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ORIGINAL RESEARCH - CLINICAL

Proteomic Profile of the *ICAM1* p.K56M HFpEF Risk Variant

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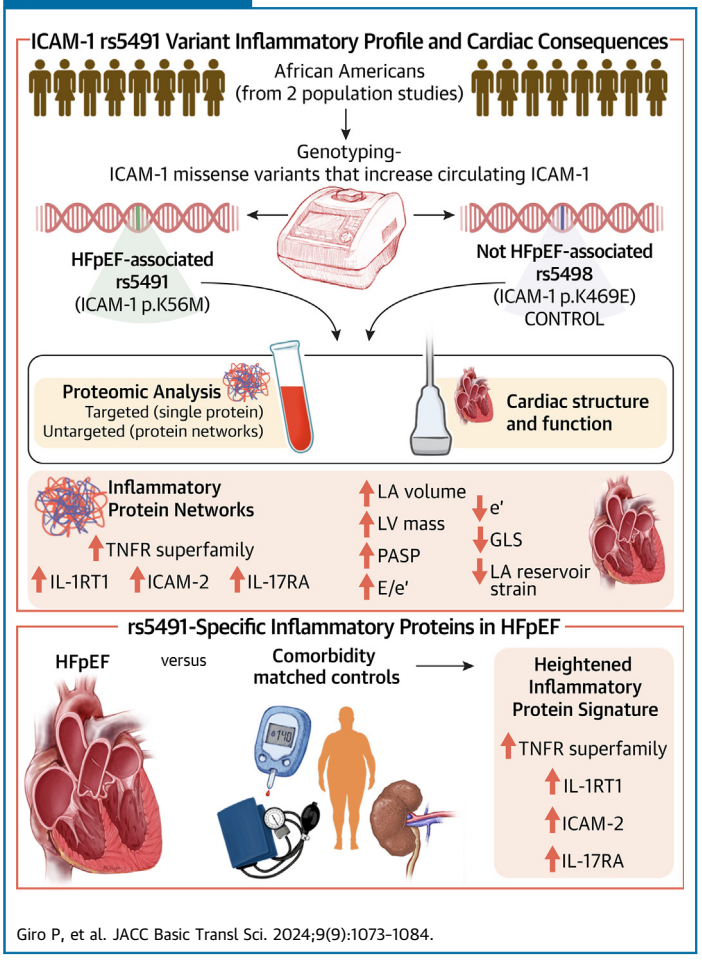
VISUAL ABSTRACT



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HIGHLIGHTS

- **The *ICAM1* pK56M (rs5491) HFpEF risk variant is associated with several individual inflammatory proteins and an inflammatory protein network, which may identify immune pathways that drive HFpEF.**
- **rs5491-specific protein networks are associated with derangements in several measures of cardiac structure and function that are commonly seen in HFpEF.**
- **Patients with HFpEF have higher levels of rs5491-specific inflammatory proteins than do comorbidity-matched control patients.**

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ABBREVIATIONS AND ACRONYMS

FDR = false discovery rate

HFpEF = heart failure with preserved ejection fraction

ICAM = intercellular adhesion molecule

LA = left atrial

LTBR = lymphotoxin β receptor

LV = left ventricular

TNF = tumor necrosis factor

WCNA = weight co-expression network analysis

SUMMARY

A common missense variant in *ICAM1* among African American individuals (rs5491; pK56M) has been associated with risk of heart failure with preserved ejection fraction (HFpEF), but the pathways that lead to HFpEF among those with this variant are not clear. In this analysis of 92 circulating proteins and their associated networks, we identified 7 circulating inflammatory proteins associated with rs5491 among >600 African American individuals. Using weighted coexpression network analysis, 3 protein networks were identified, one of which was associated with rs5491. This protein network was most highly represented by members of the tumor necrosis receptor superfamily. The rs5491 variant demonstrated an inflammatory proteomic profile in a separate cohort of African American individuals. This analysis identifies inflammatory pathways that may drive HFpEF among African American individuals with the *ICAM1* pK56M (rs5491) variant. (JACC Basic Transl Sci. 2024;9:1073–1084)

Immune dysregulation may play an important role in the pathogenesis of some heart failure (HF) syndromes, but exact mechanisms remain poorly understood.[1,2] Upregulation of cellular adhesion molecules has been hypothesized as a key biological event that leads to certain HF syndromes. Chronic comorbidity-induced systemic inflammation may increase the expression of cellular adhesion molecules, resulting in inflammatory cascades that may precipitate HF.[3,4] Intercellular adhesion molecule (ICAM)-1 is a specific cell-surface protein that has been implicated in the development of HF with preserved ejection fraction (HFpEF).[5-8]

A common missense genetic variant of *ICAM1* among African American individuals (rs5491; p.Lys56Met) has been associated with higher levels of ICAM-1 and an increased risk of incident HFpEF.[9,10] Despite these associations, the exact biological pathways by which rs5491 may increase the risk for HFpEF remain unclear. To investigate pathways potentially driving this relationship, we used several complementary methods to perform a proteomic analysis of rs5491. First, we evaluated the proteomic profile of rs5491 cross-sectionally and determined the associations of rs5491-specific protein networks with cardiac function on echocardiography among African American individuals. We next compared the proteomic profile of rs5491 with another *ICAM1* missense variant (rs5498) that has also been associated with higher ICAM-1 levels but not with HFpEF.[11,12] We subsequently evaluated the differences in rs5491-specific protein levels among patients with prevalent HFpEF compared with patients who had cardiometabolic comorbidities but were without HFpEF. We hypothesized that rs5491 is associated with an inflammatory proteomic profile.

METHODS

STUDY POPULATIONS. The MESA (Multi-Ethnic Study of Atherosclerosis) study is a cohort of community-dwelling adults (aged 45-85 years) who were enrolled from 6 field centers in the United States to better understand the risk factors and prevalence of subclinical cardiovascular disease. The details of this cohort have been described in detail elsewhere.[13] The study enrolled participants without existing clinical cardiovascular disease from 2000 to 2002.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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Cardiovascular disease was defined as a history of myocardial infarction, angina, stroke, transient ischemic attack, HF, atrial fibrillation, nitroglycerin use, angioplasty, pacemaker or implantable cardioverter defibrillator, or cardiac surgery. The rs5491 variant is common in African American individuals (minor allele frequency ~20%) but rare in other race/ethnic groups. Additionally, the association between rs5491 and HFpEF has been demonstrated in African American populations alone. As such, we analyzed only African American individuals in MESA. The MESA study had 6,814 participants at baseline, of whom 1,891 identified as African American. Among these participants, 670 individuals attended Exam 6 and had both proteomic data and genotyping data available, which constitutes the final analytic cohort. The study protocol was approved by the institutional review board of each site. All MESA participants signed informed consent.

Details regarding the Northwestern cohort and the ARIC (Atherosclerosis Risk In Communities) cohort are fully described in the Supplemental Methods and elsewhere.[14,15] Briefly, the Northwestern cohort consisted of patients with HFpEF and control patients who were enrolled from outpatient clinics at Northwestern University between November 2013 and May 2017. Details regarding HFpEF diagnostic criteria have been previously described and can be found in the Supplemental Methods. Control patients had ≥1 cardiovascular risk factor but did not have a history of HF.

GENOTYPING. Genotyping in MESA was performed at the Broad Institute using the Affymetrix Genome-Wide SNP Array 6.0 according to manufacturer's recommendations. Genotyping and quality control procedures have been previously described.[16,17] Further details regarding genotyping, including imputation methods, can be found in the Supplemental Methods. In MESA, principal component (PC) analysis was performed using EIGENSTRAT within each race/ethnic group. Both rs5491 and rs5498 were imputed.

PROTEOMICS. Plasma samples collected during MESA Exam 6 that were stored at −80 °C were used for proteomic analysis. Plasma proteomic measurements were performed using the Olink Target 96 Cardiovascular III panel (Uppsala, Sweden), which consisted of 92 unique proteins. The Olink Proseek multiplex immunoassay uses antibody pairs linked to DNA strands that specifically target certain molecules. When antibody-DNA pairs bind to their target, it triggers a polymerase chain reaction in a proximity-dependent manner. Normalized protein units are expressed on a log2 scale, where a 1-unit increase corresponds to a doubling of protein concentration. The Olink website provides information on the reproducibility and validation of the proteins analyzed in the Olink Target 96 Cardiovascular III panel. A list of the proteins included in the analysis can be found in Supplemental Table 1. The Olink Target 96 Cardiovascular III panel was also measured in the Northwestern cohort. In the ARIC cohort, proteins were measured using the SOMAScan v4 assay at visit 2, as previously described (Supplemental Methods).[18]

ECHOCARDIOGRAPHY. During MESA Exam 6, comprehensive 2-dimensional echocardiography with Doppler imaging was performed using a commercially available ultrasound system with harmonic imaging (GE EchoPAC software version 201, GE Healthcare). The frame rate for all 2-dimensional images ranged from 50 to 80 frames.

In accordance with previous societal guideline recommendations, cardiac chamber quantification was measured. To do this, left ventricular (LV) volumes, LV ejection fraction, and left atrial (LA) volumes were measured using the biplane method of disks from apical 4- and 2-chamber views, and LV mass was calculated using the Devereux formula. Tissue Doppler was conducted at the septal and lateral mitral annulus during early diastole (e′ velocities).

Speckle-tracking echocardiography of the LA and LV were carried out by a skilled sonographer who was blinded to other clinical data using GE EchoPAC software. To verify their accuracy, 2 cardiologists with expertise in echocardiography reviewed the strain curves. The endocardial border of each cardiac chamber was traced, and regions of interest spanning epicardium to endocardium were defined. Then, standard anatomical segments of each cardiac chamber were defined by the program to conduct regional speckle-tracking analysis. Subsequently, the speckle-tracking software produced curvilinear graphs of strain measurements over time that corresponded to the anatomical segments of the chamber of interest, along with a curve representing the average of the segments. All strain measurements were reported as absolute values, where lower strain values indicated worse function. LA strain was calculated using strain measurements from apical 4- and 2-chamber views, and reservoir strain was defined as the sum of booster and conduit strain. LV global longitudinal strain was determined as the average strain from apical 4-, 3-, and 2-chamber views, and LV circumferential strain was obtained from the LV midchamber short-axis view.

STATISTICAL ANALYSIS. Continuous variables are presented as the mean ± SD, and categorical variables

as count (%). Baseline characteristics were compared between participants with and without at least 1 copy of the rs5491 variant. Differences in continuous variables were assessed using 2-sample *t*-tests, and chi-square and Fisher exact tests were used for categorical variables. Normality was assessed using Shapiro-Wilk tests for continuous variables.

Targeted and untargeted approaches were used to investigate the associations between the rs5491 variant and proteomic profiles. The rs5491 was modeled in a dominant fashion for all models. In the targeted approach, we evaluated the association between rs5491 and 15 prespecified inflammatory proteins using single-protein regressions. Covariate adjustments were made for age, gender, and the first 3 principal components of ancestry. Multiple comparison corrections were applied using the false discovery rate (FDR) method. Principal component analysis (PCA) was conducted among the 15 inflammatory proteins to reduce dimensionality and identify principal components (PCs) that captured the largest proportion of the variance. Associations between rs5491 and the first three PCs were assessed using linear regression models adjusted for age, gender, and ancestry principal components.

For the untargeted approach, the unbiased protein signatures of rs5491 were explored by investigating associations between the variant and all 92 proteins. Weighted co-expression network analysis (WCNA) was used to cluster proteins based on their interrelationships using the WGCNA package in R.[19] Briefly, WCNA is a systems biology method that identifies clusters (modules) of highly correlated genes or proteins, which are assumed to be functionally related or involved in similar biological processes. The analysis involves constructing a topological overlap matrix, which measures the similarity of the co-expression relationships between pairs of proteins. The first principal component (eigengene) is then calculated for each cluster, summarizing the overall expression pattern within the module. Associations between rs5491 and each cluster's eigengene were then evaluated using linear regression models adjusted for age, gender, and the first 3 principal components of ancestry. In WCNA, protein networks are identified by color. In sensitivity analysis, we further adjusted for 6-minute walk distance and e′ septal velocity at Exam 6 in linear regression models evaluating associations of rs5491 with top inflammatory proteins and the turquoise protein network. All regression results are presented as regression coefficient (β) with standard error (SE) or 95% CI.

Differential network analysis was then conducted to identify specific protein interactions within the WCNA networks that were differentially associated with the presence of rs5491 using the iDINGO package in R.[20] This package identifies differential networks by comparing partial correlation coefficients between 2 groups (rs5491+ and rs5491−) while adjusting for covariates. It highlights specific protein interactions that are stronger or weaker between the groups, revealing potential key differences in network architecture.

To better understand how these proteins may affect cardiac function and structure, associations between individual proteins, protein networks, and echocardiographic measures of cardiac structure and function were evaluated using correlation heatmaps and linear regression models adjusting for covariates described above. Functional enrichment analysis was also performed to identify the biological themes and pathways most likely to be affected by rs5491.[21] The module membership metric was used to rank proteins, and multiple comparison corrections were applied using the FDR method.

To understand whether the associations between rs5491 and the circulating proteome were specific to the rs5491 or were reflective of its relationship to higher circulating ICAM-1 levels, we also used the methods described above to evaluate the proteomic profile of another *ICAM1* missense variant (p.Lys469Glu; rs5498). The rs5498 variant has been demonstrated to be associated with higher circulating ICAM-1 levels in several race/ethnic groups in previous investigations but is not associated with HFpEF.[11,12]

We used the ARIC cohort to validate whether rs5491 carried an inflammatory proteomic profile. Of the 92 proteins on the Olink Target 96 Cardiovascular III panel, 87 were also on the SOMAScan v4 assay. Associations between rs5491 and these 87 proteins at ARIC visit 2 were subsequently evaluated using linear regression models, adjusting for age, gender, and principal components of ancestry 1-3.

To understand whether the proteins identified to be associated with rs5491 were specific to HFpEF as opposed to cardiometabolic risk factors, we leveraged the Northwestern cohort to evaluate the associations of prevalent HFpEF with top proteins associated with rs5491, using linear regression models. Models were adjusted for age, gender, and estimated glomerular filtration rate.

All statistical analyses were performed using R statistical software v4.0.3 (R Core Team). A P value <0.05 was considered statistically significant.

RESULTS

CHARACTERISTICS OF PARTICIPANTS. The characteristics of the analytic cohort at Exam 6 stratified by rs5491 status are shown in Table 1. Of 670 participants, 250 (37.3%) had at least 1 copy of rs5491. Participants with at least 1 copy of rs5491 were younger and had slightly higher body mass index.

ASSOCIATIONS OF RS5491 WITH INFLAMMATORY PROTEINS: TARGETED APPROACH. We hypothesized that rs5491 would be most likely to carry an inflammatory proteomic profile. Using functional annotation from publicly available databases, we identified 15 of the 92 proteins as involved in inflammation to subsequently evaluate in single-protein regressions (Supplemental Table 1). Among these 15 prespecified inflammatory proteins, rs5491 was significantly associated with higher levels of 7 proteins after covariate adjustment and controlling for multiple comparisons using FDR correction (Table 2), including 4 tumor necrosis factor (TNF) receptor superfamily members, interleukin-1 receptor type 1, interleukin-17 receptor A, and intercellular adhesion molecule-2. Subsequently, principal component analysis was performed among the 15 prespecified inflammatory proteins. We assessed only the first 3 principal components (PC1-3), because additional components contributed minimal incremental variance. PC1 accounted for 40% of the variance in these 15 proteins. rs5491 was significantly associated with PC1 after adjustment for age, gender, and ancestry PC (Table 3) but was not associated with PC2 or PC3.

ASSOCIATIONS OF RS5491 WITH PROTEIN NETWORKS: UNTARGETED APPROACH. To identify an unbiased protein signature of rs5491, we next evaluated associations of rs5491 with all 92 proteins. We first visualized protein levels by rs5491 genotype status through a heatmap (Figure 1) and performed linear regression analyses between rs5491 and all 92 proteins (Supplemental Table 2), which demonstrated substantial heterogeneity in several proteins. Since pathways between rs5491 and HFpEF are likely complex and dependent on networks of proteins as opposed to individual proteins, we used weighted co-expression network analysis (WCNA) to then cluster the 92 proteins on the basis of their interrelationship. WCNA identified 3 distinct clusters of proteins, labeled as the following colors: turquoise, blue, and gray (Figure 2A). The turquoise cluster (Figure 2B) (main hub proteins: tumor necrosis factor-receptor 1 [TNF-R1] and tumor necrosis factor-receptor 2 [TNF-R2]) contained 64 proteins, many of which were reflective of systemic inflammation and included 13 of the 15 prespecified inflammatory proteins from our targeted approach. After clusters were identified, principal component analysis was used to calculate 1 eigenvalue summarizing all proteins within each cluster. The presence of at least 1 copy of rs5491 was significantly associated with the turquoise cluster (Table 3, Figure 3) but was not associated with the blue or gray cluster after covariate adjustment. Given the association between rs5491 and the turquoise protein cluster, we next aimed to identify whether

TABLE 1 Baseline Characteristics of Analytic Cohort at Exam 6 of MESA

	rs5491 - AA (n = 420)	rs5491 - AT or TT (n = 250)	*P* Value
Age, y	75 ± 9	73 ± 8	0.004
Male	189 (45.0)	115 (46.0)	0.86
BMI, kg/m^2	29.8 ± 6.1	30.8 ± 6.1	0.049
Diabetes mellitus	131 (31.1)	72 (28.8)	0.51
SBP, mm Hg	133 ± 21	134 ± 22	0.47
Antihypertensive medication use	299 (71.5)	186 (74.7)	0.43
Cigarette status			0.78
Never	172 (41.1)	107 (42.8)	
Former	210 (50.2)	125 (50.0)	
Current	36 (8.6)	18 (7.2)	
Total cholesterol	186 ± 41	184 ± 44	0.60
Hemoglobin A1c, %	6.2 ± 1.1	6.14 ± 1.0	0.90
eGFR, mL/min/1.73m^2	75 ± 21	74 ± 21	0.78

Values are mean ± SD or n (%). A 2-sample *t*-test compared continuous variables, and chi-square or Fisher exact tests compared categorical variables.

AA = no copies of minor allele; AT = 1 copy of minor allele; BMI = body mass index; eGFR = estimated glomerular filtration rate; MESA = Multi-Ethnic Study of Atherosclerosis; SBP = systolic blood pressure; TT = 2 copies of minor allele.

TABLE 2 Associations Between rs5491 and Prespecified Inflammatory Proteins

Protein	β-Coefficient[a]	95% CI	FDR Adjusted *P* Value
TNFRSF14	0.151	0.061-0.241	0.013
TNF-R2	0.137	0.051-0.223	0.013
TNF-R1	0.131	0.046-0.215	0.013
IL-1RT1	0.070	0.022-0.117	0.016
LTBR	0.091	0.022-0.159	0.028
ICAM-2	0.066	0.014-0.118	0.032
IL-17RA	0.138	0.026-0.250	0.034
IL2-RA	0.074	−0.004 to 0.152	0.12
IL-6RA	0.043	−0.007 to 0.093	0.15
MMP-3	0.062	−0.033 to 0.157	0.30
MMP-9	0.097	−0.060 to 0.255	0.31
ITGB2	0.063	−0.053 to 0.179	0.36
TNFSF13B	0.022	−0.041 to 0.084	0.54
IL-1RT2	−0.021	−0.081 to 0.039	0.54
MCP-1	0.008	−0.089 to 0.104	0.88

[a]Adjusted for age, gender, and ancestry PC 1-3.

FDR = false discovery rate; PC = principal component.

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TABLE 3 Associations Between rs5491 and Protein Networks by PCA and WCNA

Protein Network	Top Protein	β-Coefficient[a]	95% CI	*P* Value
PC1	TNF-R2	0.247	0.096-0.398	0.001
PC2	ITGB2	−0.004	−0.159 to 0.150	0.96
PC3	MMP-3	0.055	−0.094 to 0.205	0.47
Blue cluster	JAM-A	0.148	−0.011 to 0.307	0.068
Turquoise cluster	TNF-R1	0.189	0.043-0.336	0.011
Gray cluster	CPA1	0.052	−0.107 to 0.211	0.52

[a]Adjusted for age, gender, and ancestry PC 1-3.

FDR = false discovery rate; PC = principal component; PCA = principal component analysis; WCNA = weight co-expression network analysis.

there were specific protein interactions within the turquoise cluster that were stronger among those individuals with rs5491 through a differential network analysis. We identified a differential network of protein-protein interactions that were specific to those individuals with at least 1 copy of rs5491 compared with those without rs5491 (Figure 2C). For example, TNF-R1 uniquely interacts with soluble urokinase-type plasminogen activator receptor and activated leukocyte adhesion molecule among individuals with rs5491.

RELATIONSHIPS BETWEEN RS5491-SPECIFIC PROTEINS AND CARDIAC STRUCTURE AND FUNCTION. We next evaluated the cross-sectional relationships of proteins and protein networks with cardiac structure and function on echocardiography at Exam 6. Upon evaluation of individual proteins with echocardiographic measures on heatmap analysis, proteins within the turquoise cluster tended to have a phenotype of higher LV filling pressure and worse diastolic function as assessed by E/e′ average, pulmonary artery systolic pressure, and e′ septal velocity (Figure 2A). Individual proteins within the blue and gray clusters did not appear to have strong correlations with echocardiographic measures of structure and function based on heatmap analysis. On evaluation of protein networks with cardiac structure and function, the turquoise cluster was significantly associated with derangements in several measures of cardiac structure and function, including higher LA volume, LV mass, PASP, and E/e′ average, and lower e′ velocity, global longitudinal strain, and LA reservoir strain (Figure 3, Supplemental Table 3). The blue and gray networks did not have strong relationships between cardiac structure and function (Figure 3).

In sensitivity analysis, associations between rs5491 and inflammatory proteins/networks were largely consistent after further adjustment for 6-minute walk test and e′ septal velocity (Supplemental Table 4).

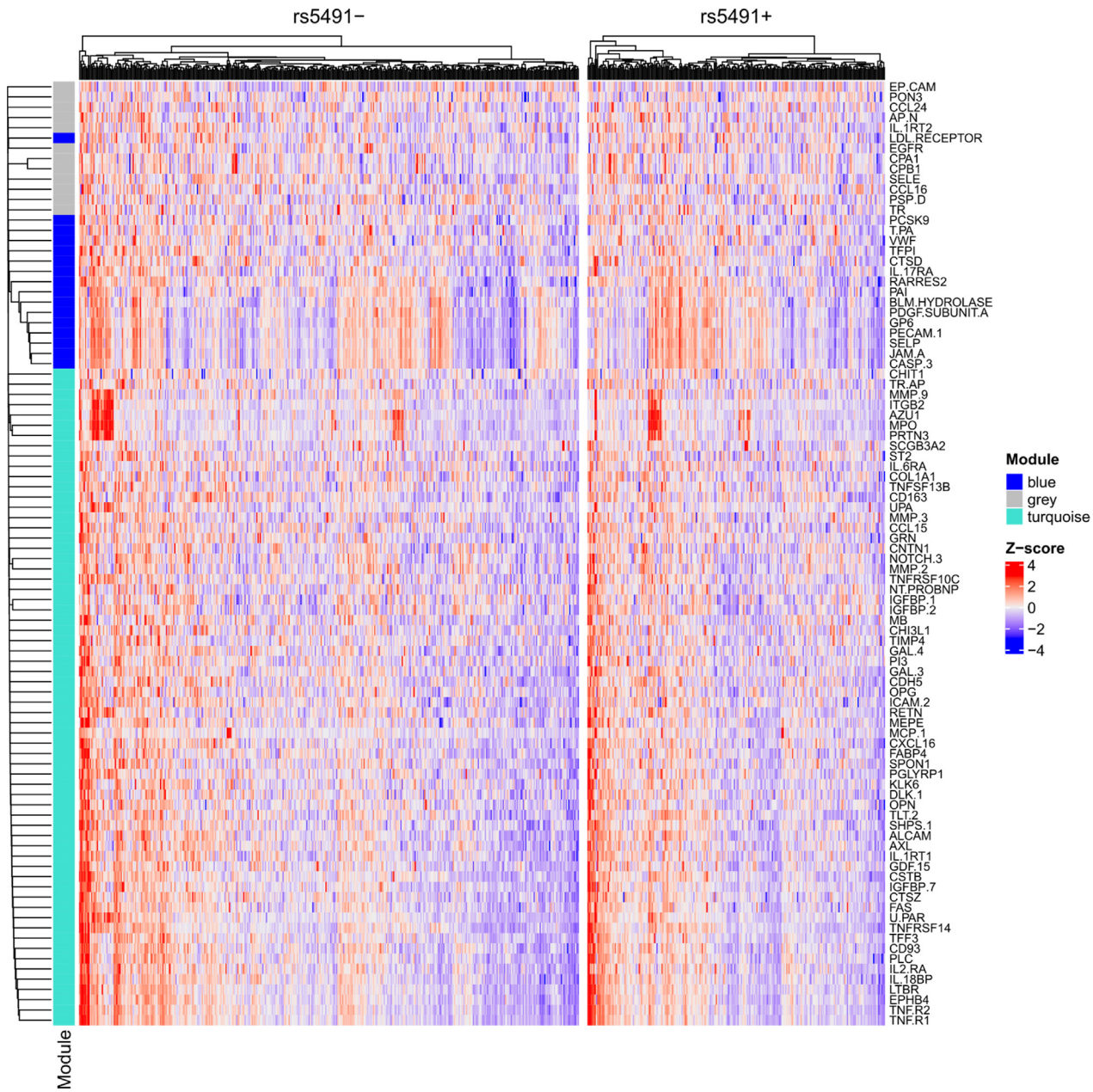
PATHWAY OVER-REPRESENTATION ANALYSIS. To better understand the pathways most likely affected by rs5491, we used a functional enrichment analysis or an over-representation analysis. To remain agnostic, we used a ranked gene set using the turquoise "module membership" as a metric to order proteins from most to least important. The module membership represents the correlation between a protein expression profile and the module eigengene (the first principal component in a given module). In other words, it represents how connected or related a particular protein is compared with all the other proteins of the module. Using this list, gene ontology terms tumor necrosis factor receptor superfamily complex and tumor necrosis factor binding were found within cellular component and molecular function, respectively.

ASSOCIATIONS OF THE RS5498 *ICAM1* MISSENSE VARIANT WITH PROTEIN NETWORKS. To understand whether the associations between rs5491 and the circulating proteome were specific to the variant itself or were reflective of its relationship to higher circulating ICAM-1 levels, we evaluated the proteomic profile of another *ICAM1* missense variant, rs5498. Notably, the effect sizes of rs5491 and rs5498 on circulating ICAM-1 levels are similar in other cohorts.[11] In the analytic cohort (n = 670), 240 (35.8%) individuals had at least 1 copy of rs5498. In contrast to rs5491, heatmap analysis of rs5498 with individual proteins did not reveal strong correlations (Figure 2A), and there were no significant associations between the presence of rs5498 and the WCNA-derived protein networks (Figure 3).

PROTEOMIC PROFILE OF RS5491 IN ARIC COHORT. Among 1,929 African American individuals in the ARIC study who had genotyping for rs5491 and protein profiling using SOMAScan v4 at visit 2, 774 (40.1%) had at least 1 copy of rs5491 (Supplemental Table 5). The presence of ≥1 copies of rs5491 was significantly associated with 10 of 87 proteins (Supplemental Figure 1, Supplemental Table 6). All 10 of these proteins are involved in inflammation and included 1 of the 7 proteins identified in MESA (LTBR) along with a different member of the TNF receptor superfamily (TNF receptor superfamily member 11B).

ASSOCIATION OF HFpEF STATUS WITH RS5491-SPECIFIC INFLAMMATORY PROTEINS. Among 147 patients in the Northwestern cohort, 117 had prevalent HFpEF, and 30 had comorbidities without HFpEF. In comparison with comorbidity-matched control patients, patients with HFpEF were more likely female, were more obese, had less coronary artery disease, and

FIGURE 1 Heatmap of Protein Levels by rs5491 Status Organized by Weight Co-expression Network Analysis Dendrogram



had worse kidney function (Supplemental Table 7). In the Northwestern cohort, patients with HFpEF exhibited significantly higher levels of all 7 rs5491-specific inflammatory proteins than did control patients with comorbidities (**Table 4**).

DISCUSSION

In a cohort of older African American individuals, we characterized the circulating proteomic profile of the *ICAM1* HFpEF risk variant rs5491 (p.Lys56Met). In single-protein regressions evaluating 15 prespecified inflammatory proteins, individuals with at least 1 copy of rs5491 had higher levels of 7 unique circulating inflammatory proteins, 4 of which involved TNF binding. In an unbiased approach of 92 proteins, we identified 3 distinct protein networks by WCNA. We demonstrated that rs5491 was uniquely associated with the turquoise cluster, within which TNF-R1 and TNF-R2 were hub proteins. In the turquoise

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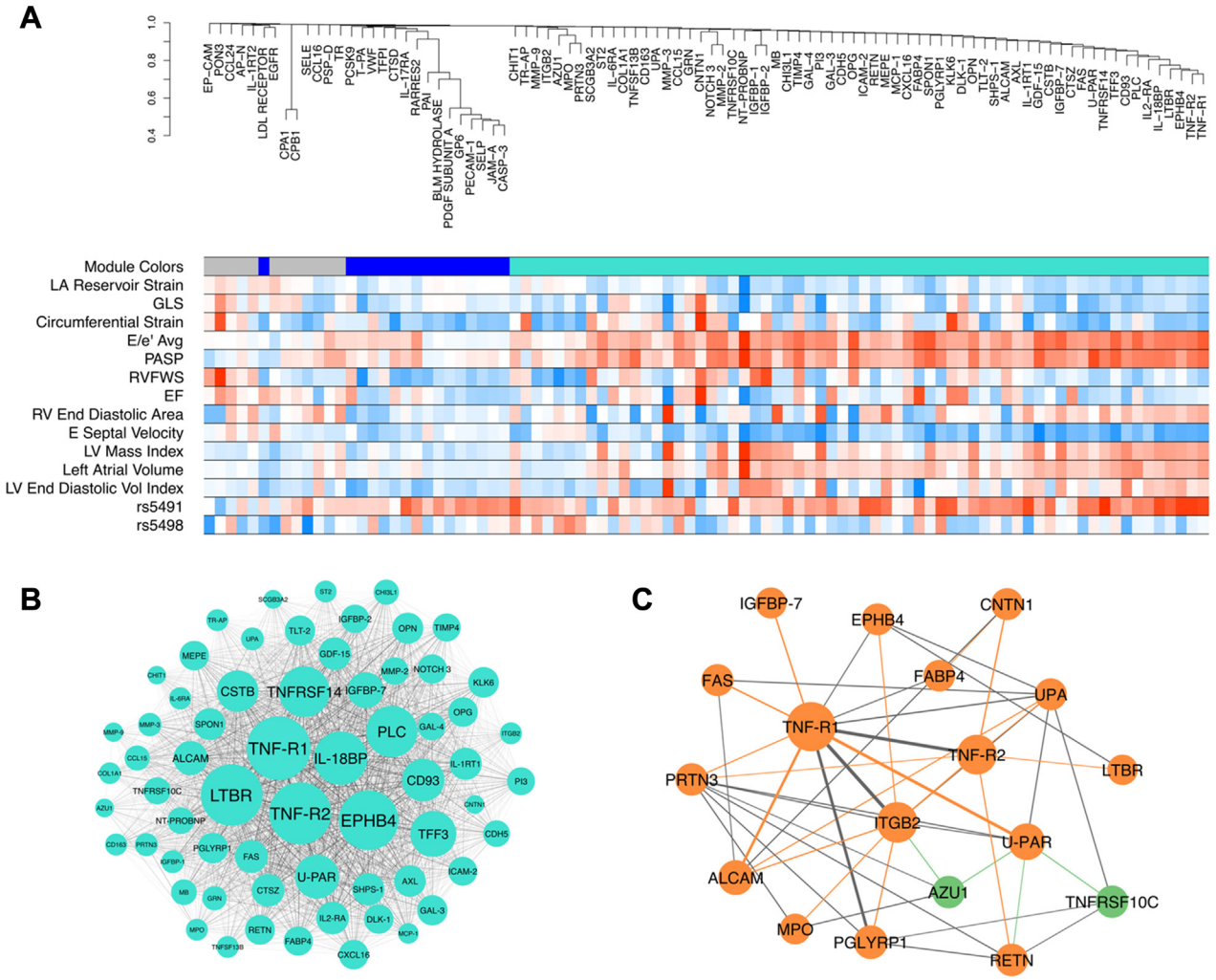
FIGURE 2 **Proteomic Profile of rs5491**



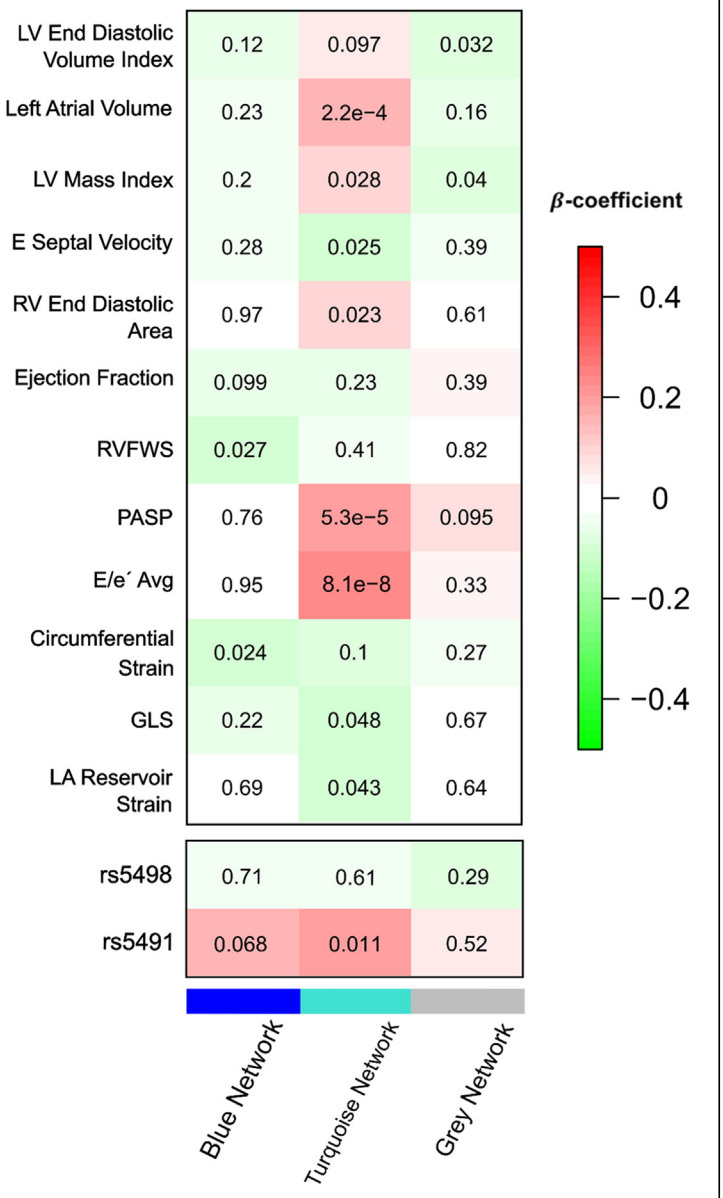
(A) Dendrogram of weight co-expression network analysis protein clusters with a heatmap of unadjusted correlations between each protein and echocardiographic measure, rs5491, and rs5498. (B) Turquoise protein cluster with larger nodes signifying proteins with higher module membership. (C) Differential network of rs5491 within the turquoise protein cluster. Gray lines represent protein-protein interactions that are shared by those with and without rs5491. Orange dots/lines represent proteins/interactions that are specific to individuals with rs5491. Green dots/lines represent proteins/interactions that are specific to individuals without rs5491.

cluster, we identified a differential network of proteins that was more strongly connected among individuals with at least 1 copy of rs5491. Additionally, the turquoise protein cluster was significantly associated with derangements in several measures of cardiac structure and function, whereas the other protein networks (blue and gray) did not carry similar associations. We demonstrated that the proteomic profile of rs5491 does not overlap with that of rs5498, a separate *ICAM1* missense variant that has been associated with higher serum ICAM-1 levels but does not confer a risk of HFpEF. Furthermore, in a separate cohort that leveraged a different proteomic platform, rs5491 carried an inflammatory proteomic profile. Finally, on evaluation of a separate cohort of patients, the 7 inflammatory proteins identified to be associated with rs5491 were higher among HFpEF patients than among control patients with cardiometabolic comorbidities. Taken together, our findings implicate specific inflammatory protein pathways that may be central to HFpEF risk among individuals who carry the rs5491 *ICAM1* missense variant.

Although the *ICAM1* missense variant rs5491 has been implicated in HFpEF development among

African American individuals, specific pathways that may promote HFpEF risk among those with the variant have been unclear. ICAM-1 itself has been hypothesized to drive HFpEF through increased leukocyte transmigration, leading to cardiac inflammation and interstitial fibrosis. Recent genetic epidemiologic analyses have further suggested a central role of ICAM-1 in HFpEF pathogenesis. The rs5491 missense variant leads to a lysine-to-methionine amino acid substitution at position 56 of ICAM-1. This variant is common among African American individuals (MAF >20%) but is relatively rare in other race/ethnic groups (MAF <5%). In 2 large cohorts of African American individuals, rs5491 conferred increased risk of HFpEF after adjustment for cardiovascular risk factors.[9,10] Functional analyses revealed that ICAM-1 protein that harbors p.Lys56Met has reduced binding to lymphocyte function-associated antigen 1 on T cells compared with wild-type ICAM-1 protein.[22] It remains unclear whether this functional effect on lymphocyte binding can explain the association of rs5491 with HFpEF. Furthermore, we are unaware of any studies evaluating the effect of the rs5498 variant on lymphocyte binding. Despite these genetic epidemiologic findings and experimental evidence that suggests a functional effect of rs5491 on T-cell avidity, exact mechanisms driving the association between rs5491 and HFpEF are not known. Individuals with rs5491 have higher circulating levels of ICAM-1,[9] suggesting that increased systemic inflammation and potentially higher ICAM-1 may ultimately promote HFpEF risk. There are other *ICAM1* missense variants (rs5498), however, that are associated with ICAM-1 levels but do not promote concomitant risk of HFpEF, suggesting that other inflammatory pathways may play important roles. Our study contributes to existing knowledge by providing evidence that the rs5491 *ICAM1* variant is significantly associated with a broad inflammatory proteomic profile, which in turn is linked to adverse changes in cardiac structure and function. These findings, therefore, provide further insight into potential inflammatory pathways relevant to HFpEF risk among individuals with the rs5491 variant.

FIGURE 3 Labeled Heatmap of Associations Between Echocardiographic Measures, *ICAM1* Missense Variants, and Protein Networks



Values in cell represent *P* values. Heatmap represents β coefficients from linear regression models.

Seven proteins involved in inflammation were significantly higher among individuals with a copy of rs5491 in single-protein regression analyses. Notably, of these 7 proteins, 4 are involved in TNFα binding (TNF-R1, TNF-R2, lymphotoxin β receptor [LTBR], and TNF receptor superfamily member 14 [TNFRSF14]). TNFα, a potent inflammatory cytokine that is produced primarily by macrophages, is responsible for the priming of the NLRP3 (NOD- LRR- and pyrin domain-containing protein 3) inflammasome and has been implicated in HFpEF.[1] Higher circulating TNFα is more strongly associated with the development of HFpEF than with HFrEF and also confers an increased risk of HF hospitalizations and mortality among individuals with prevalent HFpEF,[23,24] suggesting a role of TNF signaling in HFpEF specifically. Of the 4 TNF-related proteins we

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TABLE 4 Associations of HFpEF Status With Inflammatory Proteins Linked to rs5491 in the Northwestern Cohort

Protein (Outcome Variable)	β-Coefficient for HFpEF Compared With Comorbidity Control (SE)[a]	*P* Value
TNFRSF14	0.494 (0.112)	2.2E-5
TNF-R2	0.494 (0.134)	0.0003
TNF-R1	0.517 (0.108)	4.9E-6
IL1-RT1	0.314 (0.101)	0.002
LTBR	0.423 (0.099)	3.7E-5
ICAM-2	0.571 (0.107)	3.9E-7
IL-17RA	0.327 (0.150)	0.031

[a]Adjusted for age, gender, and estimated glomerular filtration rate.
HFpEF = heart failure with preserved ejection fraction.

identified to be associated with rs5491, TNF-R2 is uniquely higher in HFpEF than in HFrEF,[25] and LTBR appears to mediate associations between metabolic comorbidities and diastolic dysfunction in prevalent HFpEF.[14] Additionally, both TNF-R2 and TNFRSF14 have been associated with incident HF.[26] Pathway over-representation analyses also suggest that proteins associated with rs5491 are specifically related to the TNFR superfamily. Despite the association of rs5491 with HFpEF in several cohorts, we did not identify an association between rs5491 and NT-proBNP. Given that rs5491 is associated with smaller LV chamber size, it would not be expected that rs5491 is independently associated with B-type natriuretic peptide, inasmuch as natriuretic peptides are stimulated by LV wall stress/dilatation. Taken together, our findings implicate alteration in TNF signaling as a pathway that may drive HFpEF among individuals with rs5491 Further investigations are required to understand the functional effects of rs5491 on the TNF signaling pathway.

By leveraging network proteomic analysis to evaluate interrelated protein networks as opposed to single proteins, we identified that rs5491 is linked to a specific inflammatory protein cluster (turquoise). Importantly, the 4 TNFR proteins identified in single-protein regressions were large nodes within the turquoise network, suggesting stronger cluster membership and further confirming the relationship between rs5491 and alterations in TNF signaling. These findings also implicate a broader network of inflammatory proteins related to rs5491 that require further investigation through functional studies to better understand pathways of rs5491 and HFpEF. Additionally, differential network analysis within the turquoise cluster demonstrates protein-protein correlations that are specific to rs5491 (**Figure 2C**). The rs5491-differential network within the turquoise cluster effectively prunes the turquoise cluster to identify a more specific proteomic signature of rs5491 that may carry importance in future studies. For example, TNF-R1 uniquely interacts with soluble urokinase-type plasminogen activator receptor and activated leukocyte adhesion molecule among individuals with rs5491, but not among those without the rs5491 variant. Finally, we demonstrated that the rs5491-specific inflammatory turquoise protein network was associated with alterations in several measures of cardiac mechanics on echocardiography, including several measures of LV diastolic dysfunction. As such, our echocardiographic findings suggest a possible link between the rs5491 variant, inflammatory proteins, and HFpEF.

The rs5491 and rs5498 carried distinct proteomic profiles, further elucidating pathways that may drive HFpEF to exist among those with rs5491. Although rs5491 and rs5498 both lead to elevation in circulating ICAM-1 with similar effect sizes, only rs5491 has been associated with the development of HFpEF. The unique inflammatory proteomic profile of the HFpEF risk variant rs5491 compared with rs5498 suggests that pathways of inflammation independent of simply ICAM-1 elevation may play roles in the development of HFpEF.

Although rs5491 carried an inflammatory proteomic profile in both the MESA and the ARIC cohorts, only 1 of the 7 specific inflammatory proteins identified in MESA was validated in ARIC (LTBR). Despite this, rs5491 was consistently associated with other inflammatory proteins in ARIC, including a distinct TNF receptor superfamily member. Several reasons may account for these differences, including variation in correlations between protein levels measured on Olink and SOMAscan platforms. Indeed, among the 7 proteins identified in MESA to be associated with rs5491, the Spearman correlation coefficients between Olink and SOMAscan varies from as low as 0.05 to as high as 0.75.[27] Additionally, although the associations between rs5491 and LTBR were significant in both MESA and ARIC, the directions of effect were opposite. In a large study evaluating variant-protein associations across the 2 proteomic platforms, discordance in the directions of effects was common (14% of 318 variant-protein associations).[28] The discordance in direction of effect between rs5491 and LTBR may indicate platform-specific binding effects, but still support a genetic effect on LTBR levels as the most likely explanation. Overall, our findings are ultimately bolstered by these complementary methodologies, given that rs5491 was consistently associated with proteins related to inflammation in both cohorts.

STUDY LIMITATIONS. The major strengths of the study include the comprehensive proteomic profiling, which allowed for both hypothesis-driven and unbiased analyses, providing a more robust understanding of the relationships between rs5491, proteins, and cardiac structure and function. Additionally, the use of a comparator variant (rs5498) strengthens the specificity of the observed associations with rs5491. The study also benefits from a well-characterized cohort with detailed echocardiographic measures, enabling a more comprehensive understanding of the associations between protein networks and cardiac structure and function.

Limitations include the cross-sectional nature of the analysis, which precludes establishing causal relationships between the rs5491 variant, proteomic changes, and HFpEF outcomes. Longitudinal studies are needed to further elucidate the causal pathways. Another limitation is the study population, which may limit the generalizability of the results to other race/ethnic groups. Furthermore, we cannot exclude the possibility of residual confounding by unmeasured factors or the presence of other genetic variants that may influence protein levels or HF failure risk. Future studies involving larger populations are required to understand the associations between increasing copies of the rs5491 risk allele and proteomic changes. Our proteomic footprint is not completely unbiased, because proteins on the Olink Target 96 CVD III panel are selected on the basis of previous implications in cardiovascular disease. Future studies are needed to evaluate the associations of rs5491 with even broader proteomic profiles, and to understand whether pathways by which rs5491 drives HFpEF are secondary to the variant's effect on T-cell binding and/or systemic inflammation.

CONCLUSIONS

In this analysis, we demonstrate that the pK56M *ICAM1* HFpEF risk variant is associated with a distinct inflammatory proteomic profile among older African American individuals, characterized by higher levels of the TNFR superfamily proteins. The proteins and protein networks linked to rs5491 are associated with alterations in cardiac structure and function that are commonly deranged in HFpEF, and rs5491-specific proteins are higher in patients with HFpEF compared with comorbidity controls. Finally, through use of another missense *ICAM1* variant as a negative control, our findings suggest that inflammatory pathways outside of circulating ICAM-1 may confer a risk of HFpEF among those with the rs5491 variant. These findings identify inflammatory pathways to interrogate in order to characterize the functional effects of rs5941, which may yield further understanding of the molecular mechanisms that drive HFpEF among African American individuals with this variant.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: A common missense variant of ICAM-1 among African American individuals that has been associated with HFpEF appears to be associated with circulating inflammatory proteins.

TRANSLATIONAL OUTLOOK: Mechanistic studies evaluating functional effects of rs5491 are required to understand how rs5491 is associated with inflammation and HFpEF.

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APPENDIX For supplemental tables and figures, please see the online version of this paper.