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Missense Genetic Variation of *ICAM1* and Incident Heart Failure

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Abstract

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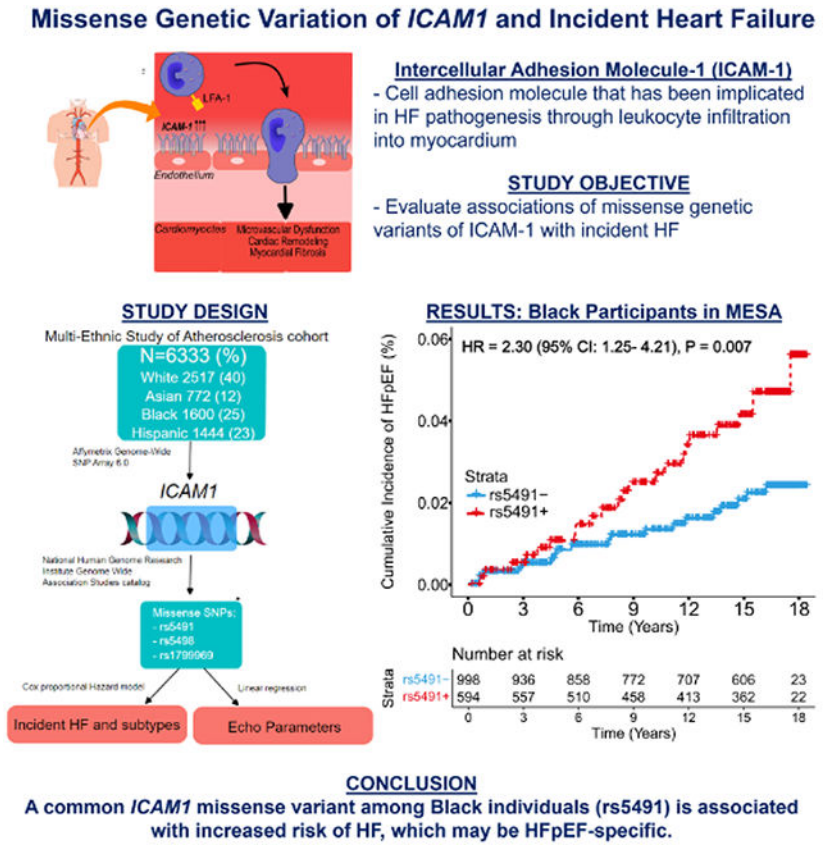
Background: Intercellular adhesion molecule-1 (ICAM-1) is a cell surface protein that participates in endothelial activation and is hypothesized to play a central role in heart failure (HF). We evaluated associations of *ICAM1* missense genetic variants with circulating ICAM-1 levels and with incident HF.

Methods and Results: We identified 3 missense variants within *ICAM1* (rs5491, rs5498, and rs1799969), and evaluated their associations with ICAM-1 levels in the Coronary Artery Risk Development in Young Adults Study and the Multi-Ethnic Study of Atherosclerosis (MESA). We determined the association between these 3 variants and incident HF in MESA. We separately evaluated significant associations in the Atherosclerosis Risk in Communities (ARIC) study. Of the 3 missense variants, rs5491 was common in Black participants (MAF >20%) and rare in other race/ethnic groups (MAF <5%). In Black participants, the presence of rs5491 was associated with higher levels of circulating ICAM-1 at 2 timepoints separated by 8 years. Among Black participants in MESA (n=1600), the presence of rs5491 was associated with an increased risk of incident HF with preserved ejection fraction (HFpEF; HR = 2.30; [95% CI 1.25 – 4.21; P=0.007]). While the other *ICAM1* missense variants (rs5498 and rs1799969) were associated with ICAM-1 levels, there were no associations with HF. In ARIC, rs5491 was significantly associated with incident HF (HR = 1.24 [95% CI 1.02 – 1.51], P=0.03), with a similar direction of effect for HFpEF that was not statistically significant.

Conclusions: A common *ICAM1* missense variant among Black individuals may be associated with increased risk of HF, which may be HFpEF-specific.

Graphical abstract



Visual Take Home Graphic. Missense Genetic Variation in ICAM1 and Incident HF. We identified 3 missense genetic variants of ICAM1. Of these 3 variants, one (rs5491) was common among individuals of Black race/ethnicity and rare in other race/ethnic cohorts. The presence of rs5491 was associated with higher circulating ICAM-1 levels. Among Black individuals in MESA, rs5491 was associated with incident HFpEF. In a separate cohort of Black individuals, rs5491 was associated with incident HF.

Lay Summary

Intercellular adhesion molecule-1 (ICAM-1) reflects endothelial activation, a process hypothesized as central to heart failure (HF). Using 3 prospective cohorts, we evaluated the associations of pre-specified missense genetic variants of *ICAM1* with circulating ICAM-1 levels and incident HF. One missense variant (rs5491) was common in Black participants, but rare in other race/ethnic groups. This variant was associated higher ICAM-1 levels. rs5491 was also associated with increased risk of HF with preserved ejection fraction in our primary cohort and with overall HF in a separate cohort. In Black individuals, a common *ICAM1* missense variant is associated with HF risk.

Tweet

New @nih_nhlbi multi-cohort study: A missense variant in ICAM1 that is common among individuals of Black race/ethnicity is associated with higher ICAM-1 levels and increased risk of HF.

Keywords

intercellular adhesion molecule-1; race/ethnicity; genetics; heart failure; rs5491

Introduction

Immune dysregulation may play a central role in the pathogenesis of certain heart failure (HF) syndromes, but precise mechanisms remain unclear (1,2). Specifically, chronic systemic inflammation driven by comorbidities may upregulate cellular adhesion molecules (CAMs) on the surface of endothelial cells, allowing for leukocyte infiltration of various tissue beds (1). This inflammatory cascade may result in microvascular dysfunction, myocardial interstitial fibrosis, and HF (3). A specific CAM, intercellular adhesion molecule (ICAM)-1, is a cell surface protein that facilitates leukocyte transmigration across the endothelium and has been implicated in both HF with preserved ejection fraction (HFpEF) and HF with reduced ejection fraction (HFrEF). ICAM-1 expression is upregulated in myocardial tissue of patients with HFpEF and murine models of HFpEF (4,5). Additionally, higher circulating ICAM-1 levels have been associated with subclinical HF at middle age (6). Finally, ICAM1-deficient mice do not develop systolic dysfunction in response to pressure overload states (7).

Despite evidence supporting a role for ICAM-1 in HF, multiple genome-wide association studies (GWAS) of HF have not identified variant associations within or near the ICAM1 locus (8,9), suggesting that ICAM-1 may not be causally related to HF. However, genetic evaluations of *ICAM1* to date have utilized limited definitions of HF, failed to evaluate HF subtypes specifically, or have not included diverse ancestral populations, which may limit understanding of the relationship between genetic variation in ICAM1 and HF. Additionally, it is possible that the role of ICAM-1 in HF is driven by environmental as opposed to genetic factors. Thus, to further understand the mechanistic role of ICAM-1 in HF, we performed a targeted assessment of associations between missense genetic variants of *ICAM1* and incident HF and its subtypes in the Multi-Ethnic Study of Atherosclerosis (MESA) with subsequent evaluation in the Atherosclerosis Risk in Communities (ARIC) Study. We separately evaluated the association between such *ICAM1* missense variants with circulating ICAM-1 levels in MESA and the Coronary Artery Risk Development in Young Adults (CARDIA) Study.

Methods

Study Populations

The Multi-Ethnic Study of Atherosclerosis (MESA) is a cohort of community-dwelling adults (age 45-85) of 4 race/ethnic groups (Black, White, Hispanic, Chinese) who were enrolled to better understand the risk factors and prevalence of subclinical cardiovascular disease. The details of this cohort have been described (10). MESA enrolled participants from 2000-2002 without existing clinical cardiovascular disease defined as myocardial infarction, angina, stroke, transient ischemic attack, HF, atrial fibrillation, nitroglycerin use, angioplasty, pacemaker or implantable cardioverter defibrillator, or cardiac surgery. Of 6,814

participants in MESA, 481 were excluded from this analysis due to missing covariate or genetic data and/or did not have any HF follow up. The final analytic cohort was 6333 and included 2517 (40%) White, 772 (12%) Chinese, 1600 (25%) Black, and 1444 (23%) Hispanic individuals.

To examine the association between certain genetic variants with circulating ICAM-1 levels, we evaluated participants in the CARDIA study. The Coronary Artery Risk Development in Young Adults (CARDIA) study is a prospective cohort of 5,115 black and white young adults, aged 18 to 30 at baseline who were recruited across 4 U.S urban sites.

For further evaluation of the significant associations between genetic variants and incident HF, we evaluated participants in the ARIC study. The ARIC cohort was comprised of 15,792 Black and White men and women aged 45–64 years at baseline from 4 U.S. communities. Full details of the MESA, CARDIA, and ARIC cohorts have been described elsewhere and further information can be found in the Supplemental Methods.

Genotyping and gene variant selection

Genotyping of the MESA, CARDIA, and ARIC cohorts were performed using the Affymetrix Genome-Wide SNP Array 6.0 according to manufacturer's recommendations. Genotyping and quality control procedures have been previously described (11,12). Further details regarding genotyping, including imputation methods, can be found in the Supplemental Methods.

We identified variants of *ICAM1* through a query of the publicly available National Human Genome Research Institute Genome Wide Association Studies catalog (13). Single nucleotide polymorphisms (SNPs) included in this analysis were required to be missense variants with minor allele frequency (MAF) >5%. Using these criteria, 3 missense SNPs (rs5491, rs5498, rs1799969) were identified and used in the analysis. SNP descriptions including base pair position, amino acid substitution, and alleles are presented in Table 1. The 3 SNPs were imputed across the 3 cohorts.

ICAM-1 Measurement

Circulating ICAM-1 levels were collected in a stratified random sample of MESA participants (n = 2396) as part of the MESA Adhesion Ancillary Study at Exam 2. Blood samples were obtained from fasting individuals and were stored at −70°C. Serum ICAM-1 was measured using Human soluble ICAM-1 (sICAM-1) Instant ELISA (Bender MedSystems GmbH, Vienna, Austria). This assay has a minimal detectable level of 2.17 ng/mL and an interassay coefficient of variation of 9.1 % at a mean concentration of 261 ng/mL.

In CARDIA, ICAM-1 soluble protein levels were measured at Year 7 and Year 15 examinations. Fasting blood samples were collected and processed within 90 minutes of blood draw and stored at −70°C. Soluble ICAM-1 concentrations were measured in serum using R&D Systems Cat No DY720. The limit of sensitivity for this assay for ICAM-1 was 15 pg/mL and the coefficient of variation was 9.4%. The correlation of 287 pairs of blinded quality control samples was 0.884.

Echocardiography

The MESA echocardiographic protocol has been described previously and can be found in the Supplemental Methods. Two-dimensional, Doppler, and speckle-tracking echocardiography was performed at Exam 6 (2016-2018) in MESA. Echocardiographic outcome variables of interest included left ventricular (LV) mass index, left atrial (LA) volume, LV end diastolic volume, right ventricular (RV) end diastolic area, RV free wall strain, pulmonary artery systolic pressure, measures of LV systolic function (ejection fraction, global longitudinal strain [GLS], and circumferential strain), indices of LV diastolic function (septal e' tissue velocity and E/e' ratio), and LA function (LA reservoir strain).

HF Assessment

As a part of the study protocol, MESA participants were screened for clinical events with telephone and in-person contacts. All hospitalizations for cardiovascular events were abstracted. MESA personnel transmitted all pertinent records to the MESA coordinating center which included records of symptoms, medical history, biomarkers, ECGs, echocardiograms, cardiac catheterization reports, other imaging studies and outpatient records if available. Incident HF events were independently adjudicated by 2 study physicians who were blinded to study data outside of hospitalization records. HF events required symptoms of HF including shortness of breath and peripheral edema and were defined as either definite or probable. In addition to shortness of breath and/or peripheral edema, definite HF also included at least one of the following criteria: pulmonary edema on chest radiograph, left ventricular dilation, decreased systolic function, or evidence of diastolic dysfunction. If these additional criteria for definite HF were not met or available, probable HF was defined as a physician diagnosis of HF in the clinical record as well as documentation of medical treatment for HF. The primary outcome of this study was incident probable or definite HF. We also evaluated for HF subtypes (incident HFrEF and HFpEF). HFpEF events were defined as HF with documentation of a left ventricular ejection fraction of 45% or greater on echocardiogram or radionucleotide study at hospitalization. In contrast, HFrEF was defined as HF with a documented left ventricular ejection fraction of less than 45% at hospitalization.

HF assessment in the ARIC cohort has been described previously and can be found in the Supplemental Methods. HF event adjudication in ARIC began in 2005; only HF events after this time were considered. In ARIC, HF subtyping was based on ejection fraction at hospitalization or, when unavailable, preadmission imaging studies performed within two years before the hospitalization.

Statistical Analysis

Demographic and clinical characteristics of MESA participants at Exam 1 (except A1c which was collected in Exam 2) were compared by race and ethnicity using chi-square test for categorical variables and Kruskal-Wallis rank sum test for continuous variables. We stratified all genetic analyses by self-identified race and ethnicity. We excluded SNP-ethnicities with MAF < 5%. We tested for Hardy Weinberg equilibrium (HWE) within each race/ethnicity stratum by using chi-square goodness of fit test. SNPs were modeled in a dominant fashion due to low HF event rates. We compared clinical characteristics at Exam 1

by the presence of at least one copy of the variant allele of each SNP using chi-square test for categorical variables and Welch Two Sample t-test for continuous variables.

We used separate multivariable least squares regression to evaluate the associations of each of the 3 genetic variants with log-transformed ICAM-1 levels in MESA or CARDIA. Previous studies have suggested that the assay used in MESA to measure ICAM-1 may be altered in the setting of the rs5491 variant (14). As such, we evaluated the association between this variant (rs5491) and log-transformed soluble levels of ICAM-1 (at both Year 7 and Year 15 exams) in the CARDIA study cohort, which used an updated assay that is not affected by this variant (14,15). We evaluated the association between the remaining 2 variants (rs5498 and rs1799969) with log-transformed serum ICAM-1 levels in MESA after excluding rs5491-T carriers who would have erroneous levels due to the aforementioned assay issue.

Multivariable Cox proportional hazard models were used to evaluate the associations of each SNP with incident HF and its subtypes. For models evaluating HF subtypes, censoring was used for opposing subtypes. The proportionality of hazards assumption was confirmed by Schoenfeld goodness-of-fit procedures. Models were adjusted for age, sex, and genetic ancestry (principal components 1-3). In MESA, CARDIA, and ARIC, principal component (PC) analysis was performed using EIGENSTRAT within each race/ethnic group. To assess potential mediators of genetic associations, we further adjusted for clinical risk factors (body mass index [BMI], diabetes, systolic blood pressure [SBP], anti-hypertensive medication therapy, eGFR, and total cholesterol). We separately evaluated significant associations between genetic variants and incident HF in ARIC. In a sensitivity analysis within ARIC, HF subtypes were reclassified based upon EF obtained within 1 week of HF hospitalization.

If a variant was significantly associated with incident HF in MESA, we evaluated its association with clinical characteristics (systolic blood pressure [SBP], body mass index [BMI], total cholesterol, hemoglobin A1c, eGFR, and urine albumin-to-creatinine ratio) and echocardiographic measures to understand potential pathways driving HF associations. These models were adjusted for age, sex, and genetic ancestry (principal components 1-3).

Statistical analysis was carried out using R version 4.0.2 (Vienna, Austria) and SAS version 9.4 (Cary, NC). Our primary objective was to assess the association of 3 missense variants with incident HF; for these analyses, two-sided P values of < 0.05 were considered statistically significant.

Results

MESA analytic cohort and missense genetic variant characteristics

Baseline characteristics of the MESA analytic cohort stratified by race and ethnicity are displayed in Supplemental Table 1. There were modest, but significant differences between age, BMI, cholesterol, diabetes, hemoglobin A1c, eGFR, SBP, and anti-hypertensive therapy between the 4 race and ethnicities. All missense variants were in HWE except rs1799969 in Whites and rs5498 in Hispanics (p value < 0.05). None of the SNPs analyzed were in strong linkage disequilibrium ($r^2 < 0.2$). MAF for the 3 *ICAM1* missense variants by ethnicity

are shown in Table 1. The MAF of rs5491 was <5% in all ethnic groups except Black participants, and therefore all analyses of this variant were carried out in Black participants alone (n = 1,600). Analyses of rs5498 were carried out in all race/ethnic groups (n = 6,333) due to MAF >5%. The MAF of rs1799969 was <5% in Chinese and Black participants, and thus analyses of this SNP were limited to White and Hispanic participants (n = 3,961).

MESA participants with at least one copy of the rs5491 variant were more likely to have a lower eGFR (Table 2). There were no other significant differences in clinical characteristics by rs5491 variant status. There were no significant differences in clinical characteristics by presence of at least one copy of the rs5498 variant (Supplemental Table 2) or the rs1799969 variant (Supplemental Table 3).

Missense ICAM1 variants and serum ICAM-1 levels

Among 636 Black participants in CARDIA with genotyping, 230 (36%) had at least one copy of rs5491. CARDIA participants with at least one copy of rs5491 were more likely to be current smokers (Supplemental Table 4). The presence of at least one copy of the rs5491 variant in Black participants was significantly associated with higher levels of circulating log-transformed ICAM-1 at Year 7 exam (5.03±0.01 ng/mL vs. 4.98±0.01 ng/mL, P=0.002) and at Year 15 exam (5.12±0.02 ng/mL vs. 5.01±0.01 ng/mL, P<0.001; Figure 1). In MESA, the presence of at least one copy of rs5498 was associated with higher ICAM-1 levels in White and Chinese participants; despite consistent direction of effect estimates among Hispanic and Black participants, there was no significant associations (Supplemental Table 5). Among Hispanic participants in MESA, the presence of at least one copy of rs1799969 was associated with lower log-transformed ICAM-1 levels; there was no significant association with rs1799969 and ICAM-1 among White participants (Supplemental Table 6).

Associations of missense variants of ICAM1 with incident HF and its subtypes

In the analytic cohort (n= 6333), the median follow up was 16.7 years (interquartile range 11.8-17.5 years). Over this period, there were 384 HF events, of which 159 (41%) were classified as HFrEF, 184 (48%) as HFpEF, and the 41 (10%) remainder as HF with unknown ejection fraction. There were 1,352 deaths among individuals who did not experience a HF event during follow-up.

Among Black participants (n = 1,600), there were 106 HF events, of which 56 were HFrEF, 42 were HFpEF, and 8 were HF with unknown EF. There was no association between the presence of at least one copy of the rs5491 and incident HF (Table 3). However, upon evaluation of HF subtypes, there was an association between the presence of the rs5491 variant with increased risk of incident HFpEF (HR = 2.30; [95% CI 1.25, 4.21; p=0.007]); Figure 2). In sensitivity analysis, the association between rs5491 with higher risk of incident HFpEF remained consistent after further adjustment for other risk factors including BMI, DM, SBP, anti-hypertensive medication therapy, eGFR, and cholesterol (HR = 2.46; [95% CI 1.36, 4.46; p=0.003]). In MESA, the presence of rs5491 was associated with a lower risk of incident HFrEF (Table 3).

There were no associations between rs5498 or rs1799969 and incident HF or its subtypes in any race/ethnic group (Supplemental Tables 7 and 8).

Associations of rs5491 with clinical characteristics and echocardiography

To better understand the pathways behind the association between rs5491 and incident HFpEF, we evaluated associations between rs5491 and pre-specified clinical characteristics and echocardiographic measures. The presence of at least one copy of rs5491 was significantly associated with lower eGFR (β coefficient = −2.3; [95% CI −3.9, −0.72; p=0.005]), higher triglycerides (β coefficient = 8.7; [95% CI 1.6, 16; p=0.016]), and lower HDL cholesterol levels (β coefficient = −1.6; [95% CI −3.0, −0.15; p=0.030]) after adjustment for age, sex, and PCs. There were no associations between rs5491 and other clinical characteristics. Upon evaluation with echocardiographic traits, rs5491 was significantly associated with smaller LV end-diastolic volumes and higher LVEF (Table 4). There were no other associations between rs5491 and echocardiographic indices of LA or LV structure/function.

Association of rs5491 with incident HF in ARIC

In the ARIC analytic cohort (n= 2,634 Black participants), the MAF of rs5491 was 22.8%. Participants with at least one copy of the rs5491 variant had similar baseline cardiovascular risk factors compared to those without (Supplemental Table 9). Over a median follow up of 12.5 years, there were 400 HF events, of which 196 (49%) were HFrEF, 158 (40%) were HFpEF, and 46 (12%) were HF with unknown EF.

At least one copy of the rs5491 allele was associated with greater risk of incident HF after adjustment for age, sex, and the first 3 principal components of ancestry (HR 1.24 [95% CI 1.02-1.51], P=0.03). Upon evaluation of HF subtypes, rs5491 was significantly associated with incident HFrEF (HR 1.39 [95% CI 1.05-1.84], P=0.02), and the direction of effect of rs5491 with HFpEF was consistent with that of the MESA cohort but was not statistically significant (HR 1.13 [95% CI 0.82-1.55], P=0.46) (Supplemental Table 10). In sensitivity analysis, HF events were reclassified based upon EF ascertainment within 1 week of hospitalization. Using this approach, 76 HFpEF and 96 HFrEF events were reclassified as HF with unknown EF (Supplemental Table 11). Effect estimates were in a similar direction for HFpEF and HFrEF, and the overall association between rs5491 and incident HF was driven by HF with unknown EF.

Discussion

We evaluated associations between 3 missense variants of *ICAM1* with circulating ICAM-1 levels and incident HF in a cohort of multi-ethnic participants, and separately evaluated associations in a distinct cohort. Among middle-age adults of Black race/ethnicity, rs5491 was associated with higher levels of ICAM-1 at 2 different timepoints separated by 8 years. We demonstrate a significant association between the presence of at least one copy of the rs5491 variant and higher risk of incident HFpEF among Black participants in MESA. This association remained consistent after further adjustment for traditional cardiovascular risk factors. We subsequently demonstrate an association between rs5491 with incident HF in

a separate cohort of Black participants. The presence of the rs5491 variant was associated with smaller LV volume and higher LV systolic function. While the other 2 variants of *ICAM1* (rs5498 and rs1799969) were associated with ICAM-1 levels in certain race/ethnic groups, there were no corresponding associations between these variants and HF risk. Taken together, our findings suggest a role of rs5491 in promoting HF risk, and highlight the variation in phenotypic effects of different protein quantitative trait loci within a single gene.

Diffuse microvascular inflammation, mediated by ICAM-1, is believed to contribute to microvascular dysfunction and HF pathogenesis. ICAM-1 mediates such inflammation by participating in the rolling, firm adhesion, and transmigration steps of leukocyte adhesion, which subsequently promotes vascular and myocardial interstitial collagen deposition, oxidative stress, and decreased nitric oxide bioavailability (1). In addition to its putative pathophysiological mechanism, several other lines of evidence have linked ICAM-1 with HF. Murine models of HF that were deficient in ICAM-1 had less proinflammatory monocyte infiltration and did not develop cardiac fibrosis or systolic dysfunction (7). Additionally, ICAM-1 is more highly expressed in myocardial tissue of both HFpEF patients and murine models of HFpEF (4,5). We have previously demonstrated that both baseline ICAM-1 levels and increase in ICAM-1 levels during young adulthood were independently associated with worse LV global longitudinal strain (6), a sensitive functional measure that is abnormal in patients with HFpEF and HFrEF (16). Our current study further highlights the potential mechanistic role of ICAM-1 in HF, and possibly HFpEF specifically, through identification of a functional genetic variant that is associated with higher ICAM-1 levels and HF.

To our knowledge, this is the first study to demonstrate a significant association between rs5491 and higher levels of soluble ICAM-1. rs5491 is a variant that is predominantly found in individuals of Black race/ethnicity. It been also termed ‘ICAM-1 Kilifi,’ as it was initially identified in the Kilifi region of Kenya (17). The relationship between rs5491 and serum ICAM-1 levels has been challenging to understand because the minor allele, which is common in Black populations, interferes with traditional assays for ICAM-1, resulting in erroneous levels of ICAM-1 among rs5491 carriers. MESA used an ELISA that appears to have less interaction with rs5491, but rs5491 still interferes with ICAM-1 measurement using this assay (14). The CARDIA study uniquely measured ICAM-1 levels using an updated assay that was designed to avoid this issue. Using this assay, we demonstrate an association between rs5491 and higher circulating ICAM-1.

We further demonstrate, for the first time to our knowledge, the association between rs5491 and incident HF. There are several possible explanations for the lack of associations of rs5491 with HF in prior, large-scale GWAS. First, the vast majority of prior GWAS of HF have been conducted in populations of European ancestry (3,18,19). Given the very low MAF of rs5491 in Europeans, it is not surprising that this variant has not been identified. Second, prior GWAS have typically grouped all HF phenotypes together (i.e., HFpEF, HFrEF, and HF with mildy reduced EF) (20). While this method increases power to detect associations due to higher case numbers, it may mask phenotype-specific associations. Our study serves an example, as there was an association between rs5491 and incident HFpEF, but not all HF, in MESA. Third, GWAS that have separately evaluated HFpEF and HFrEF

as distinct traits have been exclusively performed in European populations (3,21), or have evaluated loci of genome-wide significance in European populations and applied them to Black populations. In our study, we identified rs5491 as a variant of interest *a priori* based on prior literature, evaluated its association with HF in cohort of only Black participants, and leveraged the rigorous event adjudication of HFpEF in the MESA cohort to determine associations with HFpEF.

In the ARIC cohort, there was a significant association between rs5491 and incident HF and HFrEF, and while effect estimates were in a similar direction for HFpEF, this association did not reach statistical significance. This contrasts with MESA, in which rs5491 was associated with a higher risk of HFpEF, but a lower risk of HFrEF. There are several reasons that may account for these findings. In MESA, HF events have been rigorously and blindly adjudicated as part of initial study protocol, and thus are more likely highly specific for HF and its subtypes. In ARIC, HF events were initially captured using ICD9 codes prior to committee review, which are less specific for HF and may result in misclassification of HF subtypes (22). Additionally, while EF ascertainment at time of hospitalization was required for HF subtyping in MESA, preadmission EF assessment was used in ARIC to subtype HF events if hospitalization EF measurement was not available. It is therefore possible there is an admixture of events across HF subtypes in ARIC that explain our findings. Indeed, rs5491 was associated with higher LVEF in MESA. Furthermore, upon reclassification of ARIC HF events using EF data within 1 week of hospitalization, the association of rs5491 with HF was driven by HF with unknown EF. These findings suggest a specific association of this variant with HFpEF.

The potential mechanisms by which rs5491 drives HF require further investigation. rs5491 was associated with smaller LV end diastolic volume and higher LVEF. These echocardiographic features are typical of certain sub-phenotypes of HFpEF (i.e., those with HF with supranormal ejection fraction), a cohort that appears to have a strong heritable component (23). We did not find associations between rs5491 and LV diastolic dysfunction, LV hypertrophy, or LA dysfunction. This lack of association may be explained by survival bias, as echocardiograms were performed at Exam 6 (≈16 years after MESA recruitment), or lack of power (n=591 with echocardiographic data). Given that HFpEF specifically is a syndrome driven by co-morbidity-induced systemic inflammation that may have detrimental effects upon multiple organ systems, it is possible that rs5491 may lead to HFpEF through “extra-cardiac” endothelial dysfunction (e.g., skeletal muscle or kidney endothelial activation), and thus contribute to HFpEF when present in combination with other cardiovascular risk factors.

The rs5491 variant has previously been shown to carry functional and clinical effects. Specifically, the rs5491 variant reduces affinity for binding to the integrin lymphocyte function-associated antigen-1 (24). Additionally, rs5491 has been associated with reduced incidence of nonmalarial febrile illnesses (25), a potentially protective immune attribute that may explain the sustained high prevalence of rs5491 in Black populations. Our study suggests that this potential protective, immune regulatory effect of rs5491 may come at the expense of increased risk of HF.

Despite associations with soluble ICAM-1 levels, we did not observe any association between rs5498 and rs1799969 and incident HF/subtypes. Importantly, the effect size of rs5498 on ICAM-1 levels was similar to rs5491. These findings highlight the potential variation in functional significance of different protein quantitative trait loci. Further investigation is required to understand functional significance of each of these missense variants, which may yield further insight to HFpEF pathogenesis.

Limitations

Our study has strengths and limitations. We leveraged multiple cohorts to characterize associations of rs5491 with ICAM-1 levels and incident HF. MESA represent a large, diverse, community-based cohort that has been well characterized through longitudinal in-person examinations, accompanied by rigorous, blinded event adjudication. Our findings should be considered as pilot and associations of rs5491 with HF subtypes require confirmation in larger cohorts of individuals of Black race/ethnicity. Incident HF were relatively low in this cohort, and the requirement for hospitalization for HF adjudication will also reduce power by failing to detect mild, outpatient cases of HF. Despite the reduction in power, the requirement of hospitalization and careful adjudication by an independent coordinating center affords this study highly specific and clinically significant events, and we evaluated associations in a separate cohort. We were unable to evaluate associations of rs5491 with incident HF in CARDIA due to low event rates in this young adult population. Due to differences in cohorts by demographics at enrollment, ICAM-1 assay type, and HF adjudication, we evaluated cohorts separately. Data regarding use of certain medications, including mineralocorticoid receptor antagonists and sodium-glucose co-transporter 2 inhibitors, were not available in MESA. For all analyses, race/ethnic groups were based upon self-report and not by genetic ancestry. Finally, our primary objective was to evaluate associations of *ICAM1* missense variants with incident HF; associations between rs5491 and echocardiographic measures should be considered exploratory.

By leveraging 3 large, community-based cohorts, we demonstrate that the presence of rs5491, a common *ICAM1* missense variant among Black individuals, is independently associated with higher circulating ICAM-1 levels and increased risk of HF. This risk is consistent after adjustment for traditional cardiovascular risk factors. Taken together, these findings suggest that specific genetic variation in *ICAM1* may have a role in promoting HF risk, which may be specific to HFpEF. Further studies are needed to corroborate these findings and to elucidate the mechanism through which rs5491 may increase HF risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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This manuscript has been reviewed by CARDIA for scientific content.

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Biography

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Highlights

- Of 3 identified missense genetic variants in ICAM1, rs5491 was common among individuals of Black race/ethnicity and rare in other race/ethnic groups.
- Among Black individuals, rs5491 was associated with higher circulating ICAM-1 levels.
- In Black individuals, the presence of rs5491 was associated with an increased risk of incident heart failure with preserved ejection fraction in our primary cohort, and with overall heart failure in a separate cohort. These findings suggest a common missense variant of ICAM1 among individuals of Black race/ethnicity is associated with risk of heart failure.

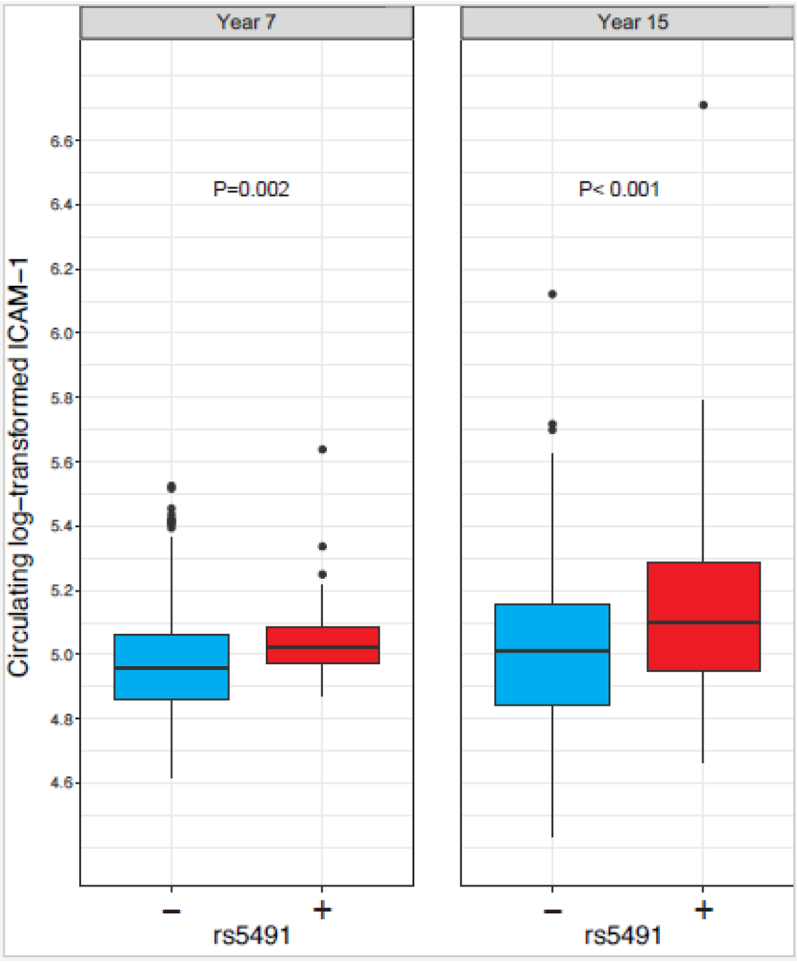

Figure 1. Association between rs5491 and circulating ICAM-1 at Year 7 and Year 15 exams among Black participants in CARDIA.

Shown are box-and-whisker plots of ICAM-1 levels among individuals with at least one copy of rs5491 (red) vs. no copies (blue).

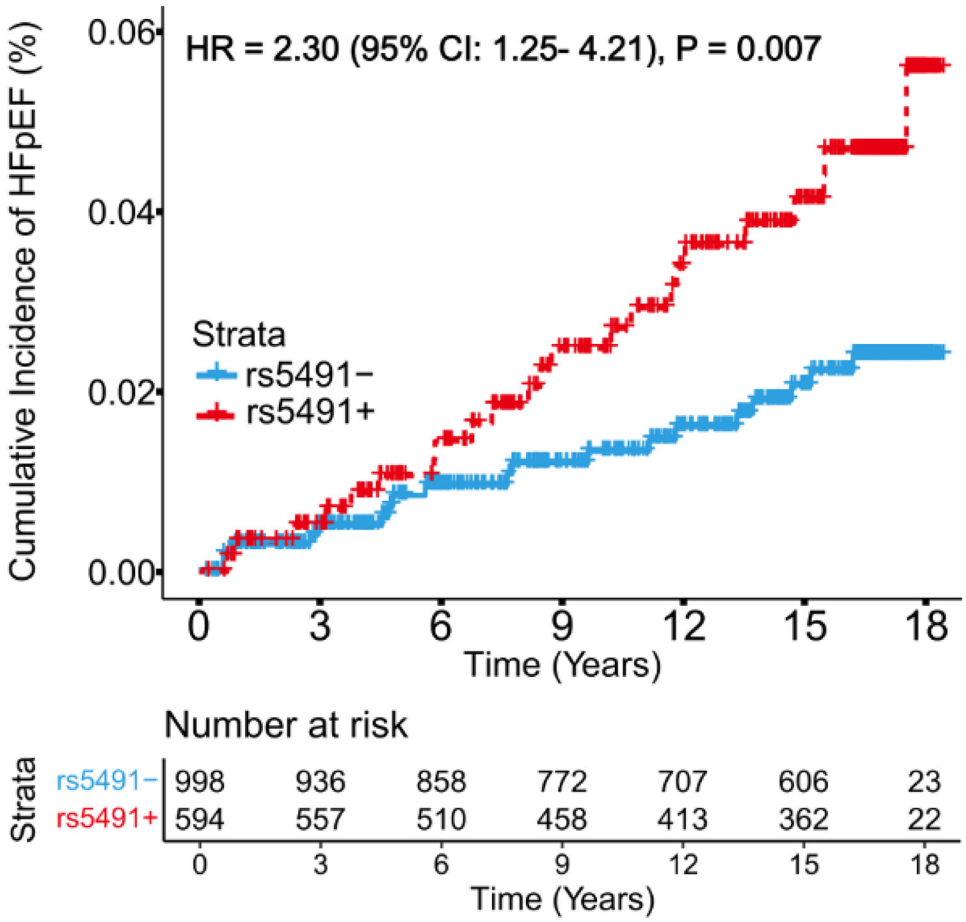

Figure 2. Cumulative incidence of heart failure with preserved ejection fraction according to presence of rs5491 among Black participants in MESA.

Shown are unadjusted cumulative incidence curves of incident HFpEF stratified by presence of at least one copy of rs5491 (red line) vs. no copies (blue line). The displayed hazard ratio (95% CI) are results from the primary model (adjusted for age, sex, and principal components 1-3).

Table 1.

Minor allele frequencies of *ICAM1* missense variants by race/ethnic group in MESA.

				Minor Allele Frequency			
Variant	**Chr:bp**	**Major, Minor Allele**	**Amino Acid Substitution**	**White (N=2517)**	**Hispanic (N=1444)**	**Chinese (N=772)**	**Black (N=1600)**
rs5491	19:10274864	A, T	K > M	0.005	0.035	0.038	0.213
rs1799969	19:10284116	G, A	K > E	0.098	0.149	0.008	0.023
rs5498	19:10285007	A, G	G > R	0.450	0.493	0.246	0.189

Table 2.

Characteristics of Black participants at Exam 1 (Baseline) by presence of rs5491 in MESA.

Characteristic	rs5491 - AA (N=1004)	rs5491 heterozygote/homozygote (N=596)	P-value
Age, years	63±10	62±10	0.15
Male sex	469 (47)	271 (45)	0.63
BMI, kg/m^2	30.0±5.9	30.4±5.8	0.22
Current smoker	183 (18)	111 (19)	0.71
Diabetes Status			0.05
Normal fasting glucose	669 (67)	407 (69)	
Impaired fasting glucose	154 (15)	88 (15)	
Untreated diabetes	26 (3)	28 (5)	
Treated diabetes	151 (15)	71 (12)	
Anti-hypertensive medication use	493 (49)	311 (52)	0.25
SBP, mmHg	132±22	131±21	0.33
Total cholesterol, mg/dL	191±36	188±37	0.12
HDL cholesterol, mg/dL	53±16	52±13	0.11
Hemoglobin A1c, %	5.94±1.16	5.90±1.06	0.49
eGFR, mL/min/1.73 m^2	81±18	79±18	0.03

Categorical variables are reported as n (%). Continuous variables are reported as mean ± SD. BMI = body mass index; HDL = high-density lipoprotein cholesterol; eGFR = estimated glomerular filtration rate; SBP = systolic blood pressure

Table 3.

Associations of rs5491 with heart failure in Black participants in MESA.

Outcome	No. of events/ No. of participants	HR for presence of at least one copy of rs5491 (95th CI)*	P-value
All heart failure	106/1600	0.95 (0.63-1.42)	0.80
Heart failure with preserved ejection fraction	42/1592	2.30 (1.25-4.21)	0.007
Heart failure with reduced ejection fraction	56/1592	0.45 (0.23-0.87)	0.017

*Adjusted for age, sex, and first 3 principal components of ancestry

Table 4.

Associations of rs5491 with cardiac mechanics on echocardiography in Black Participants in MESA.

	rs5491 - AA (N=377)	rs5491 heterozygote/homozygote (N=214)	P-value*
LV structure and function			
LV end diastolic volume index, mL/m^2	41 (35-47)	40 (34-46)	0.03
LV mass index, g/m^2	83 (69-98)	79 (68-96)	0.16
LVEF, %	62 (58-66)	63 (59-66)	0.03
GLS, %	19.4 (17.4-21.3)	19.5 (17.5-21.4)	0.53
GCS, %	18.3 (16.1-20.8)	18.2 (16.4-21.1)	0.53
e' septal, cm/s	7.2 (5.8-8.8)	7.1 (5.9-9.0)	0.79
E/e' average	9.7 (8.1-12.0)	9.4 (8.2-11.1)	0.58
LA structure and function			
LA volume, mL	54 (44-68)	54 (42-65)	0.17
LA reservoir strain, %	27 (23-31)	27 (23-30)	0.49
RV structure and function			
RV end diastolic area, cm^2	18.3 (15.9-20.9)	18.6 (16.3-21.4)	0.57
RV free wall strain, %	24.4 (21.1-27.8)	24.6 (21.0-26.9)	0.20
Hemodynamics			
PASP, mmHg	33 (29-39)	32 (28-37)	0.72

*Adjusted for age, race/ethnicity, and first 3 principal components of ancestry

EF = ejection fraction; GCS = global circumferential strain; GLS = global longitudinal strain; LA = left atrial; LV = left ventricular; PASP = pulmonary artery systolic pressure; RV = right ventricular