and copine-1, low affinity immunoglobulin gamma Fc region receptor III-A and III-B, probable non-functional immunoglobulin kappa variable 2D-24, glutathione S-transferase Mu 1, and argininosuccinate lyase were upregulated in frailty.

Conclusions Proteomic biomarkers related to the immune system, stress response, and detoxification were differentially expressed between physically frail and non-physically frail adults with HF.

Keywords Heart Failure, Frailty, Proteomics, Biomarkers

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Introduction

Heart failure (HF) affects 6.7 million adults in the United States [1]. Approximately 1 out of every 2 adults with HF also experiences physical frailty [2], which is characterized as physiological decline across multiple systems, resulting in a phenotype of weight loss, weakness, slowness, physical exhaustion, and low physical activity [3]. Physical frailty is linked with worse clinical and patient-reported outcomes in HF [4, 5] and presents with notable sex differences [6, 7]. Despite the high prevalence and prognostic significance of physical frailty in HF, it is not known why it develops in some patients vs. others. It is hypothesized that a combination of HF-related (e.g. hemodynamic instability) and non-HF-related (e.g. aging, comorbidities) factors contribute to physical frailty in HF through common biological mechanisms such as inflammation, sarcopenia, and skeletal muscle dysfunction [8, 9]. These relationships, however, are still not well understood, posing a major barrier to the development and deployment of effective interventions. Thus, there is a need to comprehensively characterize the pathophysiology of physical frailty in HF to better understand mechanisms and antecedents.

Proteomics, the large-scale evaluation of proteins in biological samples, has evolved technologically and can be used to identify and quantify proteins in complex biological mixtures such as plasma [10]. Blood circulating throughout the body is in direct contact with all organs of the body and contains information about overall physiological milieu. Proteomics is appealing because it provides an unbiased proteome evaluation of these complex biological mixtures that, in turn, can be translated into the discovery of candidate biomarkers. For example, proteomics has been used to identify novel biomarkers in human plasma that are associated with conditions such as HF [11] and frailty [12]. With the advances in proteomics technologies and the need to further characterize the pathophysiology of physical frailty in HF, the purpose of this paper was to perform a pilot discovery-based proteomic analysis of plasma samples to identify plasma proteomic biomarkers that are differentially expressed between physically frail and non-physically frail adults with HF.

Methods

Study design and sample

We performed a secondary analysis of a subset of data and plasma samples from an observational study of frailty among patients with New York Heart Association (NYHA) Functional Classification I-IV HF, as described previously [7]. The sample was comprised of patients with chronic stable HF enrolled from outpatient HF and general cardiology clinics at a single center who were enrolled between May 2018 and February 2020. Inclusion criteria were age 21 years or older, ability to read and comprehend 5th grade English, and diagnosed with NYHA functional classification I-IV HF. Exclusion criteria were documented major cognitive impairment (e.g., Alzheimer's disease) or active psychosis that would preclude study participation, prior heart transplantation or durable mechanical circulatory support, major and uncorrected hearing dysfunction, or were otherwise unable to complete the requirements of the study (e.g., life-threatening illness).

After enrollment into the study, participants were scheduled for a study visit at our laboratory where they provided a fasting blood sample and were assessed on physical frailty. For this study, we selected a subset of patients from the larger sample based on level of physical frailty and propensity scores (described in Statistical Analysis). Both the parent study and this secondary analysis were approved by the Institutional Review Board. Written informed consent was obtained from all participants, and the data reported on in this study only include those who consented to storing data and samples in a biorepository for future research.

Measurement

Sociodemographic and clinical data

Data on sociodemographics were collected using a sociodemographic questionnaire. We performed a medical record review to collect data on HF history, etiology, NYHA functional class, clinical and laboratory data, and treatment of HF. The Charlson Comorbidity Index [13] was used to summarize comorbid conditions.

Physical frailty

Physical frailty was measured using the Frailty Phenotype Criteria [3] with adaptations for the HF population [14]. We used the following five criteria: 1) unintentional weight loss of >10 pounds over the last year by self-report, 2) weakness of the lower extremities using 5-repeat chair stands, 3) slowness with gait speed assessed over four meters, 4) physical exhaustion, and 5) reduced physical activity by asking how much time was spent exercising over the past week. After completing the measures for each of the five criteria, the scores were totaled (range zero to five). Each participant was then classified as either "non-physically frail" (i.e. zero to two criteria met) or "physically frail" (i.e. ≥ three criteria met).

Proteomic analysis

Whole blood was collected in EDTA tubes from participants after fasting for 8 h and abstaining from caffeine consumption and exercise prior to the blood draw. Blood samples were immediately placed on ice and transported

to the university Research Core Lab. Plasma was aliquoted and immediately stored at − 80 °C until processing. Plasma samples were processed at Pacific Northwest National Laboratory (Richland, WA), as previously described [15] and as detailed in Supplemental File 1.

Statistical analysis

We generated propensity scores to identify the matched pairs of physically frail vs. non-physically frail patients with HF. Propensity score analysis is a method used to reduce comparative bias between non-randomized samples [16] and is designed to balance observational data such that baseline covariates are similar between "treated/exposed" and "non-treated/non-exposed" groups. In this case the "exposure" factor was physical frailty: physically frail (met three to five of the Frailty Phenotype Criteria) or non-physically frail (met zero to two of the Frailty Phenotype Criteria). We estimated the propensity scores by first selecting covariates based on significant variables identified in prior work, including our own [7, 9, 14, 17]. Specifically, we identified the following covariates as potential significant contributors to physical frailty in HF: sex (male/female), age (< 65 years/≥ 65 years), HF etiology (ischemic/non-ischemic), HF classification (HF with preserved vs. reduced ejection fraction), prescribed an angiotensin converting enzyme inhibitor or angiotensin receptor blocker or not, Charlson Comorbidity Index category (low vs. medium vs. high comorbidity burden), type 2 diabetes (presence vs. absence), and body mass index (obese vs. non-obese). Given the size of the sample from which this study is based (n = 115), we were limited to using dichotomous variables rather than continuous variables. A comparison of the generated propensity scores for physically frail vs. non-frail is presented in Supplemental Fig. 1. Using these propensity scores, we performed nearest neighbor matching based on a caliper of 0.25*standard deviation of the propensity scores. This generated 25 matched pairs. Each of the pairs was evaluated for propensity score value (closer to zero was better) and matching (≥ five out of eight criteria matched). Two pairs were removed because four out of eight criteria were mismatched, and two pairs with propensity score differences ≥ 0.40 were removed. Thus, n = 21 matched pairs were used for proteomic analysis. Post-matching comparisons between treated (frail) vs. non-treated (non-frail) showed no significant differences.

Following the proteomic experiments, one sample did not generate usable data, so the pair was removed, yielding a final sample of n = 20 matched pairs. Identified proteins were compared between frail and non-frail using two approaches. First, all the proteins were normalized to the reference pool and median centered to total intensity, and then a paired t-test (because of the propensity matched samples) was used to compare the two groups. The paired t-test p-values and associated log fold changes for each of the identified proteins were then mapped to volcano plots. Additionally, each of the three plexes was analyzed separately where each protein was median centered to total intensity, and then a Fisher's combined p-value of the three p-values was generated. We adjusted for false discovery rates using the Benjamini–Hochberg method; however, the sample size was too small to identify any significant proteins. Thus, given our exploratory approach, we chose to only focus on proteins that were significant by the volcano plot and in differential expression across all three plexes. Stata v.17 (College Station, TX) and RStudio Version 4.0.3 [18] were used for analysis. Reactome Knowledgebase (http://reactome.org) was used to map proteins to physiologic pathways [19]. GraphPad Prism 10.2 (GraphPad Software, San Diego, CA) and RStudio Version 4.0.3 were used to prepare figures.

Results

The sample (n = 40) was 62.8 ± 16.9 years old, over half female, and 80% Non-Hispanic White. The majority were non-ischemic etiology HF (73%) and NYHA Class III/IV (55%). The full sample characteristics, overall and by level of physical frailty, are presented in Table 1. By design with propensity score matching, no characteristics were significantly different. Among the non-frail group (n = 20), five did not meet any frailty criteria, six met one frailty criterion, and nine met two frailty criteria (i.e. 15 were pre-frail). Among the frail group (n = 20), 14 met three frailty criteria and six met four frailty criteria. Distribution of frailty criteria met across both groups is presented in Fig. 1.

Proteins meeting full differential criteria

The proteomic analysis of plasma samples identified 2684 proteins; of these, 67 proteins were significantly different between physically frail (n = 20) and non-physically frail (n = 20) patients using paired t-tests (Supplemental Table 1). The log2 fold changes for each of the 2684 proteins were compared with the corresponding -log10 p-values in a volcano plot depicted in Fig. 2. From this level of constraint, 27 proteins were significantly different: 10 downregulated and 17 upregulated (Table 2). Of the downregulated proteins (i.e. downregulated with physical frailty), matrix metalloproteinase-14 (MMP14) was the only protein consistently downregulated across all three plexes. Of the upregulated proteins (i.e. upregulated with physical frailty), six were consistently upregulated across all three plexes: copine-1 (CPNE1), low affinity immunoglobulin gamma

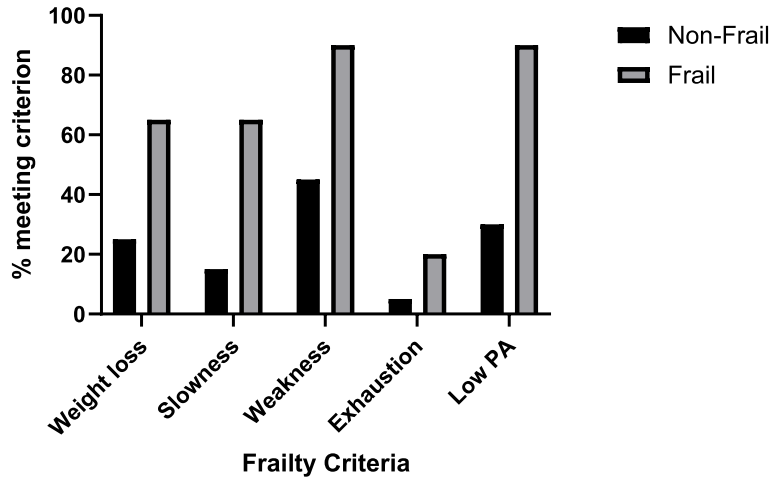

Fig. 1 Frailty criteria met by group: non-frail and frail. Percentage of patients meeting each of the five physical frailty criteria within each frailty group. *Abbreviations: PA, physical activity*

Table 1 Characteristics of the sample and by level of physical frailty

	Total ($n = 40$)	Non-Physically Frail ($n = 20$)[a]	Physically Frail ($n = 20$)	*p* value
Patient Characteristics				
Age (years)	62.8 ± 16.9	65.4 ± 14.7	60.3 ± 18.8	0.351
Female	23 (58%)	12 (60%)	11 (55%)	0.749
Non-Hispanic White	32 (80%)	16 (80%)	16 (80%)	1.00
Clinical Characteristics				
Body mass index (kg/m^2)	31.2 ± 6.6	31.1 ± 6.0	31.3 ± 7.4	0.933
Charlson Comorbidity Index (weighted)	2.6 ± 1.8	2.9 ± 2.0	2.4 ± 1.5	0.378
Stage 3 chronic kidney disease	10 (25%)	6 (30%)	4 (20%)	0.465
Type 2 diabetes	16 (40%)	8 (40%)	8 (40%)	1.00
Heart Failure Characteristics				
Time with heart failure (years)	2.9 [0.9–6.2]	3.9 [1.7–9.4]	2.7 [0.8–5.1]	0.285
New York Heart Association functional class III/IV	22 (55%)	11 (55%)	11 (55%)	1.00
Non-ischemic etiology	29 (73%)	14 (70%)	15 (75%)	0.723
Heart failure with reduced EF	28 (70%)	16 (80%)	12 (60%)	0.168
Left ventricular ejection fraction (%)	41.0 ± 16.6	39.1 ± 17.1	42.9 ± 16.3	0.476
Serum hemoglobin (g/dL)	12.8 ± 1.9	13.0 ± 1.7	12.6 ± 2.1	0.507
Serum BUN:Creatinine ratio	21.0 ± 8.1	22.3 ± 9.2	19.8 ± 6.7	0.333
Prescribed a β-blocker	34 (85%)	18 (90%)	16 (80%)	0.376
Prescribed an angiotensin-converting enzyme-inhibitor or angiotensin II receptor blocker	27 (68%)	16 (80%)	11 (55%)	0.091
SHFM one year projected survival (%)	95.5 [91–97]	96 [92–98]	93.5 [88.5–97]	0.321

Data are presented as mean±standard deviation, median [interquartile range], or N (%). Abbreviations: BUN Blood urea nitrogen, *EF* Ejection fraction, *SHFM* Seattle Heart Failure Model

[a] Non-physically frail includes both non-frail and pre-frail

Fc region receptor III-A (FCG3 A), low affinity immunoglobulin gamma Fc region receptor III-B (FCG3B), probable non-functional immunoglobulin kappa variable 2D-24 (KVD24), glutathione S-transferase Mu 1 (GSTM1), and argininosuccinate lyase (ARLY).

Proteins meeting partial differential criteria

There were also other proteins not identified in the volcano plot, but significantly different by paired t-test, Fisher's test, and consistently up- or down-regulated across all three plexes (Table 3). There were six

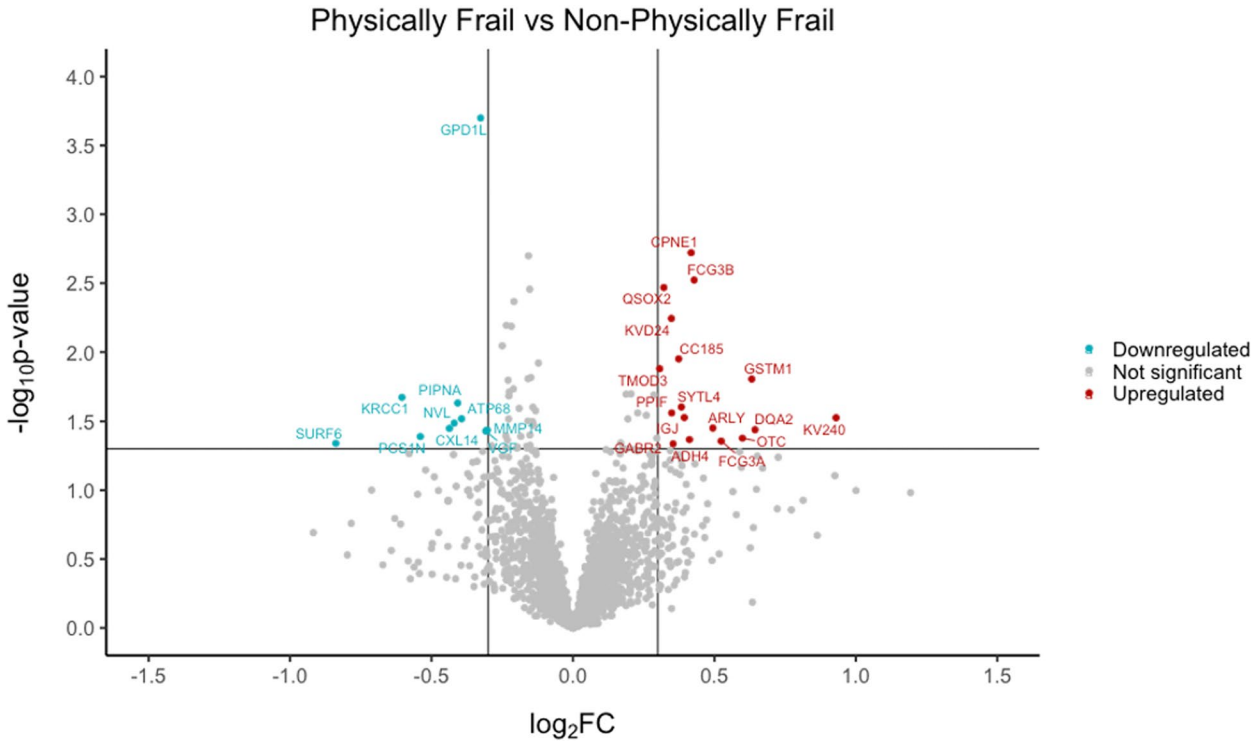

Fig. 2 Volcano plot of identified proteins comparing log2 fold change vs. -log10 p-value. Out of 2684 proteins, 27 proteins were identified as significantly differentially expressed by log2 fold change and -log10 p-value, 10 proteins down-regulated and 17 proteins up-regulated. *Abbreviations: FC, fold change*

additional down-regulated proteins beyond MMP14, which included: growth/differentiation factor 8, G patch domain-containing protein 1, dolichol-phosphate mannosyltransferase subunit 3, plasma protease C1 inhibitor, dynein regulatory complex protein 11, and insulin-like growth factor II. There were no other additional up-regulated proteins.

Proteins meeting a single differential criterion

In order to gain a better understanding of the pathways involved, there were 148 proteins significantly identified via Fisher's test, and of these, 120 proteins were mapped to pathways (Supplemental Table 2). Proteins within the immune system pathway were the most common differentially expressed markers in both up-regulated and down-regulated directions.

Discussion

In this propensity score matched discovery-based pilot study of physically frail vs. non-physically frail adults with HF, we found proteomic markers related to immune function, stress response, and detoxification were significantly differentially expressed. Specifically, proteomic analysis revealed seven proteins that were differentially expressed using full differential criteria: MMP14 was downregulated with physical frailty, and CPNE1, FCG3A, FCG3B, KVD24, GSTM1, and ARLY were upregulated with physical frailty. There were also other expressed proteins that support the pathways of immune function and cellular stress. Simply put, patients with HF who are physically frail are in a state of chronic immune dysfunction (with both upregulated and downregulated immune markers), stress response upregulation, and dysfunctional cellular activation. By identifying biomarkers associated with a clinical assessment of physical frailty, we can begin to ascertain potential biological mechanisms to target with interventions.

Proteins related to the immune system were the most prominent markers identified, indicating a potential role of immune dysfunction and inflammation, which is one of most commonly identified frailty pathways [9, 20, 21]. Immunosenescence refers to age-related alterations in the immune system and may be a possible mechanism underlying physical frailty in HF [9]. Immunosenescence results from increased proinflammatory mediators in the absence of an obvious trigger, possibly induced by damaged cells, failure of dysfunctional neutrophils and macrophages to properly remove cellular debris, and a growing number of senescent cells secreting proinflammatory cytokines [22]. For example, in our study,

Table 2 Proteins significantly differentially expressed on volcano plot (log_2 fold change vs. -log_{10} *p*-value)

Protein Abbreviation	Protein Name	Paired t test *p*-value	log2 fold change	Fisher's *p*-value	Detection patterns across plexes
Downregulated with Physical Frailty					
GPD1L	Glycerol-3-phosphate dehydrogenase 1-like protein	< 0.001	− 0.327	0.001	Detected in one plex
KRCC	Lysine-rich coiled-coil protein 1	0.021	− 0.605	0.020	Detected in one plex
PIPNA	Phosphatidylinositol transfer protein alpha isoform	0.023	− 0.408	0.015	Detected in one plex
ATP68	ATP synthase subunit ATP5MJ, mitochondrial	0.030	− 0.394	0.053	Detected in one plex
NVL	Nuclear valosin-containing protein-like	0.033	− 0.420	0.031	Detected in one plex
CXL14	C-X-C motif chemokine 14	0.036	− 0.437	0.050	Detected in two plexes, same direction
MMP14	**Matrix metalloproteinase-14**	**0.037**	**− 0.303**	**0.170**	**Detected in three plexes, same direction**
VGF	Neurosecretory protein VGF	0.037	− 0.309	0.089	Detected in two plexes, same direction
PCS1 N	ProSAAS	0.041	− 0.540	0.029	Detected in one plex
SURF6	Surfeit locus protein 6	0.046	− 0.838	0.040	Detected in one plex
Upregulated with Physical Frailty					
CPNE1	**Copine-1**	**0.002**	**0.418**	**0.025**	**Detected in three plexes, same direction**
FCG3B	**Low affinity immunoglobulin gamma Fc region receptor III-B**	**0.003**	**0.428**	**0.054**	**Detected in three plexes, same direction**
QSOX2	Sulfhydryl oxidase 2	0.003	0.321	0.005	Detected in three plexes; one plex had a different direction
KVD24	**Probable non-functional immunoglobulin kappa variable 2D- 24**	**0.006**	**0.348**	**0.048**	**Detected in three plexes, same direction**
CC185	Coiled-coil domain-containing protein 185	0.011	0.374	0.044	Detected in one plex
TMOD3	Tropomodulin-3	0.013	0.307	0.028	Detected in one plex
GSTM1	**Glutathione S-transferase Mu 1**	**0.016**	**0.632**	**0.003**	**Detected in three plexes, same direction**
SYTL4	Synaptotagmin-like protein 4	0.025	0.383	0.023	Detected in one plex
PP1 F	Peptidyl-prolyl cis–trans isomerase F, mitochondrial	0.028	0.349	0.014	Detected in one plex
IGJ	Immunoglobulin J chain	0.030	0.394	< 0.050	Detected in three plexes; one plex had a different direction
KV240	Immunoglobulin kappa variable 2–40	0.030	0.930	0.016	Detected in one plex
ARLY	**Argininosuccinate lyase**	**0.035**	**0.494**	**0.171**	**Detected in three plexes, same direction**
DQA2	HLA class II histocompatibility antigen, DQ alpha 2 chain	0.036	0.644	0.026	Detected in one plex
OTC	Ornithine transcarbamylase, mitochondrial	0.042	0.599	0.104	Detected in two plexes, same direction
ADH4	All-trans-retinol dehydrogenase [NAD(+)] ADH4	0.043	0.412	0.063	Detected in two plexes; opposite direction
FCG3 A	**Low affinity immunoglobulin gamma Fc region receptor III-A**	**0.044**	**0.524**	**0.048**	**Detected in three plexes, same direction**
GABR2	Gamma-aminobutyric acid type B receptor subunit 2	0.046	0.354	0.105	Detected in one plex

Bold indicates those proteins significant by the volcano plot and significantly up- or down-regulated across all three plexes

Table 3 Proteins consistently differentially expressed across all three plexes

Protein Abbreviation	Protein Name	Paired t test p-value	log2 fold change	Fisher's p-value
Downregulated				
GDF8	Growth/differentiation factor 8	0.004	− 0.209	0.017
GPTC1	G patch domain-containing protein 1	0.006	− 0.236	0.026
DPM3	Dolichol-phosphate mannosyltransferase subunit 3	0.007	− 0.218	0.016
IC1	Plasma protease C1 inhibitor	0.015	− 0.149	0.039
DRC11	Dynein regulatory complex protein 11	0.018	− 0.211	0.009
IGF2	Insulin-like growth factor II	0.030	− 0.228	0.045
MMP14	Matrix metalloproteinase- 14	0.037	− 0.303	0.170
Upregulated				
CPNE1	Copine- 1	0.002	0.418	0.025
FCG3B	Low affinity immunoglobulin gamma Fc region receptor III-B	0.003	0.428	0.054
KVD24	Probable non-functional immunoglobulin kappa variable 2D- 24	0.006	0.348	0.048
GSTM1	Glutathione S-transferase Mu 1	0.016	0.632	0.003
ARLY	Argininosuccinate lyase	0.035	0.494	0.171
FCG3 A	Low affinity immunoglobulin gamma Fc region receptor III-A	0.044	0.524	0.048

CPNE1 was upregulated with physical frailty. CPNE1 is a widely expressed membrane-bound protein that may be involved in the development and progression of cancer [23]. Recently, it has been proposed that CPNE1 could be involved in sarcopenia and myogenesis [24]. Moreover, despite depletion of the larger IgG components, the consistent upregulation of the immunoglobulin proteins (e.g. FCG3A, FCG3B, and KVD24) with physical frailty is notable.

Additionally, stress response and dysfunctional cellular activation and detoxification appear to be other contributing mechanisms. For example, GSTM1 was upregulated with physical frailty; GSTM1 is related to stress response, in addition to various other immune functions, and may play a role in cachexia [25]. There also appears to be a downregulation in cellular activation and detoxification. MMP14, which was consistently downregulated, is an endopeptidase that degrades components of the extracellular matrix (e.g. collagen). Matrix metalloproteinases play an important role in the progression of cardiovascular disease [26], and in turn may influence the development of physical frailty as well. There were other proteins that were downregulated across two plexes that also support a dysfunctional cellular activity and response. For example, C-X-C motif chemokine 14 and neurosecretory protein VGF, which are similar to MMP14, are involved in cellular activity and response. Downregulation of growth differentiation factor 8 and insulin-like growth factor 2 also seems to support the reduction in growth and activity related to physical frailty, similar to other studies [27, 28].

While there is no universal biomarker of frailty, previous studies using proteomics have identified potential biomarkers of frailty [12] such as vascular biomarkers [29], inflammatory glycoproteins [30], and markers of skeletal muscle derangements [31]. Liu et al. examined plasma proteins from two large cardiovascular studies in association with pre-frailty and frailty, and they found that higher levels of growth differentiation factor 15, transgelin, and insulin-like growth factor-binding protein 2 and lower levels of growth hormone receptor were associated with higher odds of prefrailty and frailty. [27] Given that inflammation, vascular dysfunction, and skeletal muscle dysfunction are common processes in HF, it is likely that these could provide the substrates for the development and/or exacerbation of physical frailty in HF. Indeed, we previously showed that markers of catabolism (i.e. insulin-like growth factor 1), adipose tissue dysfunction (i.e. adiponectin), and skeletal muscle dysfunction (i.e. myostatin) are linked with physical frailty in HF using a targeted protein approach [32].

With these common biological processes, there is considerable overlap in the presentation of HF and physical frailty, which makes it difficult to distinguish cause and effect and, in turn, appropriate management strategies. For example, HF has many overlapping symptoms with physical frailty (e.g. fatigue and dyspnea), and physical frailty in HF exacerbates the need for assistance with activities of daily living. Additionally, a major knowledge gap exists in determining the point at which physical frailty in HF becomes reversible or irreversible. Thus, there is a need to distinguish HF-related physical limitations, which perhaps could be reversed with ventricular

assist device implantation or transplantation, versus the point at which the body is in an end-stage catabolic state. Our findings provide a biological profile of physical frailty in HF, which might elucidate mechanisms that are responsive to HF management, both pharmacologically and non-pharmacologically. Additionally, given the overlap of physical frailty with other related concepts (e.g. cognitive dysfunction, nutritional deficiencies) [33], these biomarkers might also facilitate our biological understanding of these aspects. In sum, these exploratory results are initial steps towards identifying a biomarker or biomarker panel that can be used in clinical settings to 1) develop personalized frailty interventions based on the biological profile and 2) track biological response to interventions.

There are several limitations to this study. First, our sample was small, and it was difficult for the quantitative identifications to pass a multiple testing threshold, including adjusting for false discovery rate. However, the directionality of the results are fairly consistent and biologically relevant and serve as a starting point for future research. Second, while our sample was over half female, we were unable to identify sex-specific markers of physical frailty given the small sample and the focus on frail vs. non-frail. Third, given the small numbers of participants who did not meet any frailty criteria in the parent study, we had to combine non-frail with pre-frail, as previously described [7]. Thus, we were unable to examine the three categories of frailty by the original Frailty Phenotype Criteria: non-frail, pre-frail, and frail [3]. Finally, while we used propensity scores to create matched pairs of frail vs. non-frail, there is the possibility of other influential factors not measured in our study, including other markers and comorbidities (e.g. prealbumin, kidney dysfunction).

There is a significant need for future research based on our findings. First and most importantly, validation studies should be performed in independent, larger samples to confirm or refute the proteomic biomarkers identified in this discovery-based analysis. Sample sizes of $n > 200$ (assuming equal groups of frail vs. non-frail) would provide 80% power to detect moderate effect sizes while adjusting for multiple comparisons. Moving forward for larger scale validation, more targeted mass spectrometry approaches (e.g. selective reaction monitoring) can be used to address potential variability across discovery platforms and analysis timelines. Current technologies enable a wide range of targets, up to several hundred, to be included, so any potential protein target identified, such as those in the current study, can easily be incorporated and quantitatively standardized for larger scale validation. Second, since causal mechanisms cannot be inferred from this cross-sectional study, longitudinal studies are needed to track causal pathways, including determining if these identified proteins are causing frailty or are the effects of frailty. Finally, any future clinical applications will need to address standardization of proteomic analysis alongside cost effectiveness considerations, keeping implementation science principles in mind.

Conclusion

Proteomic biomarkers related to the immune system, stress response, and cellular detoxification and activation were differentially expressed between physically frail and non-physically frail adults with HF. Patients with HF who are frail are in a state of chronic immune and stress response upregulation coupled with downregulation of cellular activation. Future work is needed to better determine the biological fingerprint of physical frailty in HF, which will likely help drive management strategies.

Abbreviations

ARLY	Argininosuccinate lyase
CPNE1	Copine-1
GSTM1	Glutathione S-transferase Mu 1
FCG3 A	Low affinity immunoglobulin gamma Fc region receptor III-A
FCG3B	Low affinity immunoglobulin gamma Fc region receptor III-B
HF	Heart failure
KVD24	Probable non-functional immunoglobulin kappa variable 2D-24
MMP14	Matrix metalloproteinase-14
NYHA	New York Heart Association

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12872-025-04725-5.

Supplementary Material 1. Proteomic Analysis Methods.

Supplementary Material 2. Histograms of estimated propensity scores by frailty group: non-frial and frail.

Supplementary Material 3.

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None.

Authors' contributions

Q.E.D. conceived and designed the study, collected the data and plasma samples, led the statistical analysis, and led the development of the manuscript. N.V.P. and M.R.D. performed statistical analyses and prepared figures. C.S.L. provided expert consultation on the statistical analyses and substantive feedback on the interpretation of findings. J.M.J., S.M.P., and M.G. performed the proteomic experiments, prepared the data for analyses, performed statistical analyses, and provided substantive feedback on the interpretation of the findings. S.M.J. provided expert review on the content and clinical interpretation of the findings. B.A.H. provided senior guidance on all aspects of the project from conception to interpretation. All authors read and approved the final manuscript.

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Data availability
Partial data are provided within the supplementary information files, and the full dataset is available upon reasonable request to the corresponding author.

Declarations

Ethics approval and consent to participate
The Oregon Health & Science University Institutional Review Board approved this study (IRB #17785 and #21950). All participants gave written informed consent to participate in the study.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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