THE BIDIRECTIONAL ASSOCIATION BETWEEN FRAILTY AND CARDIAC DYSFUNC

THE BIDIRECTIONAL ASSOCIATION BETWEEN FRAILTY AND CARDIAC DYSFUNCTION

By
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I have reviewed this thesis. It represents work done by the author under my supervision.
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OVERVIEW

Frailty and Heart Failure (HF) are both highly prevalent in older adults, and frequently coexist. Frailty is a highly morbid, albeit reversible[1] condition characterized by a loss of functional capacity, and an increased vulnerability to internal and external stressors.[2] Individuals who are frail are at increased risk of adverse outcomes including fractures, cardiovascular morbidity, and death.[3] Although there are many scoring criteria designed to measure frailty, the one published by Fried et al. in 2001 is most frequently used in epidemiology studies and incorporates data on (1) Low energy expenditure; (2) Low walking speed; (3) Lack of energy (exhaustion); (4) Low grip strength; and (5) Unintentional weight loss.[2] Frail individuals have higher prevalence of both HF and subclinical decline in cardiac structure and function, are associated with an increased risk of incident HF.[4,5] Inflammation has been hypothesized as a common pathobiology linking frailty and HF. However, mechanisms potentially linking frailty and HF, particularly in late life, are incompletely understood. Two genome-wide association studies (GWASs) have identified several genes that may be associated with frailty,[6] but these do not overlap with genes identified in HF GWAS. As the products of gene expression, proteomics hold the potential to identify possible shared biomarkers of risk and molecular pathways common the frailty and HF.

Our work builds upon prior clinical evidence of the relationship between cardiac dysfunction and frailty and aims to further characterize the association between cardiac structure and function, which underlies HF, and the frailty phenotype. We achieve this by using longitudinal data, and high-throughput proteomics to disentangle the components and better understand the pathobiology that link frailty and cardiac dysfunction.

MANUSCRIPT 1

Bidirectional Association Between Frailty and Structural and Functional Echocardiographic Measurements: The Atherosclerosis Risk in Communities Study

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Abstract

Background: Frailty is associated with greater risk of developing heart failure (HF) in late-life, and prevalent HF is associated with a high prevalence of frailty. However, little is known regarding the temporal and bidirectional relationship between subclinical alterations in cardiac structure and function and the development of frailty.

Methods: This analysis included 2,574 participants in the community-based Atherosclerosis Risk in Communities (ARIC) study who attended study Visit 5 (2011-2013), 6 (2016-2017), and/or 7 (2018-2019), and were free of HF at visit 5. Frailty was assessed using the Fried frailty phenotype at each study visit, and protocol echocardiography was performed at Visits 5 and 7. The associations of frailty at visit 5, and longitudinal changes in frailty status from visits 5 to 7, with longitudinal changes in cardiac structure and function from visits 5 to 7 were assessed using multivariable linear regression. Among 1,648 HF-free robust participants at Visit 5, we further assessed the association of measures of cardiac structure and function at visit 5 with progression in frailty status using multivariable ordinal logistic regression. Models were adjusted for demographics and comorbidities.

Results: Among 2,574 HF-free participants with frailty and echocardiographic assessments at Visits 5 and 7 (mean age 74 ± 4 years at Visit 5 and 81 ± 4 years at Visit 7; 57% women; 22% self-reported Black race), 3% (n=83) were frail. Frailty at Visit 5 was significantly associated with greater left atrial volume index (LAVi) and E/e' ratio at both Visits 5 and 7, but not with greater changes in cardiac structure and function between visits. Participants who transitioned from robust at visit 5 to frail at visit 7 demonstrated greater increases in left ventricular (LV)

mass index, LAVi, and E/e' over the same period. Among 1,648 robust participants at Visit 5, 49 developed frailty at Visit 6. Greater LVMi and MWT, lower TDI e', and higher E/e' ratio were associated with a higher odds of progression in frailty status.

Conclusion: Among robust, HF-free, older adults in the community, a bidirectional relationship exists between the development of frailty and subclinical LV remodeling and diastolic dysfunction. As both frailty status and cardiac structure and function are modifiable, future studies should evaluate whether interventions to modify one may exert beneficial effects on the other.

Introduction

Increasing life expectancy is expected to result in greater numbers of older individuals and an increase in the attendant challenges of late-life.[7] Older individuals are at heightened risk of frailty, a geriatric clinical syndrome characterized by loss of homeostatic reserves, and increased vulnerability to internal and external stressors.[2] Frailty is a reversible[1], albeit burdensome condition, affecting approximately 15% of individuals 65 years or older.[8] Older individuals are also at a heightened risk for heart failure (HF), and ageing – independent of cardiovascular risk factors [9,10] is associated with alterations in cardiac structure and function which underlie the development of HF.[11–13] Frailty is common in patients with prevalent HF[14,15], among whom it associates with higher risk of adverse outcomes.[16] In addition, frail individuals are at higher risk of cardiovascular morbidity and mortality, including incident HF[5], compared to robust individuals.[17,18] Cross-sectional studies demonstrate subclinical alterations in cardiac structure and function in frail individuals.[19] In addition to being common in late life, both frailty and cardiac dysfunction may share systemic inflammation as a common pathobiology.[20–22] Therefore, one condition can theoretically increase the risk of developing the other in later life. However, the temporal relationship between alterations in cardiac function and the development of frailty is unclear.

The objective of this study is to investigate the bidirectional associations between frailty and cardiac dysfunction among older adults in the community. Leveraging longitudinal cardiac and echocardiographic phenotyping in the community-based Atherosclerosis Risk in Communities (ARIC) Study, we first evaluated the association of frailty – and progression in frailty status – with subsequent changes in cardiac structure and function over 6 years in late life.

We then assessed the extent to which measures of cardiac structure and function at a single timepoint independently predict the progression to frailty over 4 years in late life.

Methods

Study Population

The ARIC study design and procedures have been previously described.[23,24] ARIC is an ongoing, prospective observational cohort study that originally enrolled 15,792 participants aged 45–64 years recruited between 1987 and 1989 (Visit 1) from four communities in the United States: Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland. Three subsequent study visits occurred between 1989 and 1998, followed by Visit 5 (2011-2013), Visit 6 (2016-2017), and Visit 7 (2018-2019). Standardized frailty assessment and echocardiography were first performed in ARIC at study visit 5 (2011–2013). Frailty assessment was repeated at Visit 6 and Visit 7, and echocardiography was repeated at Visit 7. Among the 10,040 surviving participants by the end of Visit 5, 6,538 participants attended that visit. We excluded 398 participants with prevalent HF at Visit 5, and 623 additional participants with a missing frailty assessment. A total of 5,517 participants were included in the study before analysis-specific inclusion criteria were applied. To evaluate the association of frailty – and progression in frailty status – with changes in cardiac structure and function over 6 years in late life, participants with concomitant echocardiographic and frailty assessment at both Visits 5 and 7 were included (n= 2,574; Visit 6 frailty data was not included in this analysis). To assess the association of measures of cardiac structure and function at a single timepoint with risk of incident frailty over 4 years, participants who were robust at Visit 5, underwent echocardiography at Visit 5, and had frailty assessment at Visit 6 were included (n= 1,648; Visit

7 frailty and echocardiographic data was not included in this analysis to minimize participant attrition). The complete consort diagram of the study sample is displayed in Figure 1.

Frailty Assessment

Frailty was defined using the Fried criteria, a previously validated measure [25] implemented in several other cohort studies,[2,19,26] which consists of five binary criteria: (1) Low energy expenditure, based on gender-specific 20th percentile rank of the Baecke leisure sports activity index; (2) Low walking speed, based on the bottom gender and height-specific quintile of time to walk 15 feet; (3) Lack of energy (exhaustion), defined as present if the participant responded "some of the time" or "most of the time" to either of the following questions: "I felt everything I did was an effort" or "I could not get going"; (4) Low grip strength, based on the bottom gender- and body mass index-specific grip strength quintile; (5) Unintentional weight loss, defined as 10% unintentional weight loss between Visits 4 (1996– 1998) and 5 or body mass index <18.5 kg/m2 at Visit 5. Frailty was defined by the presence of at least three of these criteria, participants with 1 or 2 criteria were classified as pre-frail, and participants with 0 criteria were classified as robust.

Echocardiography

Detailed protocols and procedures for echocardiography at ARIC Visit 5, including reproducibility metrics, have been previously described in detail, and equivalent procedures were employed at study Visit 7.[27] Briefly, all studies were performed by trained study sonographers certified in performance of the study-specific imaging protocol using uniform echocardiography equipment. Quantitative analysis at both visits was performed by the same central

echocardiography reading center in accordance with American Society of Echocardiography guidelines [28,29] by analysts blinded to clinical information.

Assessment of Covariates

Coronary heart disease (CHD) was defined as having history of prior adjudicated myocardial infarction (MI) or coronary intervention,[30] or a regional wall motion abnormality on echocardiography at Visit 5. Hypertension was based on self-report, antihypertensive medication, or blood pressure ≥140/90 mmHg at study Visit. Diabetes mellitus was defined based on self-report, fasting glucose ≥126 mg/dL, or any glucose measurement ≥200 mg/ dL. Prevalent HF was defined as hospitalization with an International Classification of Diseases, Ninth Revision (ICD-9) code of 428 before 2005,[31] with additional physician adjudication since 2005 as previously described.[32] AF diagnosis was based on ECG at visits 1 through 5, and hospital discharge records as previously reported.[33]

Statistical Analysis

Continuous variables are expressed as mean ± standard deviation for normally distributed data or median (25th, 75th percentiles) for non-normally distributed data. Categorical variables are expressed as frequency and proportions. Clinical and demographic differences between non-frail, pre-frail and frail groups were evaluated by t-test and chi-square test.

To assess the association of prevalent frailty at Visit 5 with changes in cardiac structure and function from Visits 5 to 7, change in each echocardiographic measure was calculated as the Visit 7 measure minus the Visit 5 measure. Clinical characteristics and echocardiographic measures were described by frailty category at Visit 5. Echocardiographic data for each variable

was normally or near-normally distributed. Analyses were performed using multivariable linear regression models adjusted for age, sex, race, field center, blood pressure (BP) and heart rate (HR) at both echo visits, hypertension, diabetes, BMI, eGFR, prevalent CHD, history of MI and prevalent stroke assessed at Visit 5. Adjustment variables were chosen based on their relationship with the exposure and the outcome, and their statistical significance difference across frailty categories. Additional covariates possibly linked to frailty and cardiac dysfunction like CKD, lipid profile, and medication use were not included as covariates as they may be highly colinear with other covariates in the model. Values from Visit 5, Visit 7 and change values were compared among Visit 5 frailty categories using multivariable linear regression. We then assessed the association of changes in frailty status between Visit 5 and Visit 7 with concomitant changes in echocardiographic measures. Participants were classified as: robust at Visit 5 to robust at Visit 7, robust to pre-frail, robust to frail. Comparisons between groups stratified by Visit 5 frailty status were performed using multivariable linear regression, with stable frailty status as the reference group.

To assess the association of alterations in cardiac structure and function at Visit 5 with the progression in frailty status between Visits 5 and 6 among participants who were robust at Visit 5, we employed multivariable ordinal logistic regression models to assess the association between a 1-standard deviation increment in each Visit 5 echocardiographic measurement and the odds of progressing to pre-frailty and frailty at V6 as an ordinal, three-level outcome (robust, pre-frail, and frail). Two models were generated, the first adjusted demographic covariates (age, sex, race, and field center). The second additionally adjusted for blood pressure (BP) and heart rate (HR), hypertension, diabetes, BMI, eGFR, prevalent CHD, history of MI and prevalent

stroke. Sensitivity analysis was performed to assess the associations of echocardiographic measures with incident frailty or pre-frailty using separate logistic regression models.

A two-sided p-value <0.05 was considered significant. Statistical analysis was performed using Stata software Version IC-16.1 (Stata Corp LP, College Station, TX).

Results

Frailty status and longitudinal changes in cardiac structure and function

Among the 2,574 participants with echocardiography and frailty assessments at Visits 5 and 7, and free of HF at Visit 5, mean age was 74.5 ± 4.4 at Visit 5 and 80.5 ± 4.4 at Visit 7, 58% were female, and 22% reported Black race (Table 1). At Visit 5, 1,393 (54%) were robust, 1,098 (43%) were pre-frail, and 83 (3%) were frail. Frailty at Visit 5 was associated with older age, female gender, and higher prevalence of cardiovascular co-morbidities (Table 1), and with greater LV mass index (LVMi), higher E/e', and larger LV end diastolic dimension (LVEDD), and LA volume index (LAVi) cross-sectionally at Visit 5 (Table 2). Frailty at Visit 5 was also associated with greater LVMi, mean wall thickness (MWT), higher E/e', and larger LAVi at Visit 7. Worse frailty status at Visit 5 associated with greater increase in E/A ratio between Visits 5 and 7, but not with changes in other echocardiographic measures (Table 2). Participants who were excluded because of a missing frailty assessment at Visit 5 or Visit 7 (n=3,351) had higher comorbidities (Supplementary Table 1)

Of 1,393 participants who were robust at Visit 5, 683 (49%) and 43 (3%) progressed to pre-frailty and frailty at Visit 7, respectively. Participants who transitioned from robust to frail demonstrated greater increases in LV mass index, end-diastolic dimension, and wall thickness, and greater increases in measures of LV filling pressure (LAVi, E/e' ratio) compared to those

who remained robust (Figure 2). No differences were observed in change in LVEF. Participants who transition from robust to pre-frail demonstrated modestly greater increases in MWT and LAVi compared to those who remain robust. Supplementary Table 2 and 3 additionally show percent change between visits and comparisons between groups for robust at baseline, and pre-frail at baseline, respectively.

Cardiac structure and function and incident frailty

Among 2,549 HF-free participants who were robust and had an echocardiographic assessment at visit 5, 213 died between visit 5 and visit 6 and frailty at visit 6 was not assessed in 688 participants. Among the 1,648 participants who were included in this analysis, 754 (45%) developed pre-frailty at Visit 6 and 49 (3%) developed frailty. Baseline characteristics of this population at visit 5 and comparisons by their frailty status at visit 6 are shown in Supplementary Table 4. In models adjusted for demographics and clinical co-morbidities, greater LVMi, lower LVEF and TDI e', and higher LAVi and E/e' ratio were associated with a higher odds of progressing in frailty status (Figure 3). These associations appeared driven by associations with incident frailty, with smaller magnitudes of effect for associations with incident pre-frailty (Supplementary Figure 1).

Discussion

Ageing is an important risk factor for frailty, HF, and alterations in cardiac structure and function underlying HF. By leveraging longitudinal cardiovascular and frailty phenotyping in a community-based cohort of older adults, we describe the following novel findings on the intersection of frailty and cardiac function among robust older-adults. First, the presence of greater LV mass index, worse diastolic function, and higher E/e' ratio is associated with a greater

likelihood of progressing to a worse frailty status over a ~5-year follow-up. Second, over ~6-year follow-up, frailty was associated with greater LV mass index and measures of LV filling pressure (E/e' ratio, LAVi) at baseline and follow-up, and the transition from robust to frail during this timeframe was characterized by greater increases in LV size, wall thickness, and mass index and by greater increases in measures of LV filling pressure including LAVi, and E/e' ratio. Transition from robust to prefrail was associated with greater increases in wall thickness and LAVi, but of lesser magnitude. Finally, these associations persisted in models accounting for traditional cardiovascular risk factors. Together, these findings suggest bidirectional associations between frailty and subclinical LV remodeling and diastolic dysfunction among older adults. Importantly, frailty status and cardiac structure and function are modifiable. Our findings raise the hypothesis that interventions to modify one may exert beneficial effects on the other.

The inter-relationship between frailty and HF is well described.[34,35] Among patients with HF, frailty is common and is associated with higher risk of adverse outcomes compared to those without frailty. [16,36,37] Moreover, prevalent HF is associated with a heightened risk of incident frailty in later life,[38] as are subclinical measures of arteriosclerosis based on carotid intima-media thickness.[39] Conversely, among older adults free of HF, prevalent frailty is associated with greater risk of incident HF, independent of traditional cardiovascular risk factors.[4,5,40] The mechanisms underlying these associations are unclear. Both frailty and HF are systemic disorders, and impairments in several organ functions are likely shared. Alterations in cardiac structure and function underlie the development of HF and are robust risk factors for HF development. In this context, among older adults free of HF, frailty is also cross-sectionally associated with greater LV remodeling and dysfunction – specifically greater LV hypertrophy, diastolic dysfunction, and subtle impairments in LV deformation by strain imaging.[19,41–43]

However, these findings are limited by potential reverse-causation, and there are few data to-date regarding the temporal sequence between development of the frailty phenotype and of alterations in cardiac structure and function.

Recent data from the Medical Research Council British National Survey of Health and Development indicate an association between severity of frailty ascertained by the frailty index in mid-life and greater LV mass index and lower LVEF in later-life.[44] Our findings now demonstrate that greater increases in LV mass index and metrics of LV filling pressure accompany transitions from robust to frail, and suggest a potential mechanism underlying the association of frailty with HF incidence. That these associations persisted after accounting for traditional cardiovascular risk factors suggest that this association may not be simply the result of shared risk factors for frailty and LV diastolic dysfunction in late life. Notably, among robust older adults, we also found that greater LV mass index and worse diastolic measures were associated with higher likelihood of developing frailty, consistent with at least one prior study suggesting an association of subclinical cardiovascular disease in the form of carotid atherosclerosis with the development of frailty.[39] These findings extend on existing cross-sectional studies to suggest bidirectional associations between LV diastolic dysfunction and frailty in late-life, whereby the presence of one promotes development of the other.

The mechanisms linking frailty and cardiac alterations are unclear. Both share common risk factors,[15,45] and residual confounding may account for the adjusted associations in our analysis. Alternatively, frailty and HF have also been associated with systemic inflammation,[21] and co-morbidity driven inflammation may be an important common pathobiology.[20,22,46] Given the systemic, multi-organ nature of both frailty and HF, future studies leveraging high throughput –omics data (proteomics, metabolomics) hold promise in more deeply interrogating

the potential role of inflammation and in discovering novel underlying molecular pathways. Importantly, frailty is modifiable[3] and potentially reversible.[1] Transitions from frailty to pre-frailty or robust are well described in observational studies,[47] and may be promoted by interventions mainly related to physical exercise.[48,49] Notably, exercise interventions appear efficacious for relevant clinical outcomes in HF, and may promote improvements in LV function in HFrEF.[50] Whether interventions aimed at improving frail individuals to non-frail status will also prove effective at preventing the development of HF – and the underlying alterations in cardiac structure and function – is unknown.

This study has several limitations. This is an analysis of an observational cohort of community-dwelling participants, making our analyses subject to residual confounding. Therefore we can only identify associations but cannot draw conclusions regarding causality. Attrition between V5 and V7 due to death or non-attendance was differential with respect to frailty categories, which limited the statistical power. In addition, this likely biased our results toward the null as participants more likely to develop frailty were less likely to have follow-up data. Survivor bias from attrition may underestimate our findings, as sicker participants could not be included in the analysis. Thus, this limitation makes our findings robust in that despite likely underestimated results, the observed effect of frailty on cardiac structure and function, and vice versa, persists. Frailty status was determined using one particular metric [2], and whether these results would be consistent using other metrics of frailty remains unknown. Nonetheless, the Fried frailty phenotype has been previously operationalized and validated in our cohort [25], and is commonly employed in other community-based cohorts.[19,26,51] The generalizability of our study is limited in that the population is from a single cohort. However, this cohort is considerably heterogeneous and from four different enrollment centers. The relative small number of

participants who demonstrated regression in frailty category between Visit 5 and Visit 7 (i.e. from frail to pre-frail [n=54] or to robust [n=6]) limited our ability to assess for changes in cardiac structure and function associated with improvements in frailty status.

Conclusions

Among robust, HF-free, older adults in the community, a bidirectional relationship exists between the development of frailty and subclinical LV remodeling and diastolic dysfunction. As both frailty status and cardiac structure and function are modifiable, future studies should evaluate whether interventions to modify one may exert beneficial effects on the other.

Tables and figures

Table 1: Baseline characteristics of the study population for changes in echo analysis at Visit 5 by frailty category, The Atherosclerosis Risk in Communities Study Cohort (2011-2013, N=2,574).

	Robust n=1393	**Pre-frail n=1098**	**Frail n=83**	**P for trend**
Age, mean ± SD, years	73.4 ± 4.1	74.7 ± 4.6	75.3 ± 4.8	< 0.001
Male, n (%)	638 (45.8%)	430 (39.2%)	25 (30.1%)	< 0.001
Black, n (%)	273 (19.6%)	271 (24.7%)	27 (32.5%)	< 0.001
Hypertension, n (%)	917 (66.1%)	803 (73.7%)	67 (80.7%)	< 0.001
Diabetes, n (%)	307 (22.0%)	353 (32.1%)	35 (42.2%)	< 0.001
BMI, mean ± SD, kg/m^2	28.3 ± 4.8	29.2 ± 5.5	31.5 ± 7.2	< 0.001
eGFR, mean ± SD, $ml/min/1.73m^2$	73.6 ± 14.7	72.4 ± 16.3	70.0 ± 17.0	0.014
CHD, n (%)	123 (9.0 %)	130 (12.1%)	10 (12.0%)	0.014
MI, n (%)	97 (7.3 %)	105 (10.0%)	9 (11.2%)	0.015
Stroke, n (%)	23 (1.7 %)	34 (3.1 %)	3 (3.6 %)	0.014

P values assess for trend for each variable across all categories. Data is shown as frequency and proportion or mean ± standard deviation.

Abbreviations: BMI= body mass index. eGFR= estimated glomerular filtration rate by CKD-EPI. CHD= coronary heart disease. MI= myocardial infarction.

Table 2: Echo measurements at Visit 5 and Visit 7 and their change, by frailty category at Visit 5.

		Visit 5 Frailty Category			P for trend	
		Robust	Pre-frail	Frail	Model 1	Model 2
LVMI (g/m^2)	n=	1366	1054	78		
	Visit 5	76.43 ± 0.47	77.26 ± 0.54	82.90 ± 1.97	0.014	0.003
	Visit 7	80.89 ± 0.53	81.90 ± 0.61	89.47 ± 2.22	0.005	0.002
	Delta	4.46 ± 0.42	4.64 ± 0.48	6.58 ± 1.74	0.42	0.55
LVEDD (cm)	n=	1375	1065	81		
	Visit 5	4.39 ± 0.01	4.40 ± 0.01	4.49 ± 0.05	0.12	0.029
	Visit 7	4.33 ± 0.01	4.31 ± 0.01	4.45 ± 0.05	0.93	0.52
	Delta	-0.05 ± 0.01	-0.09 ± 0.01	-0.04 ± 0.04	0.07	0.06
MWT (cm)	n=	1380	1077	82		
	Visit 5	0.97 ± 0.00	0.98 ± 0.00	1.00 ± 0.01	0.06	0.06
	Visit 7	1.02 ± 0.00	1.03 ± 0.00	1.06 ± 0.02	0.002	0.003
	Delta	0.05 ± 0.00	0.05 ± 0.00	0.06 ± 0.01	0.07	0.11
LVEF (%)	n=	1313	1006	77		
	Visit 5	65.75 ± 0.16	65.58 ± 0.18	65.07 ± 0.64	0.28	0.43
	Visit 7	63.31 ± 0.19	63.39 ± 0.22	61.85 ± 0.78	0.51	0.75
	Delta	-2.44 ± 0.18	-2.19 ± 0.20	-3.22 ± 0.74	0.82	0.73
LAVI (ml/m^2)	n=	1351	1043	78		
	Visit 5	24.85 ± 0.20	25.05 ± 0.23	27.30 ± 0.83	0.06	0.006
	Visit 7	27.44 ± 0.25	27.96 ± 0.28	30.70 ± 1.02	0.009	0.002
	Delta	2.58 ± 0.19	2.91 ± 0.21	3.39 ± 0.78	0.17	0.25
e' lateral (cm/s)	n=	1374	1079	82		
	Visit 5	7.26 ± 0.05	7.18 ± 0.06	7.10 ± 0.22	0.24	0.36
	Visit 7	6.63 ± 0.05	6.63 ± 0.06	7.01 ± 0.21	0.4	0.38
	Delta	-0.64 ± 0.06	-0.55 ± 0.06	-0.10 ± 0.23	0.06	0.09
E/e' lateral (ratio)	n=	1366	1006	78		
	Visit 5	9.57 ± 0.09	9.97 ± 0.10	10.17 ± 0.36	0.002	0.003
	Visit 7	11.94 ± 0.13	12.47 ± 0.14	11.99 ± 0.51	0.026	0.031
	Delta	2.37 ± 0.11	2.51 ± 0.12	1.83 ± 0.43	0.94	0.95

E/A (ratio)	n=	1297	999	76		
	Visit 5	0.87 ± 0.01	0.86 ± 0.01	0.84 ± 0.03	0.36	0.98
	Visit 7	0.85 ± 0.01	0.87 ± 0.01	0.87 ± 0.03	0.19	0.05
	Delta	-0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.03	0.031	0.04

P-values provide significance for trend across frailty categories. Data is shown as mean ± standard error. Model 1 adjusts for demographics (age, sex, race, and field center); Model 2 adjust for demographics and heart rate and blood pressure at Visit 5 and Visit 7.

Abbreviations: LVMi= left ventricular mass index, MWT= mean wall thickness, LVEDD= left ventricular end diastolic dimension, LVEF= left ventricular ejection fraction, LAVi= left atrial volume index.

Figure 1: Consort diagram summarizing the study population of each analysis. Red arrows indicate that the subset of participants was excluded from analysis.

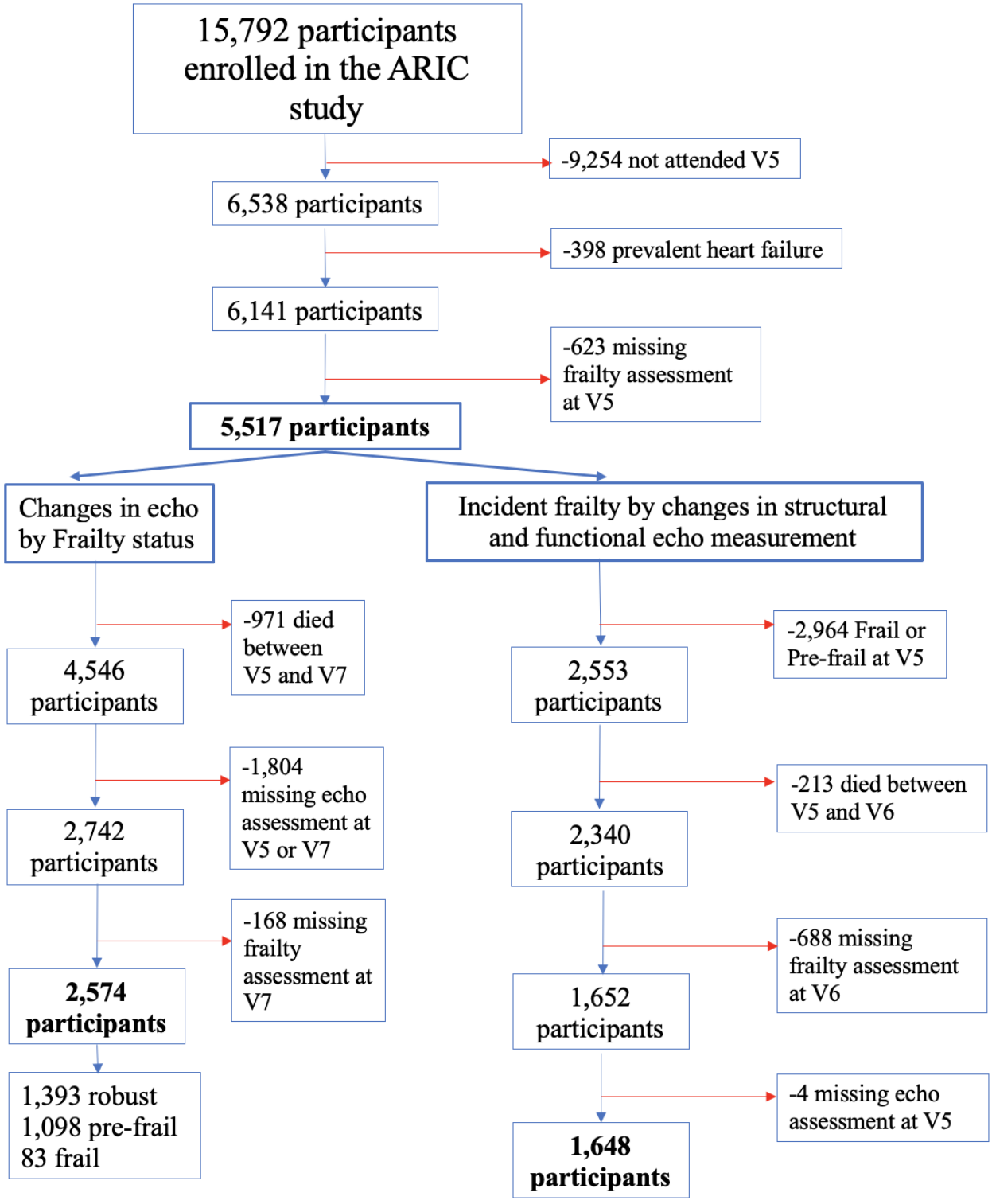


Figure 2: Associations of transitions in frailty status from Visit 5 to Visit 7 with concomitant changes in echocardiographic measures. Plot demonstrates model beta coefficients and 95% CI for echocardiographic measurements by changes in frailty status. Model 1 adjusts for demographics (age, sex, race, and field center). Model 2 adjusts for demographics, and HR and BP at study visits. Model 3 adjusts for demographics, HR and BP at study visits, and comorbidities (hypertension, diabetes, BMI, eGFR, prevalent CHD, prevalent stroke) at V5. No change in frailty status between visits is taken as the reference value for each comparison (X line at 0). *Indicate significant at p <0.05.

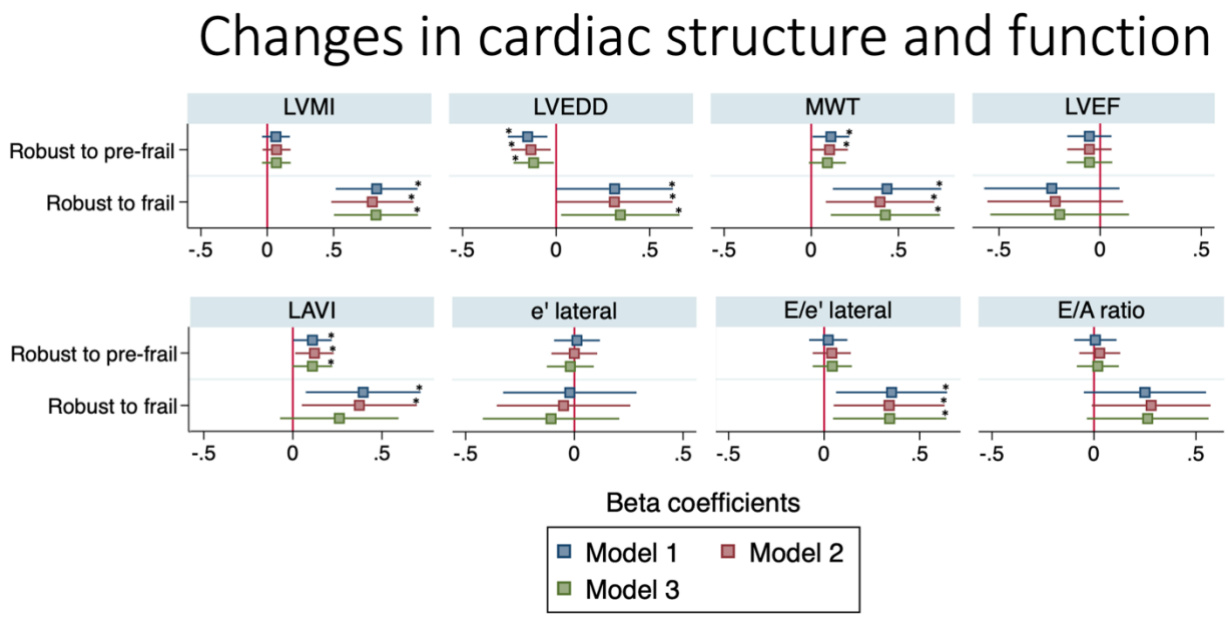
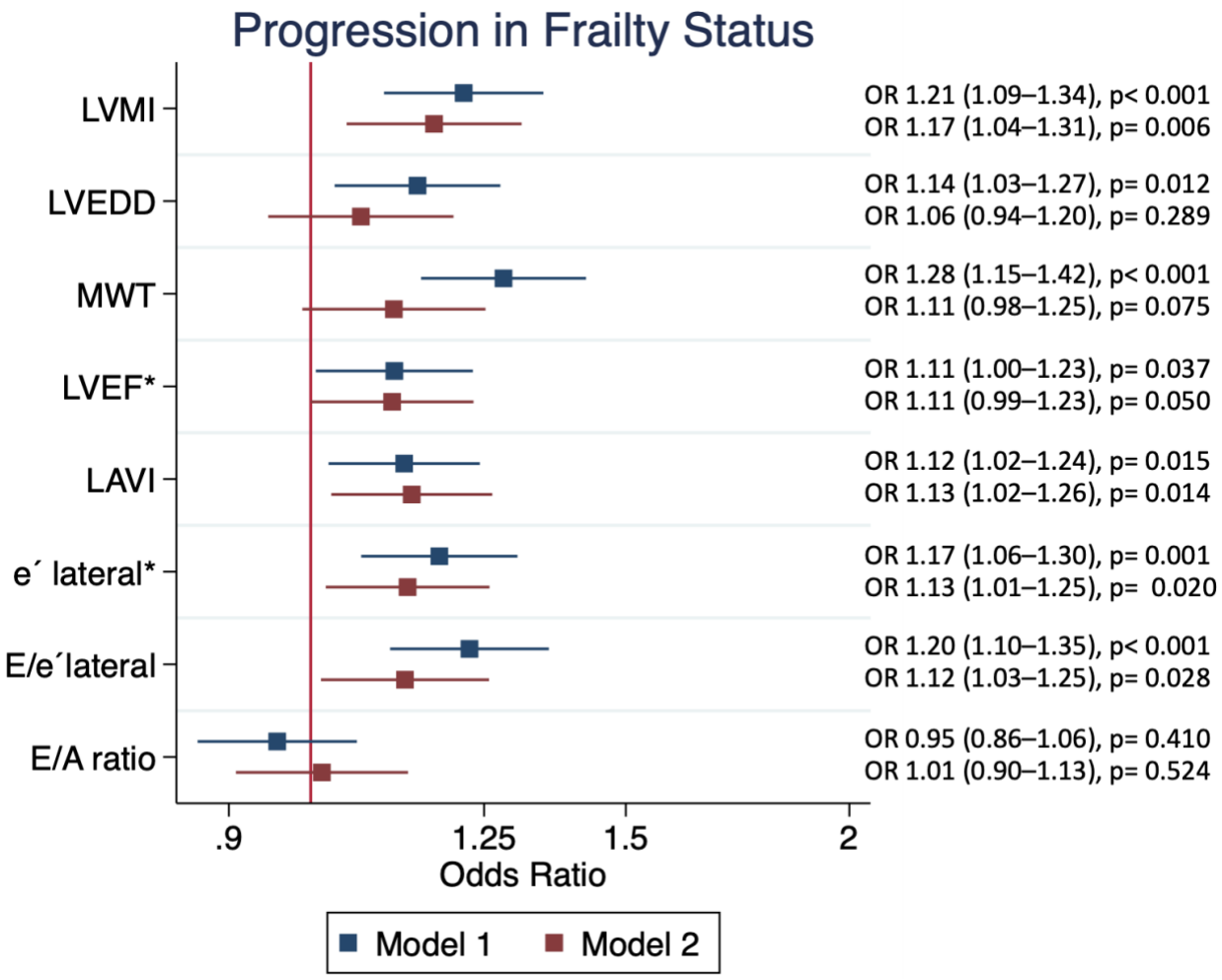


Figure 3: Association of measures of cardiac structure and function at Visit 5 with the progression in frailty status at Visit 6. Model 1 adjusts for demographics (age, sex, race, and field center). Model 2 adjusts for demographics, HR and BP at visits 5 and 7, and comorbidities (hypertension, diabetes, BMI, eGFR, prevalent CHD, prevalent stroke) at Visit 5. *Indicates odds associated with lower values.



Supplement

Supplementary Table 1. Comparison of baseline characteristics at Visit 5 of participants who had and who did not have frailty assessments at Visit 5 and Visit 7. (2011-2013, N= 6,140).

Variable	Frailty assessment at Visit 5 and Visit 7 n=2789	No frailty assessment at Visit 5 or Visit 7 n=3351	P-value
Age	74.1 ± 4.5	77.0 ± 5.4	<0.001
Male	1174 (42.1%)	1323 (39.5%)	0.038
Black	616 (22.1%)	800 (23.9%)	0.100
Hypertension	1954 (70.4%)	2534 (77.2%)	<0.001
Diabetes	769 (27.6%)	1166 (34.8%)	<0.001
BMI	28.8 ± 5.4	28.5 ± 6.0	0.013
eGFR	72.7 ± 15.7	68.0 ± 18.0	<0.001
CHD	296 (10.8%)	488 (14.8%)	<0.001
MI	238 (8.9 %)	381 (12.3%)	<0.001
Stroke	65 (2.3 %)	158 (4.7 %)	<0.001

Supplementary Table 2. Comparison of echo measurements at Visit 5 and Visit 7, Delta and percent change across changes in frailty status with robust at baseline. Visit 7 measurements adjusted for Visit 5 measurements are also provided. P values compare between each change in frailty group, and trend. All measurements are adjusted for demographics (age, gender, race, and field center), HR and BP at Visits 5 and 7, and comorbidities (hypertension, diabetes, history of smoking, BMI, eGFR, CHD, stroke). LVMi= left ventricular mass index, LVEDD= LV end diastolic dimension, MWT= mean wall thickness, LVEF= LV ejection fraction, LAVi= left atrial volume index. RR= robust at Visit 5 and robust at Visit 7, RP= robust at Visit 5 and pre-frail at Visit 7, RF= robust at Visit 5 and Frail at Visit 7.

		Frailty category			p-value for between groups			P for Trend
		Robust-Robust	Robust-Prefrail	Robust-Frail	RR vs RP	RP vs RF	RR vs RF	
LVMI (g/m²)	n=	657	668	41				
	Visit 5	76.14 ± 0.64	76.60 ± 0.63	76.93 ± 2.57	0.690	0.680	0.990	0.590
	Visit 7	79.51 ± 0.72	81.03 ± 0.71	92.94 ± 2.89	0.170	0.001	0.001	0.002
	Visit 7adj	79.69 ± 0.56	80.88 ± 0.56	92.56 ± 2.27	0.150	0.001	0.001	0.001
	Delta	3.37 ± 0.59	4.43 ± 0.59	16.01 ± 2.40	0.220	0.001	0.001	0.001
	% change	0.06 ± 0.01	0.07 ± 0.01	0.24 ± 0.03	0.400	0.001	0.001	0.003
LVEDD (cm)	n=	661	673	41				
	Visit 5	4.39 ± 0.02	4.41 ± 0.02	4.33 ± 0.07	0.540	0.260	0.300	0.930
	Visit 7	4.36 ± 0.02	4.33 ± 0.02	4.42 ± 0.07	0.230	0.210	0.550	0.560
	Visit 7adj	4.36 ± 0.01	4.32 ± 0.01	4.47 ± 0.05	0.032	0.008	0.090	0.400
	Delta	-0.03 ± 0.01	-0.08 ± 0.01	0.09 ± 0.06	0.026	0.004	0.050	0.410
	% change	-0.00 ± 0.00	-0.02 ± 0.00	0.02 ± 0.01	0.021	0.003	0.048	0.390
MWT (cm)	n=	662	677	41				
	Visit 5	0.98 ± 0.00	0.97 ± 0.00	1.00 ± 0.02	0.320	0.090	0.530	0.830
	Visit 7	1.01 ± 0.01	1.02 ± 0.01	1.08 ± 0.02	0.500	0.002	0.003	0.039
	Visit 7adj	1.01 ± 0.00	1.02 ± 0.00	1.07 ± 0.02	0.140	0.009	0.002	0.008

	Delta	0.04 ± 0.00	0.05 ± 0.00	0.09 ± 0.02	0.090	0.050	0.008	0.010
	% change	0.04 ± 0.00	0.05 ± 0.00	0.09 ± 0.02	0.100	0.070	0.016	0.015
LVEF (%)	n=	654	658	39				
	Visit 5	65.59 ± 0.22	65.69 ± 0.22	66.31 ± 0.93	0.810	0.410	0.770	0.570
	Visit 7	63.39 ± 0.27	63.15 ± 0.27	62.80 ± 1.14	0.480	0.920	0.530	0.460
	Visit 7adj	63.43 ± 0.24	63.13 ± 0.24	62.44 ± 1.02	0.360	0.580	0.420	0.270
	Delta	-2.19 ± 0.26	-2.54 ± 0.26	-3.50 ± 1.10	0.340	0.430	0.400	0.210
	% change	-0.03 ± 0.00	-0.04 ± 0.00	-0.05 ± 0.02	0.300	0.600	0.560	0.220
LAVI (ml/m^2)	n=	658	673	43				
	Visit 5	24.48 ± 0.28	25.04 ± 0.28	25.02 ± 1.15	0.200	0.910	0.700	0.180
	Visit 7	26.59 ± 0.35	27.92 ± 0.35	28.95 ± 1.46	0.009	0.560	0.090	0.005
	Visit 7adj	26.83 ± 0.27	27.69 ± 0.27	28.74 ± 1.12	0.020	0.510	0.060	0.011
	Delta	2.11 ± 0.28	2.87 ± 0.27	3.93 ± 1.14	0.040	0.520	0.080	0.025
	% change	0.11 ± 0.01	0.14 ± 0.01	0.20 ± 0.05	0.060	0.330	0.070	0.031
e' lateral (cm/s)	n=	658	674	42				
	Visit 5	7.29 ± 0.08	7.34 ± 0.08	7.26 ± 0.31	0.560	0.820	0.720	0.730
	Visit 7	6.69 ± 0.08	6.70 ± 0.07	6.43 ± 0.30	0.840	0.440	0.290	0.790
	Visit 7adj	6.70 ± 0.07	6.69 ± 0.07	6.46 ± 0.27	0.960	0.450	0.320	0.640
	Delta	-0.60 ± 0.08	-0.64 ± 0.08	-0.82 ± 0.32	0.720	0.600	0.530	0.570
	% change	-0.04 ± 0.01	-0.05 ± 0.01	-0.06 ± 0.05	0.540	0.900	0.770	0.550
E/e' lateral (ratio)	n=	657	670	43				
	Visit 5	9.48 ± 0.12	9.48 ± 0.12	10.04 ± 0.48	0.880	0.230	0.270	0.640
	Visit 7	11.67 ± 0.18	11.84 ± 0.17	13.62 ± 0.70	0.560	0.021	0.008	0.080
	Visit 7adj	11.68 ± 0.15	11.85 ± 0.14	13.18 ± 0.58	0.420	0.048	0.015	0.080
	Delta	2.19 ± 0.15	2.36 ± 0.15	3.58 ± 0.59	0.420	0.070	0.025	0.090
	% change	0.28 ± 0.02	0.30 ± 0.02	0.36 ± 0.06	0.500	0.410	0.270	0.290
E/A (ratio)	n=	627	630	40				
	Visit 5	0.88 ± 0.01	0.86 ± 0.01	0.91 ± 0.04	0.049	0.090	0.940	0.190
	Visit 7	0.86 ± 0.01	0.84 ± 0.01	0.96 ± 0.04	0.180	0.006	0.070	0.900
	Visit 7adj	0.86 ± 0.01	0.85 ± 0.01	0.94 ± 0.04	0.690	0.027	0.047	0.540
	Delta	-0.02 ± 0.01	-0.02 ± 0.01	0.05 ± 0.04	0.690	0.110	0.070	0.290
	% change	0.01 ± 0.01	0.01 ± 0.01	0.06 ± 0.04	0.990	0.270	0.260	0.660

Supplementary Table 3. Comparison of echo measurements at Visit 5 and Visit 7, Delta and percent change across changes in frailty status with pre-frail at baseline. Visit 7 measurements adjusted for Visit 5 measurements are also provided. P values compare between each change in frailty group, and trend. All measurements are adjusted for demographics (age, gender, race, and field center), HR and BP at Visits 5 and 7, and comorbidities (hypertension, diabetes, history of smoking, BMI, eGFR, CHD, stroke). LVMi= left ventricular mass index, LVEDD= LV end diastolic dimension, MWT= mean wall thickness, LVEF= LV ejection fraction, LAVi= left atrial volume index. PR= pre-frail at Visit 5 and robust at Visit 7, PP= pre-frail at Visit 5 and pre-frail at Visit 7, PF= pre-frail at Visit 5 and frail at Visit 7.

		Frailty category			p-value for between groups			P for Trend
		Prefrail-Robust	Prefrail-Prefrail	Prefrail-Frail	PR vs PP	PP vs PF	PR vs PF	
LVMI (g/m²)	n=	229	722	103				
	Visit 5	75.36 ± 1.15	77.70 ± 0.64	78.74 ± 1.82	0.060	0.630	0.230	0.060
	Visit 7	79.24 ± 1.32	82.37 ± 0.74	84.66 ± 2.09	0.032	0.300	0.070	0.015
	Visit 7adj	80.68 ± 1.00	82.05 ± 0.56	83.55 ± 1.58	0.230	0.340	0.160	0.110
	Delta	3.88 ± 1.04	4.67 ± 0.58	5.92 ± 1.64	0.520	0.430	0.290	0.300
	% change	0.06 ± 0.01	0.07 ± 0.01	0.09 ± 0.02	0.440	0.410	0.260	0.240
LVEDD (cm)	n=	230	730	105				
	Visit 5	4.41 ± 0.03	4.38 ± 0.02	4.36 ± 0.04	0.460	0.530	0.380	0.340
	Visit 7	4.34 ± 0.03	4.28 ± 0.02	4.24 ± 0.05	0.080	0.480	0.100	0.044
	Visit 7adj	4.32 ± 0.02	4.28 ± 0.01	4.26 ± 0.04	0.080	0.690	0.160	0.070
	Delta	-0.07 ± 0.02	-0.11 ± 0.01	-0.12 ± 0.04	0.180	0.900	0.300	0.180
	% change	-0.01 ± 0.01	-0.02 ± 0.00	-0.02 ± 0.01	0.200	0.990	0.340	0.230
MWT (cm)	n=	234	736	107				
	Visit 5	0.96 ± 0.01	0.98 ± 0.00	1.00 ± 0.01	0.016	0.310	0.034	0.007
	Visit 7	1.01 ± 0.01	1.04 ± 0.01	1.06 ± 0.01	0.002	0.230	0.006	0.001
	Visit 7adj	1.02 ± 0.01	1.04 ± 0.00	1.05 ± 0.01	0.035	0.430	0.060	0.018
	Delta	0.05 ± 0.01	0.06 ± 0.00	0.06 ± 0.01	0.270	0.720	0.400	0.230
	% change	0.05 ± 0.01	0.06 ± 0.00	0.07 ± 0.01	0.290	0.600	0.320	0.200
LVEF	n=	228	715	100				

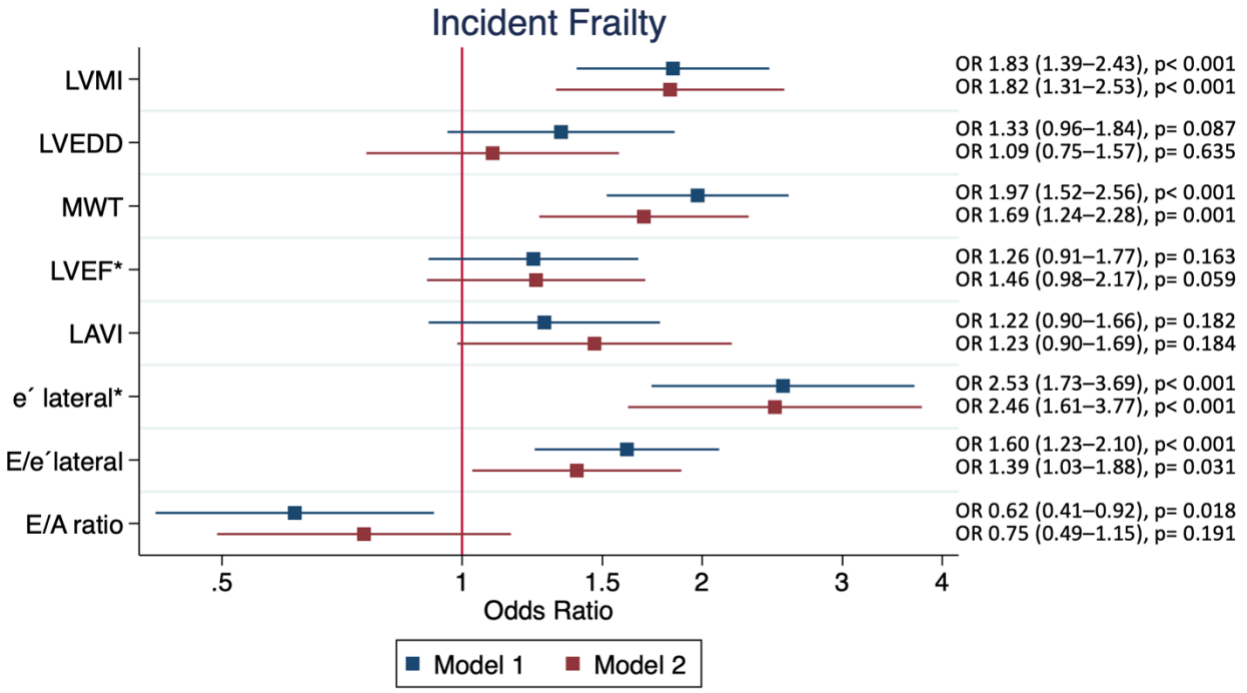
(%)	Visit 5	65.48 ± 0.38	65.77 ± 0.22	64.71 ± 0.61	0.540	0.100	0.490	0.630
	Visit 7	63.09 ± 0.46	63.77 ± 0.26	61.44 ± 0.74	0.240	0.002	0.260	0.410
	Visit 7adj	63.17 ± 0.41	63.68 ± 0.23	61.96 ± 0.65	0.320	0.010	0.360	0.500
	Delta	-2.39 ± 0.44	-2.00 ± 0.25	-3.27 ± 0.70	0.470	0.080	0.530	0.660
	% change	-0.03 ± 0.01	-0.03 ± 0.00	-0.05 ± 0.01	0.440	0.110	0.710	0.770
LAVI (ml/m^2)	n=	228	715	100				
	Visit 5	24.53 ± 0.49	25.35 ± 0.28	25.34 ± 0.78	0.120	0.880	0.440	0.210
	Visit 7	26.97 ± 0.60	28.30 ± 0.34	29.50 ± 0.96	0.042	0.290	0.040	0.016
	Visit 7adj	27.48 ± 0.46	28.15 ± 0.26	29.36 ± 0.73	0.170	0.120	0.050	0.036
	Delta	2.44 ± 0.47	2.95 ± 0.26	4.15 ± 0.74	0.310	0.110	0.090	0.070
	% change	0.14 ± 0.02	0.14 ± 0.01	0.20 ± 0.03	0.850	0.090	0.220	0.260
e' lateral (cm/s)	n=	232	735	103				
	Visit 5	7.12 ± 0.13	7.10 ± 0.07	7.18 ± 0.21	0.990	0.830	0.850	0.900
	Visit 7	6.55 ± 0.13	6.57 ± 0.07	6.68 ± 0.21	0.890	0.660	0.660	0.650
	Visit 7adj	6.54 ± 0.12	6.57 ± 0.07	6.65 ± 0.19	0.880	0.700	0.690	0.660
	Delta	-0.57 ± 0.14	-0.54 ± 0.08	-0.50 ± 0.22	0.900	0.830	0.800	0.760
	% change	-0.05 ± 0.02	-0.04 ± 0.01	-0.02 ± 0.03	0.670	0.570	0.370	0.420
E/e' lateral (ratio)	n=	231	732	103				
	Visit 5	9.93 ± 0.23	10.01 ± 0.13	10.29 ± 0.36	0.800	0.480	0.300	0.460
	Visit 7	12.44 ± 0.32	12.49 ± 0.18	13.07 ± 0.51	0.900	0.240	0.360	0.420
	Visit 7adj	12.51 ± 0.27	12.50 ± 0.15	12.86 ± 0.42	0.990	0.350	0.670	0.630
	Delta	2.51 ± 0.27	2.48 ± 0.15	2.78 ± 0.43	0.950	0.440	0.780	0.730
	% change	0.31 ± 0.03	0.31 ± 0.02	0.33 ± 0.05	0.850	0.600	0.780	0.680
E/A (ratio)	n=	220	690	89				
	Visit 5	0.86 ± 0.02	0.84 ± 0.01	0.93 ± 0.03	0.350	0.002	0.090	0.230
	Visit 7	0.88 ± 0.02	0.85 ± 0.01	0.91 ± 0.03	0.230	0.130	0.820	0.880
	Visit 7adj	0.88 ± 0.02	0.86 ± 0.01	0.87 ± 0.03	0.370	0.890	0.640	0.490
	Delta	0.03 ± 0.02	0.01 ± 0.01	-0.02 ± 0.03	0.630	0.340	0.260	0.270
	% change	0.10 ± 0.03	0.05 ± 0.02	0.03 ± 0.05	0.150	0.680	0.410	0.130

Supplementary Table 4. Baseline characteristics at Visit 5 of the study population for the incident frailty analysis, overall and by subsequent frailty category at Visit 6.

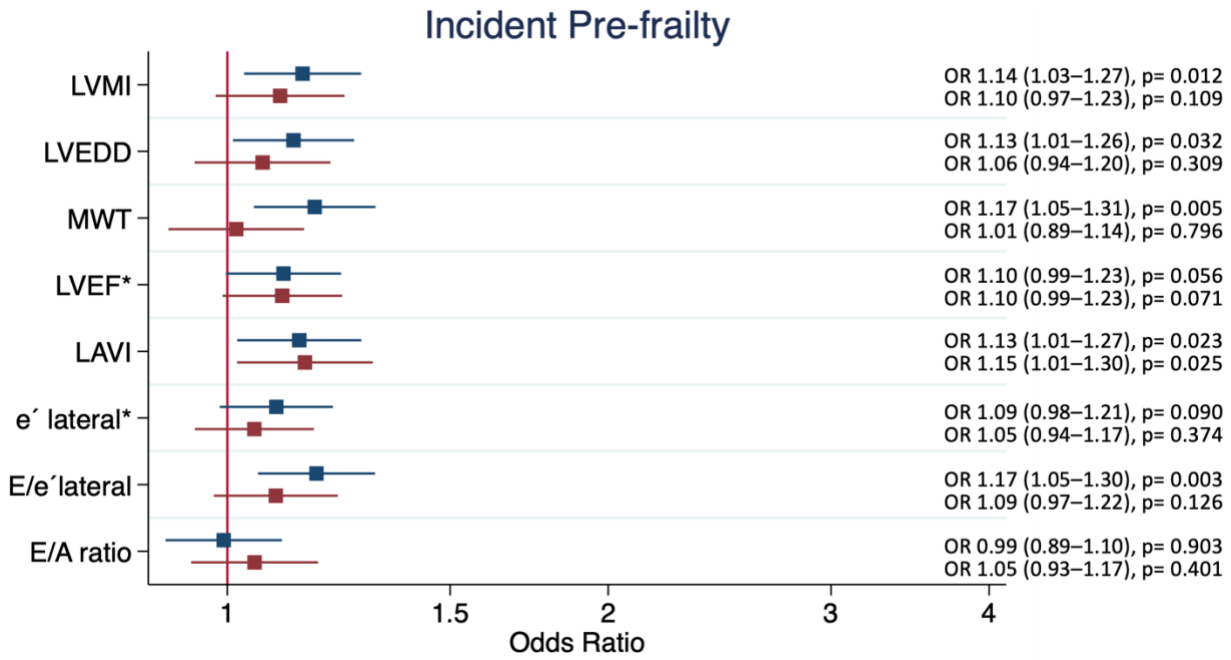
Variable at V5	Overall at V5 (n= 1,648)	Frailty category at V6			p-value (trend)
		Robust (n=845)	Pre-frail (n=754)	Frail (n=49)	
Age	73.6 ± 4.2	72.9 ± 3.9	74.2 ± 4.4	74.5 ± 4.1	<0.001
Male	755 (45.8%)	401 (47.5%)	331 (43.9%)	23 (46.9%)	0.24
Black	333 (20.2%)	155 (18.3%)	164 (21.8%)	14 (28.6%)	0.029
Hypertension	1126 (68.6%)	541 (64.4%)	543 (72.2%)	42 (85.7%)	<0.001
Diabetes	381 (23.1%)	161 (19.1%)	197 (26.1%)	23 (46.9%)	<0.001
BMI	28.4 ± 5.1	27.6 ± 4.3	29.0 ± 5.4	32.9 ± 6.9	<0.001
eGFR	73.1 ± 15.2	73.9 ± 14.3	72.5 ± 16.2	69.2 ± 15.9	0.011
CHD	171 (10.5%)	77 (9.3 %)	87 (11.7%)	7 (14.6%)	0.07
MI	127 (8.1 %)	55 (6.8 %)	67 (9.4 %)	5 (10.9%)	0.049
Stroke	32 (1.9 %)	14 (1.7 %)	18 (2.4 %)	0 (0.0 %)	0.64
Echocardiographic measurements					
LVMI (g/m^2)	76.85 ± 17.89	75.5 ± 17.0	77.6 ± 18.1	87.4 ± 25.4	< 0.001
LVEDD (cm)	4.40 ± 0.49	4.4 ± 0.5	4.4 ± 0.5	4.5 ± 0.6	0.12
MWT (cm)	0.98 ± 0.13	1.0 ± 0.1	1.0 ± 0.1	1.1 ± 0.2	< 0.001
LAVI (ml/m^2)	24.98 ± 8.42	24.4 ± 6.9	25.6 ± 9.8	26.2 ± 7.4	0.003
e' lateral (cm/s)	7.22 ± 1.95	7.4 ± 1.9	7.1 ± 1.9	5.9 ± 1.7	< 0.001
E/e' lateral (ratio)	9.60 ± 3.33	9.2 ± 3.1	9.9 ± 3.5	11.2 ± 4.0	< 0.001
E/A (ratio)	0.87 ± 0.25	0.9 ± 0.2	0.9 ± 0.3	0.8 ± 0.3	0.025

P values assess for trend for each variable across all categories. Data is shown as frequency and proportion or mean ± standard deviation. BMI= body mass index, eGFR= estimated glomerular filtration rate by CKD-EPI, CHD= coronary heart disease, MI= myocardial infarction, LVMi= left ventricular mass index, MWT= mean wall thickness, LVEDD= left ventricular end diastolic dimension, LAVi= left atrial volume index.

Supplementary Figure 1: Association of measures of cardiac structure and function at Visit 5 with Incident Frailty (Panel A) or incident Pre-frailty (Panel B) at Visit 6. Model 1 adjusts for demographics (age, sex, race, and field center). Model 2 adjusts for demographics, HR and BP at visits 5 and 7, and comorbidities (hypertension, diabetes, BMI, eGFR, prevalent CHD, prevalent stroke) at Visit 5.



LVMi= left ventricular mass index, MWT= mean wall thickness, LVEDD= left ventricular end diastolic dimension, LAVi= left atrial volume index. *Indicates odds associated with lower values.



LVMi= left ventricular mass index, MWT= mean wall thickness, LVEDD= left ventricular end diastolic dimension, LAVi= left atrial volume index. *Indicates odds associated with lower values.

MANUSCRIPT 2

High Throughput Plasma Proteomic Analysis Linking Risk of Heart Failure and Frailty in Late Life: The Atherosclerosis Risk in Communities Study

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Word count: 3,507

Abstract

Background The incidence and prevalence of heart failure (HF) and frailty is high among older individuals, and frequently co-exist. While HF and frailty have several common pathobiologic features, limited date exist regarding potential shared mechanisms between the two conditions.

Methods Among participants in the Atherosclerosis Risk in Communities (ARIC) study, an ongoing community-based cohort study, 4,877 plasma proteins were measured using an aptamer-affinity assay (SomaScan v4) at study Visit 3 (V3; 1993-1994; n=10,368, age 60 ±6 years; 822 incident HF events) and at study Visit 5 (V5; 2011-2013; n= 3,908, age 75 ±5 years; 336 incident HF events), and frailty was assessed using Fried criteria at V5 and Visit 6 (V6; 2016-2018; n= 2,358, age 79 ±5 years, 152 incident frailty events). Multivariable Cox proportional hazard regression models were used to identify proteins consistently associated with incident HF post-V3 and post-V5. Among these HF-associated proteins, multivariable logistic regression was used to identify proteins associated with both prevalent frailty at V5 (n=223 cases) and incident frailty between V5 and V6 (n=152 incident cases). All models adjusted for age, sex, race, hypertension, diabetes, cardiovascular disease, BMI, and atrial fibrillation. HF- and frailty-associated proteins were evaluated for associations with cardiac function at V5, incident HF with preserved (HFpEF) or reduced ejection fraction (HFrEF) post-V5, individual frailty components at V6, enriched biologic pathways using the g:profiler toolkit, and for potential causal associations using two-sample Mendelian randomization.

Results: A total of 289 proteins were associated with incident HF post-V3 at $p < 1.0 \times 10^{-5}$ (0.05/4,877) and 84 were significantly associated with incident HF post-V5 at $p < 1.7 \times 10^{-4}$

(0.05/289). Among 4,131 HF-free participants at V5, 48 of these 84 HF-associated proteins associated with prevalent frailty at $p< 5.9 \times 10^{-4}$ (0.05/84). Among 3,908 frailty and HF-free participants,18 of these proteins were significantly associated with incident frailty at $p< 1.0 \times 10^{-3}$ (0.05/48). At V5, these candidate proteins associated most consistently with larger left ventricular and atrial size and higher E wave velocity and tended to be more strongly associated with incident HFpEF than HFrEF post-Visit 5frailty than HF. Mendelian randomization identified potential causal associations between *WFDC1,* and frailty and HF. Pathway enrichment analysis identified pathways linked to collagen metabolism and inflammation as enriched.

Conclusions We identified 18 proteins that independently associate with incident HF and incident frailty in late life, supporting the presence of shared biologic pathways underlying frailty and HF in late life. These proteins highlight collagen metabolism and immune pathways as potential shared biologic pathways for frailty and HF and deepen our understanding of the interplay between frailty and HF in late-life.

Introduction

Heart failure (HF) and frailty are common in late life and frequently co-exist.[14] Frailty is a clinical syndrome characterized by a loss of functional reserve and is associated with an increased risk of several adverse outcomes[2] including death[3,25]. Risk factors for the development of frailty include advanced age, obesity, sedentarism[52], and cardiovascular disease.[19,39] The association between HF and frailty is well described, with a prevalence of 36-53% among persons with established HF.[53] In addition, frailty is associated with higher prevalence and incidence of HF in cross-sectional and longitudinal[4,5,40] analyses. Inflammation has been hypothesized as a common pathobiology linking frailty and HF. [20,21,54] Indeed, interleukins, tumor necrosis factor alpha (TNFα), and C-reactive protein (CRP) are known biomarkers for both frailty and HF.[46,55] However, a deeper understanding of the potential shared pathways between frailty and HF in late life has been limited by the restricted number of candidate biomarkers that have been studied in this context to-date.

Investigating the association of high throughput proteomics with incident frailty and HF in a deeply phenotyped longitudinal cohort holds promise to identify novel biomarkers of shared risk and to identify shared pathways for HF and frailty in late life. Among participants in the Atherosclerosis Risk in Communities (ARIC) study, a community-based longitudinal cohort study, we leveraged serial high-throughput aptamer-affinity proteomics (n=4,877 protein aptamers), prospective ascertainment of HF events, and serial frailty assessments to identify circulating proteins associated with risk of both incident HF and frailty.

Methods

Study Population

The design and procedures of the ARIC study has been previously described.[23] Beginning between 1987 and 1989 (Visit 1), 15,792 participants aged 45-64 years enrolled in this ongoing, prospective observational cohort from four communities across the United States: Forsyth County, North Carolina; Jackson, Mississippi; Minneapolis, Minnesota; and Washington County, Maryland. Three study visits were subsequently conducted between 1990 and 1999, followed by Visit 5 in 2011-2013, Visit 6 in 2016-2018, and Visit 7 in 2018-2019. Ascertainment of HF events was performed since study inception while frailty was assessed at Visits 5, 6, and 7. This analysis included participants attending Study Visits 3 and 5, at which time plasma proteomics were measured. Among 13,688 participants who were alive at the time of Visit 3, 12,877 attended the study visit of whom 10,638 were included in this analysis after excluding those with missing proteomic data (n=1,406) and prevalent HF (n=833). Of the 8,637 surviving participants at the time of Visit 5, 6,538 participants attended that visit of whom 4,131 were included in this analysis after excluding those with missing proteomic data (n=1,345), prevalent HF (n=710), and missing frailty assessment (n=352).

Protein measurement

A total of 5,284 modified aptamers were used to measure plasma protein concentrations from samples collected at ARIC Visits 3 and 5 (SomaScan Platform, SomaLogic, Boulder CO).[56] Blood was collected at study field centers using a standardized protocol and shipped to the ARIC central laboratory as previously described.[56] Protein level quantification by the SomaScan assay has been previously described in detail, including normalization and quality control procedures.

[56–60]. Relative fluorescence against healthy controls was used as the measurement unit of protein levels and standardized thereafter for this study. After excluding proteins not passing quality control, this analysis included 4,877 modified aptamers measuring 4,697 individual proteins.

Frailty Assessment

Frailty was ascertained at study Visits 5, 6, and 7 using the Fried criteria which has been previously validated[25] and operationalized in the ARIC and other cohorts[2,19,26]. The Fried criteria integrates five measures: (1) Low energy expenditure, based on gender-specific 20th percentile rank of the Baecke leisure sports activity index; (2) Low walking speed, based on the bottom gender and height-specific quintile of time to walk 15 feet; (3) Lack of energy (exhaustion), defined as present if the participant responded "some of the time" or "most of the time" to either of the following questions: "I felt everything I did was an effort" or "I could not get going"; (4) Low grip strength, based on the bottom gender- and body mass index-specific grip strength quintile; (5) Unintentional weight loss, defined as 10% unintentional weight loss or body mass index <18.5 kg/m2. Participants who met three criteria were classified as frail, those who met one or two criteria were classified as pre-frail and those meeting none were classified as robust.

Heart Failure Ascertainment

HF was ascertained through active surveillance of hospital discharges and annual follow-up calls to participants to identify hospitalizations HF-associated International Classification of Diseases (ICD) codes. Prior to 2005, HF hospitalization was defined as a hospitalization with an ICD Ninth Revision code of 428 as previously described.[31] After January 1, 2005, HF hospitalization was based on chart abstraction and ARIC physician adjudication of all

hospitalizations with a broader set of HF-related ICD codes as previously described.[32] For adjudicated HF events, HF with preserved ejection fraction (LVEF, HFpEF) was defined based on an abstracted LVEF of ≥50% at the time of hospitalization while HF with reduced LVEF (HFrEF) was defined by an LVEF <50% at the time of hospitalization. Prevalent HF at each visit was defined based on an incident HF event occurring before the date of that visit.

Echocardiography

Detailed protocols and procedures for echocardiography at ARIC Visit 5, including reproducibility metrics have been previously described.[27] All studies were performed by trained sonographers certified by the ARIC study-specific imaging protocol and using consistent echocardiography equipment. Quantitative analysis was performed at a central echocardiography reading center by analysts were blinded to clinical information, and in accordance with American Society of Echocardiography guidelines [28,29].

Assessment of Covariates

Age, race, and sex were self-reported. Coronary heart disease (CHD) was defined as meeting any of the following: (1) self-reported CHD at Visit 1 (2) having a history of myocardial infarction (MI) or (3) having had a coronary artery intervention.[30,61] Hypertension was based on self-report of antihypertensive medications, or having blood pressure ≥140/90 mmHg at the current or a previous study visit. Diabetes mellitus was defined based on self-report or having a serum fasting glucose ≥126 mg/dL, or any capillary glucose measurement ≥200 mg/ dL. AF diagnosis was based on ECG at visits 1 through 5, and hospital discharge records as previously reported.[33] eGFR at Visit 3 was estimated by CKD-EPI using Visit 2 creatinine. Visit 5 eGFR was estimated by CKD-EPI using Visit 5 creatinine. Smoking status at each visit was defined as

being a current smoker. Anthropometric measurements of weight, height and fat mass were obtained. Fat mass was obtained using bioelectric impedance with the Tanita Body Composition Analyzer, TBF-300A.[62] Spirometry at Visit 5 was performed using SensorMedics model 1022 (OMI, Houston, TX). [63,64] This study uses precent predicted forced vital capacity (ppFVC) and precent predicted forced expiratory volume per second ($ppFEV_1$).

Statistical Analysis

We first identified proteins consistently associated with risk of incident HF in mid-life (post-Visit 3) and late-life (post-Visit5). Among participants free of HF at Visit 3, we employed multivariable uniprotein Cox proportional hazard regression models to assess the association of the 4,877 proteins measured at Visit 3 (1993-1994) with incident HF occurring after Visit 3 (median follow-up 10.0 years [IQR 0, n=822 incident HF events) at a Bonferroni-adjusted level of significance ($p< 1.0 \times 10^{-5}$ [0.05/4,877]). For proteins significantly associated with risk of incident HF post-Visit 3, we assessed the association of their values measured at Visit 5 (2011-2013) with incident adjudicated HF occurring after Visit 5 (median follow-up 7.3 years [IQR 2, n=336 incident HF events) at a Bonferroni-adjusted level of significance ($p< 1.7x10^{-4}$ [0.05/289]). This analysis excluded participants with prevalent HF or frailty at Visit 5. The resulting proteins comprised our 'HF-associated proteins'.

To define those proteins also associated with frailty, we then identified the sub-set of HF-associated proteins that were cross-sectionally associated with prevalent frailty (n=223 prevalent frailty cases) at Visit 5 using multivariable logistic regression and a Bonferroni-adjusted level of significance ($p< 5.9 \times 10^{-4}$ [0.05/84]). Finally, among those HF-associated proteins also associated with prevalent frailty, we identified the sub-set associated with incident frailty

between Visit 5 and Visit 6 (median time interval 4.9 years [IQR 0.8] years; n=152 incident frailty events) at a Bonferroni-adjusted level of significance ($p< 1.0 \times 10^{-3}$ [0.05/48]). This analysis excluded participants with prevalent frailty or HF at Visit 5, and missing frailty assessments at Visit 5 or Visit 6. These proteins comprised our candidate HF- and frailty-associated proteins. For these candidate proteins, we assessed their association with cardiac structure and function and non-cardiac measures at Visit 5, and with individual frailty components at Visit 6. All analyses adjusted for age, sex, race, field center, hypertension, diabetes, smoking status, BMI, eGFR, prevalent CHD, prevalent AF, and history of MI assessed at each visit accordingly.

Multiple imputation by chained equations was performed to account for missing covariate values with a frequency of less than 5%. Statistical analysis was performed using Stata software Version IC-16.1 (Stata Corp LP, College Station, TX).

To estimate the potential causal effect of candidate proteins on frailty, HF, and cardiac structure and function, we used single nucleotide polymorphisms (SNPs) and summary statistics from GWAS studies on frailty[65], HF[66], and three cardiac magnetic resonance imaging (CMR) phenotypes[67] to perform two-sample Mendelian randomization. Analyses were run using the TwoSampleMR package in R.[68] Pleiotropy was assessed using MR-Egger regression.[69] P-values less than 0.05 represent a significant causal association.

To evaluate the biological role of identified protein related to frailty and HF, we used the g:Profiler toolkit (https:/biit.cs.ut.ee/gprofiler).[70] G:Profiler performs a functional enrichment analysis of multiple proteins. Functional profiling of our frailty- and HF-related proteins was conducted using the Reactome knowledgebase (https://reactome.org). Reactome is a manually

curated molecular archive of biological processes that systematically links proteins to known molecular functions. In addition, it facilitates the discovery of novel functional relationships between genes and pathophysiologic molecular pathways.[71,72] To better characterize the enrichment pathways involved in our analysis, we used a threshold of <0.05 instead of Bonferroni significance on the last stage of the protein derivation i.e., proteins associated with incident HF and incident frailty.

Results

Proteins Reflecting Shared Risk of HF and Frailty

The 10,638 HF-free participants with proteomics data at Visit 3 (1993-1994) had a mean age of 60±6 years, 46% were men, and 21% reported Black race (Table1). Over a mean follow-up time of 9.3 ±2.0 years between Visit 3 and Visit 5, 822 participants developed incident HF. Baseline characteristics by frailty status at Visit 5 are summarized in the Supplementary table 1. In multivariable Cox proportional hazard models adjusting for demographics and cardiovascular co-morbidities, 289 of the 4,877 proteins measured at Visit 3 were associated with post-Visit 3 incident HF at $p< 1.0 \times 10^{-5}$. The 3,908 HF-free and frailty-free participants with proteomic data who attended Visit 5 (2011-2013) had a mean age of 75±5 years, 42% were men, and 17% reported Black race (Table 1). At a median follow-up of 6.4±1.9 years after visit 5, 336 participants developed incident HF. When measured at Visit 5, 84 of the 289 proteins associated with incident HF post-Visit 3 were also associated with incident HF post-Visit 5 at $p< 1.7x10^{-4}$ in multivariable Cox proportional hazards models.

Among 4,131 HF-free participants at Visit 5 who had a frailty assessment, 223 (5%) were classified as frail. Of the 84 HF-associated candidate proteins, 48 proteins were cross-sectionally

associated with frailty status at $p< 5.9 \times 10^{-4}$ in multivariable logistic regression models. Among 2,358 participants free of HF and frailty at Visit 5 who had frailty assessments at visits 5 and 6, 152 (4%) developed incident frailty by Visit 6. 18 of these 48 proteins were also associated with incident frailty at Visit 6 at $p< 1.0 \times 10^{-3}$ in multivariable logistic regression models and comprised our final set of proteins reflecting shared risk of HF and frailty (Table 2).

Associations of Candidate Proteins with Cardiac and Non-Cardiac Organ Function, HF Phenotype, and Frailty Components

Among 3,908 participants free of HF and frailty at Visit 5, candidate proteins were cross-sectionally associated with measures of cardiac structure and function, arterial function, pulmonary function, and body composition (fat mass; Figure 3A). The most consistent associations were observed across proteins with larger LV and LA size (greater LV end-diastolic volume index, greater LV mass index, larger LA volume index), higher E wave velocity, and lower fat mass. Hierarchical clustering identified three clusters of proteins. Cluster 1 consisted of 7 proteins and demonstrated generally modest associations with cardiac and non-cardiac measures. Cluster 2 consisted of 5 proteins and was associated with higher E wave velocity, large LAVi, worse pulmonary function and (lower percent predicted FEV1 and FVC). Cluster 3 consisted of 6 proteins and demonstrated robust associations with larger LVEDV index, LV mass index, and TDI e', in addition to E wave velocity and LAVi.

Among these 3,908 HF-free and frailty-free participants at Visit 5, 161 participants developed incident HFpEF during the follow-up period, 132 developed incident HFrEF, and 43 developed HF with unknown LVEF. The magnitude of association of the 18 HF- and frailty-associated proteins tended to be higher for incident HFpEF compared to incident HFrEF (Figure

3b). Thirteen proteins were significantly associated with only incident HFpEF, while four were significantly associated with both incident HFpEF and HFrEF and one was significantly associated with only incident HFrEF. Notably, three of the four proteins associated with both incident HFpEF and HFrEF belonged to the protein cluster demonstrating associations with LV systolic measures.

Among 2,358 HF-free and frailty-free participants at Visit 5 with frailty assessment at Visit 5 and Visit 6, the HF- and frailty-associated candidate proteins demonstrated associations with development of each frailty component: low gait speed, low energy, weight loss, low grip strength and exhaustion at Visit 6 (Figure 3C). Hierarchical clustering identified three protein clusters based on associations with frailty components at Visit 6. Cluster 1 consisted of 7 proteins and demonstrated associations primarily with low gait speed, low energy, and low grip strength. Cluster 2 consisted of 8 proteins and demonstrated additional associations with weight loss. Cluster 3 consisted of 3 proteins and demonstrated associations with all frailty components.

Mendelian Randomization to Identify Potentially Causal Associations

Results of Mendelian randomization analyses for frailty, HF, and CMR-based LV structure and function are shown in Figure 4. Multi-marker MR found nominal associations between *COL28A1* and *lower* LV end diastolic volume (p= 0.025), and between *TREM1* and *lower* LVEF. (p= 0.010). Single SNP MR showed nominal associations between two uncorrelated trans-pQTLs of the protein *WFDC1* with frailty (rs28664709, p= 0.021) and HF (rs7172696, p= 0.013). Additionally, cis-pQTLs for *GDF15* (rs45543339, p=0.020) and *TREM1* (rs3789204, p=0.025) were associated with lower LVEF, while a single trans-pQTL (rs1260326) for both *CST3* and *EFEMP1* was associated with LV end diastolic (p= 1.96E-05) and end

systolic volume (p= 0.00053). Single SNP associations between pQTLs for *LEFTY2* and CMR phenotypes as well as the multi-marker association between *GDF15* and LVEF were found to be highly significant in bi-directional MR, leading us to conclude that the direction of causality is opposite.

Biological Pathways

Enriched biological pathways were identified based on 31 HF- and frailty-associated candidate proteins at $p < 0.05$ significance. Pathway enrichment analysis using the Reactome knowledgebase identified collagen biosynthesis, formation, and trimerization pathways (COL28A1, COL6A3, EFEMP1), as well as cytokine immune pathways and TNF receptor binding (TNFRSF1A and B, VEGFA, B2M, and HAVCR2). These data suggest collagen metabolism and immune pathways may be shared biological pathways of frailty and HF in late life. Full results of the functional profiling from enrichment pathways in the Reactome knowledgebase are depicted in Supplementary Figure 1.

Discussion

Using high throughout proteomics in a longitudinal prospective cohort study, we identified 18 proteins that independently associate with incident HF and incident frailty in late life consistently across several analyses and after correcting for multiple testing. While most commonly associated with higher LVEDV index, E wave velocity, and LAV index, these 18 proteins demonstrated differential associations with cardiac structure and function, and also with pulmonary function. The large majority of these proteins also demonstrated more robust associations with risk on incident HFpEF as opposed to HFrEF in late life. The association

between the candidate proteins and incident frailty was driven mainly by associations with three of the frailty components: gait speed, low energy expenditure and grip strength. Mendelian randomization analysis suggested possible causal association between WFDC1 and both frailty and HF, while pathway analysis suggested collagen metabolism and immune pathways as potential shared biologic pathways for frailty and HF.

Frailty and HF frequently coexist, and their relationship is both cross-sectional[15,34,41] and longitudinal[4,5,38,40]. Patients suffering from HF suffer more adverse events when frail,[16,36,37] while frailty is associated with subclinical alteration in cardiac structure and function[19,41–43] and a higher risk of both incident HFpEF and HFrEF in late life.[4 73] Conversely, pro-brain natriuretic peptide (pro-BNP), a biomarker of myocardial stress and potent prognostic risk factor for HF, was independently associated with frailty in a recent meta-analysis of three separate cohorts.[74] Furthermore, impaired skeletal muscle function – a prominent feature of frailty – is frequently observed in HFpEF patients. [34,75] Indeed, a murine model of HFpEF demonstrated skeletal muscle dysfunction at the molecular, functional, and histological levels. Together, these findings suggest shared clinical features, and possibly underlying mechanisms, for frailty and HF. We identified numerous plasma proteins associated with both incident HF and frailty, 18 of which were robustly associated with both endpoints across several analyses and accounting for multiple testing. While not definitive, the presence of shared circulating biomarkers of risk supports the hypothesis that some common pathophysiologic mechanisms exist.

Inflammation has been hypothesized as a common pathobiology underlying both the development of frailty and HF.[21] Inflammatory biomarkers including C-reactive protein (CRP), various interleukins (ILs), tumor necrosis factor (TNF), and myeloperoxidase (MPO) have been implicated in the pathophysiology of frailty.[76,77] These same biomarkers, CRP, ILs, and TNF,

have also been associated with heighted risk of incident HF in community-based studies.[78] While targeting inflammation has not been effective in HFrEF,[79,80] substantial interest currently exists in the role of systemic inflammation in the pathophysiology of HFpEF.[81,82] Small studies of human myocardial tissue biopsies suggest particularly important roles for inflammatory cytokines including IL-6, TNF and reactive oxygen species (ROS), cardiomyocyte and vascular endothelial damage, and resulting myocardial fibrosis through collagen synthesis. [83] Notably, the frailty- and HF-associated proteins we identified in this analysis enriched for collagen metabolism pathways and for immune pathways. These markers also tended to associate more strongly with risk of incident HFpEF than HFrEF, and with frailty components reflecting impaired skeletal muscle function including grip strength and gait speed. These findings therefore support and expand on prior studies suggesting shared pathways between HF and frailty in late life. Further studies are necessary to determine whether these proteins, alone or in combination, can effectively identify older persons at heightened risk for both frailty and HF to target for preventative interventions, such as exercise training.

Results of Mendelian randomization analyses for frailty, HF, and CMR-based LV structure and function are shown in Figure 4. Multi-marker MR found nominal associations between *COL28A1* and *lower* LV end diastolic volume (p= 0.025), and between *TREM1* and *lower* LVEF. (p= 0.010). Single SNP MR showed nominal associations between two uncorrelated trans-pQTLs of the protein *WFDC1* with frailty (rs28664709, p= 0.021) and HF (rs7172696, p= 0.013). Additionally, cis-pQTLs for *GDF15* (rs45543339, p=0.020) and *TREM1* (rs3789204, p=0.025) were associated with lower LVEF, while a single trans-pQTL (rs1260326) for both *CST3* and *EFEMP1* was associated with LV end diastolic (p= 1.96E-05) and end systolic volume (p= 0.00053). Single SNP associations between pQTLs for *LEFTY2* and CMR

phenotypes as well as the multi-marker association between *GDF15* and LVEF were found to be highly significant in bi-directional MR, leading us to conclude that the direction of causality is opposite.

This study has several limitations. As an observational study, unmeasured confounding may impact our findings and precludes causal inference. We attempted to mitigate this limitation by performing two-sample Mendelian Randomization for key candidate proteins with available genetic instruments. The Fried score is a validated and commonly employed criteria to measure frailty in epidemiologic studies, but frailty likely comprises complex and potentially heterogeneous biological systems which may limit our ability to identify molecular pathways associated with frailty and HF. We employed high throughput aptamer-affinity proteomics which enable broad sampling of the proteome, but which may have limited target specificity for some proteins. Differential attrition between visits may introduce selection bias to our study, as sicker participants are at higher risk of censoring. The ARIC study participants tend to be healthier, have higher socioeconomic status, and have more access to healthcare than the average American. Furthermore, we did not perform replication in an independent cohort. Therefore, whether these findings are generalizable to other populations remains unclear.

Conclusions

We identified 18 proteins that independently associate with incident HF and incident frailty in late life. These proteins tended to be more strongly associated with incident HFpEF than HFrEF, with structural and Doppler-based measures suggestive of diastolic – as opposed to systolic – dysfunction, and with measures of peripheral muscle strength including gait speed and grip strength in addition to low energy and weight loss. These proteins highlight collagen

metabolism and immune pathways as potential shared biologic pathways for frailty and HF. These findings deepen our understanding of the interplay between frailty and HF in late-life.

Tables and Figures

Table 1. Baseline characteristics at Visit 3 and Visit 5. Visit 3 includes participants who attended Visit 3 and were free of HF. Visit 5 includes participants who attended Visit 5 and were free of frailty and HF.

	Visit 3	Visit 5
Variable	n= 10,638	n= 3,908
Age, mean ± SD, years	60 (6)	75.11 (5)
Black, n (%)	2201 (21)	763 (17)
Male, n (%)	4886 (46)	1861 (41)
BMI, mean ± SD, kg/m2	28 (5)	28.34 (5)
Smoke, n (%)	1873 (18)	226 (6)
CAD, n (%)	671 (6)	566 (14)
DM2, n (%)	1899 (18)	1324 (34)
A-fib, n (%)	112 (1)	184 (5)
Hypertension, n (%)	4768 (45)	3117 (80)
eGFR, median ± IQR, kg/m^2	86 [75, 95]	72.18 [60, 84]

Table 2. Point estimates of HF-and frailty-associated proteins with incident HF after Visit 3 and Visit 5, prevalent frailty at Visit 5, and incident frailty after Visit 5. Estimates are expressed hazards ratio (HR) and odds ratio (OR), as appropriate.

	Incident HF post Visit 3	Incident HF post Visit 5	Prevalent Frailty at Visit 5	Incident Frailty post Visit 5
	HR (95%CI) p-value	HR (95%CI) p-value	OR (95%CI) p-value	OR (95%CI) p-value
COL28A1	1.327 (1.253 - 1.405) p=2.46e-22	1.298 (1.143 - 1.474) p=5.90e-05	1.752 (1.492, 2.059) p=8.19e-12	1.533 (1.254, 1.880) p=3.23e-05
COL6A3	1.275 (1.219 - 1.335) p=1.21e-25	1.359 (1.199 - 1.539) p=1.44e-06	1.820 (1.553, 2.135) p=1.53e-13	1.397 (1.140, 1.703) p=1.01e-03
WFDC2	1.448 (1.354 - 1.548) p=2.06e-27	1.521 (1.328 - 1.741) p=1.27e-09	1.719 (1.438, 2.051) p=2.30e-09	1.787 (1.423, 2.242) p=5.38e-07
PXDN	1.344 (1.246 - 1.450) p=1.96e-14	1.549 (1.326 - 1.809) p=3.32e-08	1.896 (1.558, 2.313) p=2.17e-10	1.663 (1.315, 2.116) p=2.83e-05
LEFTY2	1.193 (1.119 - 1.273) p=7.97e-08	1.269 (1.123 - 1.435) p=1.38e-04	1.476 (1.260, 1.729) p=1.38e-06	1.396 (1.146, 1.699) p=9.01e-04
TAGLN	1.258 (1.176 - 1.346) p=2.27e-11	1.568 (1.380 - 1.780) p=4.40e-12	1.944 (1.637, 2.311) p=4.04e-14	1.573 (1.266, 1.956) p=4.32e-05
GABARAP	1.303 (1.241 - 1.368) p=1.46e-26	1.241 (1.135 - 1.357) p=2.25e-06	1.355 (1.185, 1.547) p=6.26e-06	1.306 (1.116, 1.510) p=3.89e-04
CST3	1.307 (1.222 - 1.397) p=3.47e-15	1.461 (1.250 - 1.707) p=1.96e-06	2.074 (1.696, 2.538) p=1.28e-12	1.773 (1.385, 2.271) p=5.35e-06
TNFRSF1A	1.322 (1.240 - 1.410) p=1.23e-17	1.469 (1.273 - 1.696) p=1.42e-07	1.907 (1.588, 2.294) p=5.73e-12	1.486 (1.177, 1.877) p=8.75e-04
NBL1	1.265 (1.203 - 1.330) p=3.20e-20	1.356 (1.191 - 1.544) p=4.21e-06	1.771 (1.504, 2.087) p=7.16e-12	1.477 (1.194, 1.822) p=2.84e-04
FSTL3	1.358 (1.270 - 1.452) p=2.88e-19	1.637 (1.439 - 1.863) p=7.48e-14	1.422 (1.194, 1.687) p=6.61e-05	1.560 (1.259, 1.926) p=3.91e-05
GDF15	1.511 (1.412 - 1.616) p=2.94e-33	1.444 (1.276 - 1.634) p=5.96e-09	1.518 (1.306, 1.762) p=4.38e-08	1.443 (1.180, 1.761) p=3.23e-04
TMED10	1.226 (1.156 - 1.299) p=9.53e-12	1.366 (1.228 - 1.520) p=9.57e-09	1.380 (1.185, 1.603) p=2.26e-05	1.434 (1.165, 1.749) p=3.97e-04
RNASE1	1.354 (1.275 - 1.437) p=2.38e-23	1.507 (1.308 - 1.737) p=1.45e-08	1.786 (1.486, 2.148) p=6.29e-10	1.613 (1.293, 2.009) p=1.90e-05
EFEMP1	1.332 (1.254 - 1.414) p=5.60e-21	1.348 (1.198 - 1.516) p=6.56e-07	1.485 (1.273, 1.729) p=4.20e-07	1.518 (1.241, 1.856) p=4.74e-05
TWSG1	1.232 (1.167 - 1.300) p=3.53e-14	1.343 (1.204 - 1.499) p=1.32e-07	1.508 (1.300, 1.745) p=4.21e-08	1.467 (1.217, 1.763) p=5.05e-05
TREM1	1.254 (1.176 - 1.338) p=6.69e-12	1.346 (1.202 - 1.508) p=2.68e-07	1.573 (1.357, 1.822) p=1.57e-09	1.495 (1.246, 1.792) p=1.37e-05
WFDC1	1.253 (1.168 - 1.344) p=3.33e-10	1.370 (1.204 - 1.558) p=1.68e-06	1.491 (1.266, 1.758) p=1.78e-06	1.591 (1.298, 1.951) p=7.97e-06

Figure 1. Analysis outline. Each step analyzes proteomics as the exposure. The first stage assesses proteomics and incident HF post Visit 3 and then post Visit 5. The second stage assesses HF-associated proteins with prevalent and then incident frailty. Lastly, HF- and frailty associated proteins are assessed cross-sectionally with echo and non-echo phenotypes, longitudinally with incident HFpEF and HFrEF, and longitudinally for incident frailty by components (Fried score). Proteins meeting Bonferroni level significance for each step are included in the next step.

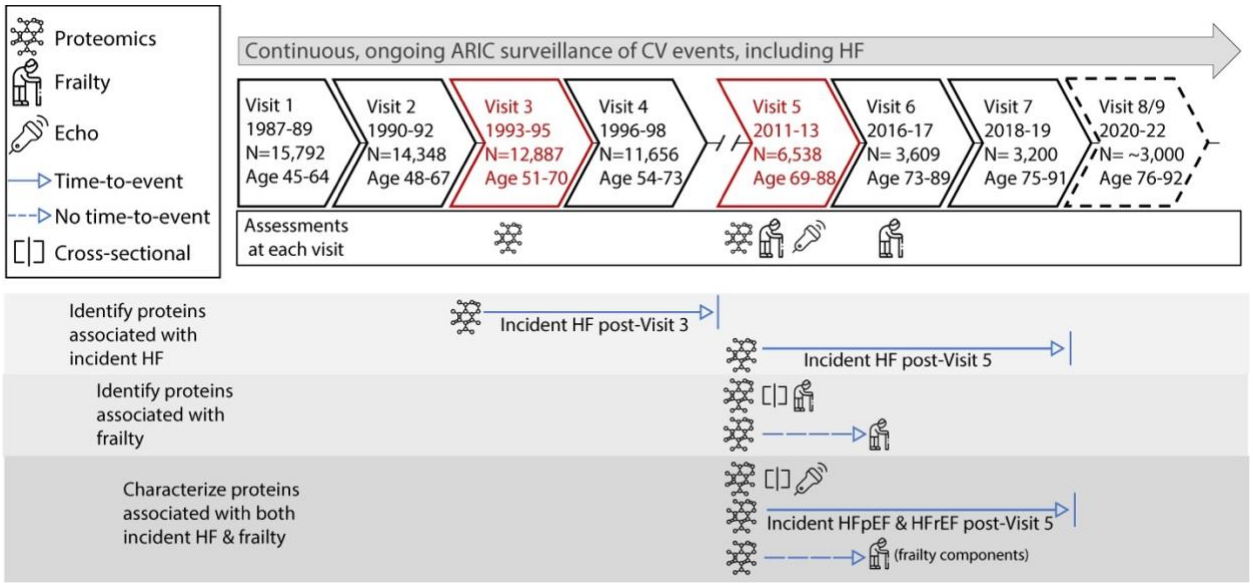


Figure 2. Panel A: Volcano plot showing the final 18 HF-associated candidate proteins and their association with incident frailty with cutoffs at <0.05 and Bonferroni significance. Panel B: Hazard Ratio- Odds Ratio plot showing risk for incident HF (after visit 5) and frailty (from visit 5 to visit 6). Risk for incident HF is expressed as hazard ratio and risk for incident frailty as odds ratio.

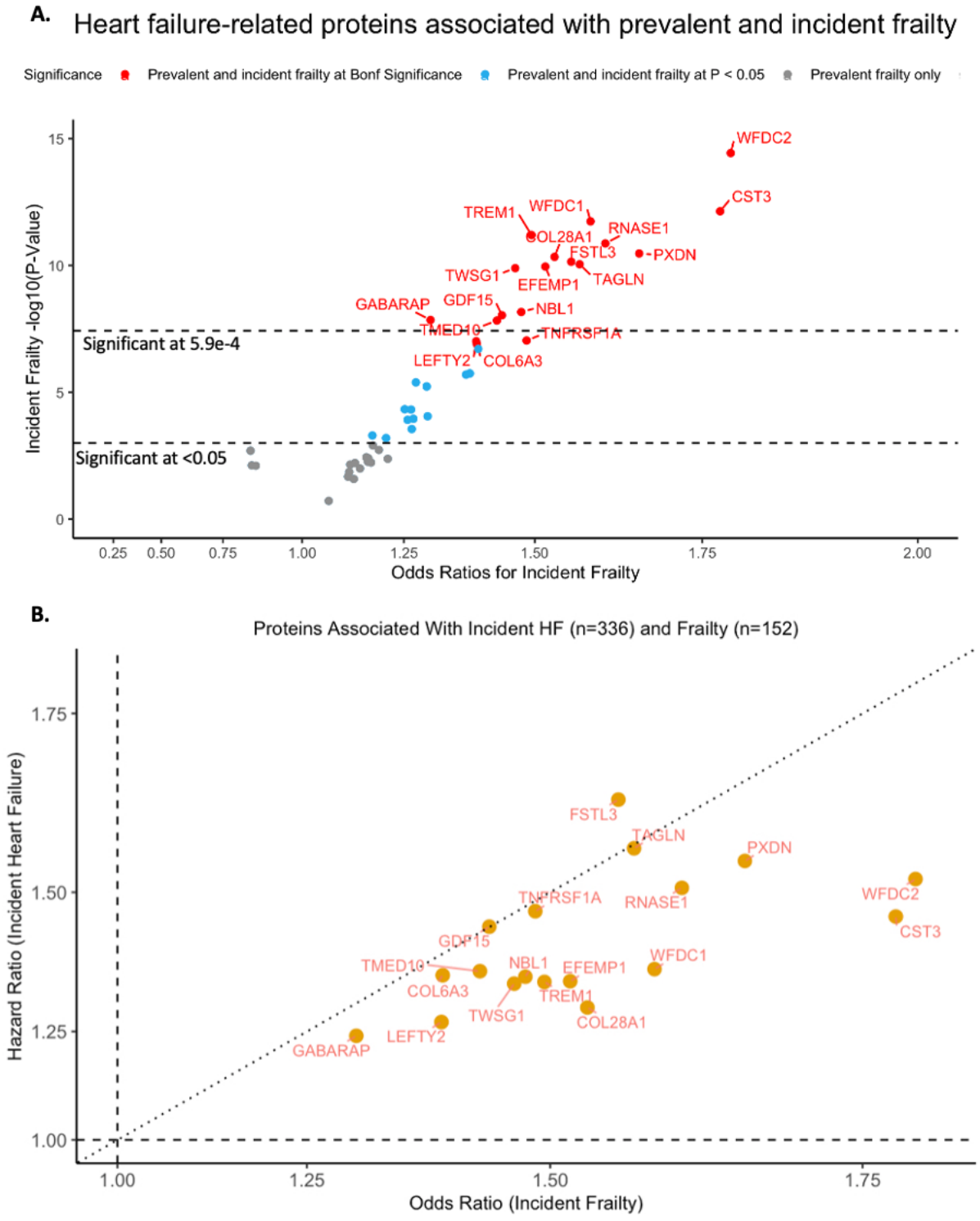
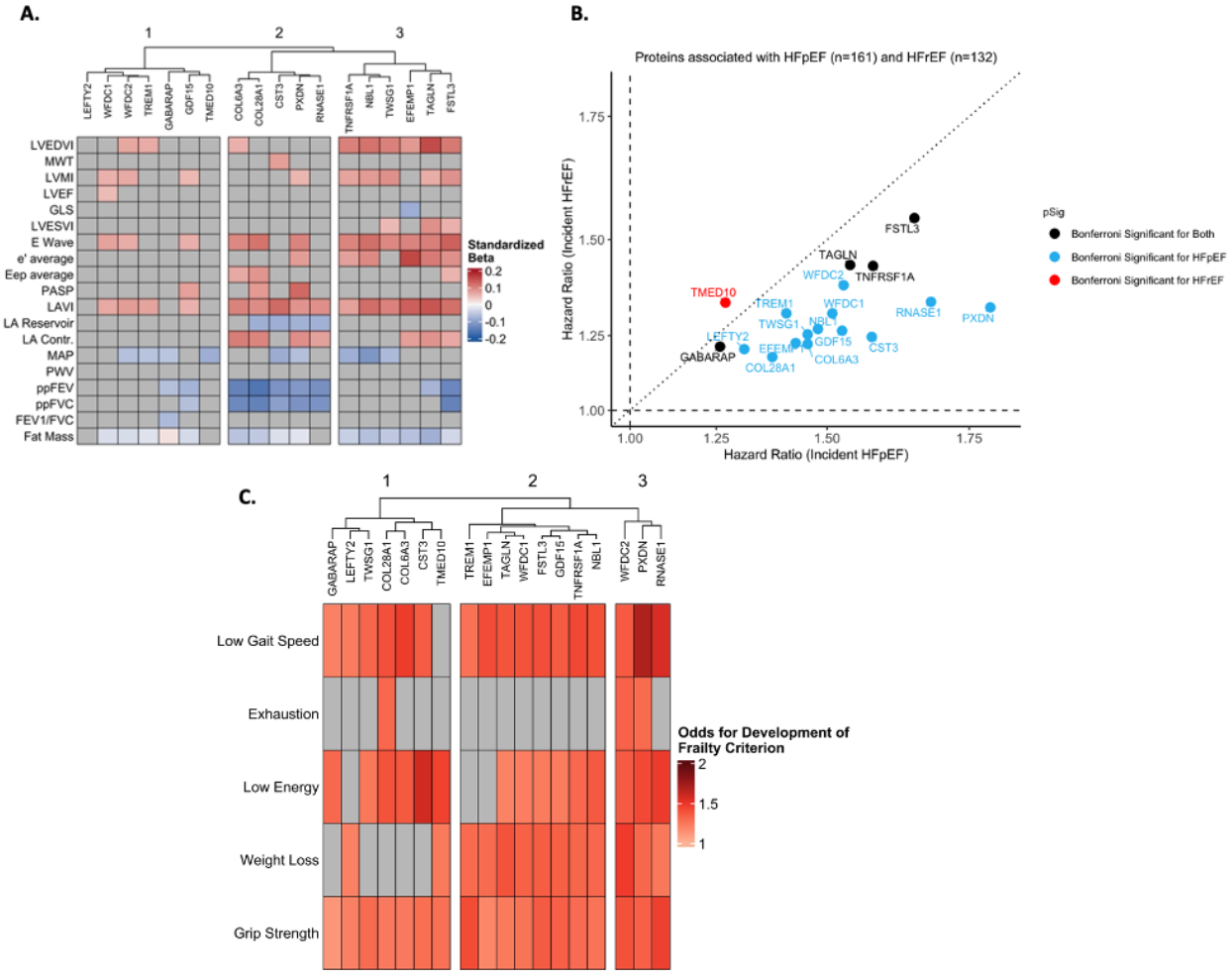
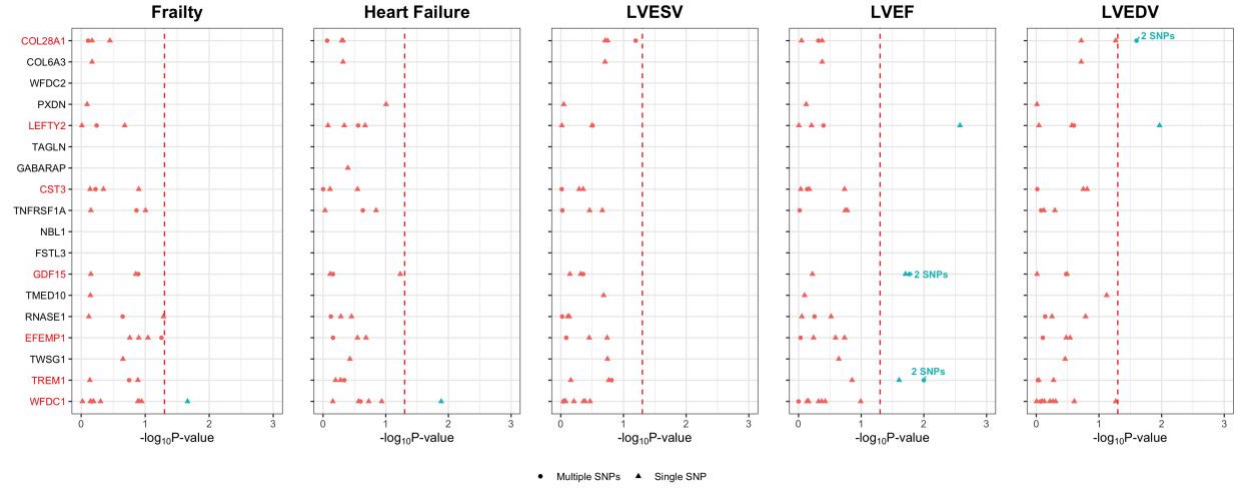


Figure 3. Panel A: Heatmap of the cross-sectional association of candidate proteins and echocardiographic and non-echocardiographic phenotypes at visit 5. Panel B: Hazards Ratio-Hazards Ratio plot using the 18 candidate proteins and their association with incident HFpEF and HFrEF after visit 5. Panel C: Heatmap of incident frailty (visit 5 to visit 6) by each component's association with candidate proteins.



LVEDVI= Left Ventricular End Diastolic Volume Index, MWT= Mean Wall Thickness, LVMI= Left Ventricular Mass Index, LVEF= Left Ventricular Ejection Fraction, GLS= Global Longitudinal Strain, LVESVI= Left Ventricular End Systolic Volume Index, e' average= average measurement between lateral and medial e', Eep average = E/e' average between lateral and medial E/e', PASP= Pulmonary Artery Systolic Pressure, LA Reservoir= Left Atrium Reservoir, MAP= Mean Arterial Pressure, PWV= Pulse Wave Veloicity, ppFEV= % predicted Forced Expiratory Volume, ppFVC= % predicted Forced Vital Capacity.

Figure 4. Manhattan-type plot showing the causal association between SNPs, and frailty, heart failure, and cardiac structure and functional measures single SNP and multimarker association were found.



LVESV= left ventricular end systolic volume, LVEF= left ventricular ejection fraction, LVEDV= left ventricular end diastolic volume. Red gene indicates causal signal found.

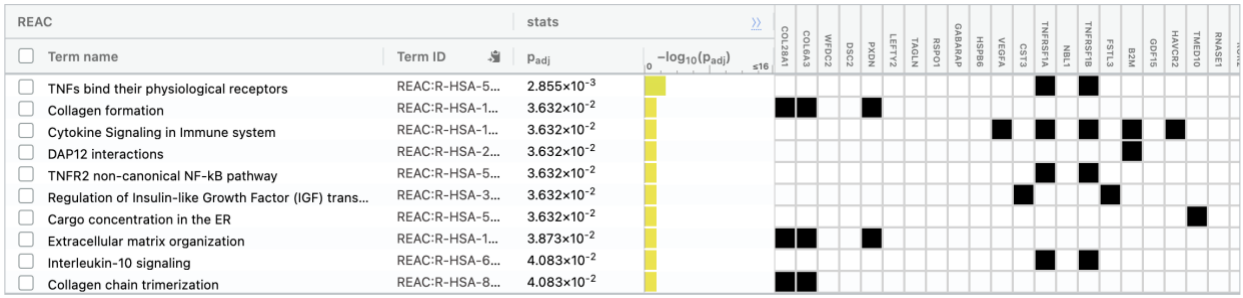
Supplement

Supplementary Table 1. Visit 5 baseline characteristics by frailty status. P-value indicates trend across frailty categories.

Variable	Robust (n=1989)	Pre-frail (n=1919)	Frail (n=223)	p-value (trend)
Age, mean (SD), years	77.7 (5.47)	76.20(5.19)	74.1 (4.40)	<0.0001
Black Race, n(%)	293 (14.73)	345 (17.98)	54 (24.22)	0.0002
Male, n(%)	909 (45.70)	755 (39.34)	69 (30.94)	<0.0001
BMI, mean, (SD) kg(m^2)	29.7 (7.03)	28.5 (5.61)	28.1 (4.82)	0.0001
Smoke, n(%)	103 (5.18)	121 (6.31)	20 (8.97)	0.0449
CAD, n(%)	253 (12.72)	312 (16.26)	40 (17.94)	0.0027
DM2, n(%)	590 (29.66)	734 (38.25)	111 (49.78)	<0.0001
A-fib, n(%)	85 (4%)	99 (5%)	12 (5%)	0.3857
Hypertension, n(%)	1531 (77%)	1586 (83%)	202 (91%)	<0.0001
*eGFR, mean ± SD, ml/min/1.73m^2	73 [62, 84]	72 [58, 83]	64 [54, 81]	<0.0001

*Indicates median and interquartile range (IQR).

Supplementary Figure 1. Functional profiling of the 31 frailty- and HF-associated candidate proteins.



SUMMARY OF CONCLUSIONS

There is an intricate interplay between frailty and cardiac dysfunction that may be perpetrated by shared underlying mechanisms. Our analyses characterize and disentangle this complex association by leveraging subsequent visits from the ARIC cohort. The study design facilitates the understanding and supports the hypothesis of a common pathobiology between frailty and cardiac dysfunction.

While the cross-sectional association between cardiac dysfunction in the form of subclinical decline in heart function or overt HF is well documented, our data additionally suggests a longitudinal and bidirectional relationship between these two entities. Our study supports the previous literature describing the co-existence of subclinical cardiac dysfunction and frailty. Moreover, our investigation adds that frailty is associated with worse cardiac structure and function in later life. However, contrary to what we had hypothesized, frailty likely results in little or no difference in the rate of cardiac structural and functional decline, as exemplified by the lack of difference in change from Visit 5 to Visit 7 across frailty categories. Nonetheless, our findings indicate that individuals who progress to worse frailty category over time have a simultaneous worsening of cardiac structure and function. The reverse longitudinal association between cardiac dysfunction and frailty was also evident in our findings. Our analysis reveals that subclinical structural and functional decline measured by echocardiography results in an increased risk for progression in frailty status in later life independently of other traditional cardiovascular risk factors. Together, these findings provide novel insights and builds on prior knowledge regarding the interrelation between frailty and cardiac dysfunction.

High throughput proteomic analysis provides a unique opportunity to identify specific molecular mechanisms and pathways that permeate the undermining common pathophysiology linking frailty and cardiac dysfunction. We analyzed ~5,000 serum proteins at two timepoints during late life using a statistically rigorous approach to pinpoint candidate proteins that associate with both incident HF and incident frailty. This investigation discovered 18 proteins that robustly associate with both outcomes. Ingenuity pathway analysis revealed these proteins associate with pathways of inflammation and collagen synthesis, suggesting these may be instrumental in the common pathobiology of frailty and HF. There was a stronger magnitude in the relationship with incident frailty, compared to incident HF. Moreover, increased serum levels of most of these proteins result in a heightened risk for incident HFpEF, and to a lesser extent HFrEF. This finding was further reflected by a strong association of candidate proteins with individual measurements of cardiac structure and diastolic function. Through Mendelian randomization, a causal inference tool, we uncovered associations between single nucleotide polymorphisms (SNPs) in the gene WFDC1 gene with both incident frailty and HF, as well as other genes associated with measures cardiac structure and function.

DISCUSSION AND PERSPECTIVES

There are limitations to our investigation that must be considered to interpret the results. Our study design is that of an observational prospective cohort. Therefore, our study is subject to unmeasured confounding that may bias our results towards or away from the null hypothesis. In addition, this study design impedes causal inference in our results and conclusions. We attempted to address this limitation in the second study by incorporating Mendelian Randomization into our results, which utilizes natural random variability in genetic recombination and allows for causal or near-causal conclusions. Another important limitation relies on the definition of frailty we used. While the Fried score is the most widely cited in the scientific literature, it is unknown if our findings are replicable in cohorts where a different definition was applied. Nonetheless, the validity of this tool in our cohort is robust, as it has been previously operationalized and widely studied. Another limitation stems from the generalizability of our results. Our analysis only includes persons from the ARIC study, where participants are healthier and have better access to healthcare than other populations. Therefore, it is unclear if our findings are replicable in other populations outside the ARIC cohort. Nonetheless, ARIC has five study centers and has a wide variety of ethnicity, socioeconomic status, and geographic location within the United States. Participant follow-up consistent throughout study visits. A significant proportion of individuals were censored between visits either because of non-survival or because of non-attendance. Presumably, censoring is differential towards a more morbid population. This limitation may introduce selection bias into our study and potentially bias our results towards the null. Yet, our study identified robust associations within our exposures and outcomes despite this limitation.

Our study sets a novel paradigm of association between frailty and cardiac dysfunction. However, there are many questions that have yet to be answered, and new questions arise. Our study revealed that older individuals who progress in frailty also have faster decline in cardiac function. Whether individuals who regress in frailty category have a slower progression in cardiac functional decline than those who remain in the same category was not captured by our study. This clinical question may be evaluated by future studies with higher sample size and a higher proportion of individuals who regress in frailty status. Another question that arises from our findings is whether there are effect modifying factors that may play a role in this association, for example gender, habits, or medication use. Lastly, our study explored potential mechanistic pathways that may explain a common pathobiology between frailty and cardiac dysfunction. These findings provide a foundation for interventional studies to hypothesize and develop drug targets to improve patient outcomes.

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