Large-Scale Proteomic Profiling of Incident Heart Failure and Its Subtypes in Older Adults

Joyce N. Njoroge, MD[1]; Sandra Sanders van Wijk, MD, PhD[2]; Thomas R. Austin, PhD[3]; Jennifer A. Brody, BA[4]; Colleen M. Sitlani, PhD[5]; Emily Hamerton, MS[6]; Joshua Bis, PhD[3]; Albert Henry, MD, MSc[7,8]; R. Thomas Lumbers, MB, BChir, PhD[8,9] The HERMES Consortium[10]; Talia Seshaiah, MS[5]; Ali Shojaie, PhD[5]; Yimin Yang, PhD[10]; Victoria Lamberson, PhD[11] Bing Yu, PhD[12] ; Amil M. Shah, MD, MPH[11]; Nisha Bansal, MD, MAS[13]; Sanjiv Shah, MD[14]; Russell P. Tracy, PhD[15]; Robert E. Gerszten, MD[16]; Lori L. Jennings, PhD[17]; Valborg Gudmundsdottir, PhD[18,19]; Vilmundur Gudnason, MD,PhD[18,19]; Valur Emilsson, PhLic, PhD[18,19]; Bruce M. Psaty, MD, PhD[3,4]; Jorge R. Kizer, MD, MSc[6,20]

1. Division of Cardiology, Department of Medicine, Stanford University, Palo Alto, CA, USA.
2. Division of Cardiology, Department of Medicine, Zuyderland Medical Center, Heerlen, Netherlands.
3. Cardiovascular Health Research Unit, Epidemiology and Health Systems and Population Health, University of Washington, Seattle, WA, USA.
4. Cardiovascular Health Research Unit, Department of Medicine, University of Washington, Seattle, WA, USA.
5. Department of Biostatistics, University of Washington, Seattle, WA, USA.
6. Cardiology Section, San Francisco Veterans Affairs Health Care System and Department of Medicine, University of California, San Francisco, San Francisco, CA, USA.
7. Institute of Cardiovascular Science, University College London, London, UK.
8. Institute of Health Informatics, University College London, London, UK.
9. The National Institute for Health Research University College London Hospitals Biomedical Research Centre, University College London, London, UK.
10. Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA.
11. Division of Cardiovascular Medicine, Department of Medicine, University of Texas Southwestern Medical Center, Houston, TX, USA
12. School of Public Health, University of Texas Health Science Center at Houston, Houston, TX, USA.
13. Division of Nephrology, Department of Medicine, and Kidney Research Institute, University of Washington, Seattle, WA, USA
14. Division of Cardiology, Department of Medicine, Northwestern University School of Medicine, Chicago, IL, USA.
15. Department of Pathology and Laboratory Medicine, Larner College of Medicine, University of Vermont, Burlington, VT, USA.
16. Department of Medicine, Division of Cardiology, Beth Israel Deaconess Hospital and Harvard Medical School, Boston, MA, USA.
17. Novartis Institute for Biomedical Research, Cambridge, MA, USA.
18. University of Iceland, Faculty of Medicine, Reykjavik, Iceland.
19. Icelandic Heart Association, Kopavogur, Iceland.
20. Department of Epidemiology and Biostatistics, University of California San Francisco, CA, USA.

Short Title: Proteomics of Incident Heart Failure in Elders

Corresponding Author:
Jorge R. Kizer, MD, MSc
Cardiology Section, San Francisco Veterans Affairs Health Care System
4150 Clement Street, Mail Code 111C
San Francisco, CA, 94121, USA.
E-mail: jorge.kizer@ucsf.edu
Phone: 415-221-4810

ORCID ID: 0000-0001-9936-7803

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Abstract

Background: Heart failure (HF) and its main subtypes, preserved (HFpEF) and reduced ejection fraction (HFrEF), impose an enormous health burden on elders. Assessment of the circulating proteome to illuminate pathogenesis could open new opportunities for treatment.

Methods: We conducted a plasma proteomics screen of incident HF and its subtypes in two older population-based cohorts, the Cardiovascular Health Study (CHS) and the Aging, Gene/Environment Susceptibility – Reykjavik Study (AGES-RS). The two studies used SomaLogic platforms, with 4,404 aptamers in common. Multivariable Cox models were fit to evaluate individual-protein associations with HF, HFpEF and HFrEF separately in each cohort, and study-specific associations combined by fixed-effects meta-analysis. Replication was performed in the Atherosclerosis Risk in Communities (ARIC) cohort. Two-sample Mendelian randomization (MR) of HF and its subtypes, along with colocalization analysis, was performed to support causal inference.

Results: Among 8,599 participants, 1,590 experienced incident HF (536 HFpEF, 471 HFrEF). There were 119 proteins associated with HF, 15 proteins with HFpEF, and 11 proteins with HFrEF, at Bonferroni-corrected significance. Among these, 9 have never previously been identified for cardiovascular diseases, and another 61 represent new associations with incident HF or its subtypes. Of these 70 proteins, 55 of the 66 available replicated externally. MR analysis revealed 7 proteins genetically associated with HF at nominal significance; 2 were separately associated with HFpEF, and another 2 with HFrEF. Seven of these 9 proteins (NCDP1, APOF, LMAN2, ADIPOQ, CD14, ARHGAP1, C9) showed new, possibly causal associations, although we did not detect evidence for colocalization.

Conclusions: In this large-scale proteomic study involving three longitudinal cohorts of older adults, we identified and replicated 55 novel protein markers of HF or its subtypes, and 7 new, possibly causal proteins. These proteins may enhance risk prediction, improve understanding of pathobiology, and help prioritize targets for therapeutic development of these foremost disorders in elders.

Key Words: Heart Failure, Proteomics, Mendelian randomization analysis

Non-standard Abbreviations and Acronyms

AGES-RS=Aging Gene/Environment Susceptibility – Reykjakiv Study, AF=Atrial fibrillation, ARIC=Atherosclerosis Risk in Communities, CHD=Coronary heart disease, CHS=Caardiovascular Health Study, CVD=Cardiovascular disease, GWAS=Genome-wide association study, HF=Heart failure, HFpEF-Heart failure with preserved ejection fraction, HFrEF=Heart failure with reduced ejection fraction, MR=Mendelian randomization, pQTL- Protein quantitative trait loci

Clinical Perspective

What is New?

- 119 proteins associated with incident HF were identified, of which 68 are novel and 8 never previously linked to HF precursors. For HF subtypes, 15 and 11 proteins were identified for incident HFpEF and HFrEF, respectively, with 1 novel protein identified for each subtype. Of the 70 novel proteins, 55 replicated in an external cohort.
- Mendelian randomization analysis of incident HF and its subtypes using newly available HERMES2 GWAS data identified genetic associations for 9 proteins with incident HF or its subtypes. This included 5 novel proteins as possibly causal candidates for HF (AdipoQ, CD14, NCDP1, APOF, LMAN2), 2 for HFpEF (AdipoQ, CD14), and 2 for HFrEF (ARHGAP1, C9), although their corresponding pQTLs were not supported as single causal variants by colocalization analysis.

What are the Clinical Implications?

- HF imposes outsized morbidity and mortality, with treatment options lacking or only partly effective. The heterogeneity of HF, particularly HFpEF, challenges understanding of their molecular determinants as targets for effective therapeutics.
- The 55 newly identified and replicated protein markers and 7 new causal candidates offer to enhance risk prediction, advance biological understanding, and help streamline development and testing of novel therapies of HF and its subtypes in older adults, the segment of the population most affected by these disorders.

Introduction

Heart failure (HF) represents a global healthcare burden, which predominantly falls upon older adults.[1] With aging of the population, the overall prevalence of HF is expected to triple by 2060, underscoring the crucial need for improved HF prevention efforts.[2-4] The disorder is classified into two major subtypes, HF with reduced ejection fraction (HFrEF) and HF with preserved ejection fraction (HFpEF),[5] which carry similarly pronounced morbidity and mortality.[6] Although therapeutic advances have been achieved for HFrEF, proven treatments for HFpEF remain limited.[7,8] This therapeutic gap likely reflects the distinct pathophysiologies of the two HF subtypes. While HFrEF is characterized by myocardial injury, cardiomyocyte loss, and neurohormonal activation, HFpEF is thought to result from microvascular dysfunction in the setting of comorbidities, with attendant cardiomyocyte stiffness and myocardial interstitial fibrosis.[9,10] Improved prevention and treatment of the two subtypes requires better understanding of these disorders' development and targetable pathophysiological pathways.

Genome-wide association studies (GWAS) have sought to identify common and rare susceptibility variants for HF, exposing associations with coronary heart disease (CHD), atrial fibrillation (AF), and obesity.[11,12] However, the identified loci thus far explain a minor proportion of HF risk and only a limited number of these have been linked to a targetable protein or pathway for therapies.[13-15] High-throughput plasma proteomics offers a powerful tool to evaluate the molecular determinants of HF and its subtypes, since circulating proteins released from cells integrate genetic and environmental inputs, and constitute gene products more directly targetable by therapeutic interventions.[14] Recent proteomic studies discovered several proteins to be associated with incident HF,[11,12,16-19] with associations extended to HFpEF and HFrEF.[13] Existing proteomic studies in elders have included only moderate numbers of HF events, however, and been limited in their capacity to evaluate HF subtypes. Nor have such studies been in a position to pursue causal inference specifically for HF subtypes through Mendelian randomization (MR) approaches.

We undertook large-scale plasma proteomics in a U.S. population-based cohort study of older adults using SomaLogic's high-throughput aptamer technology, and meta-analyzed our findings with those of a European population-based cohort study of elders that applied a similar proteomic platform, in order to enhance power to identify protein markers associated with overall HF, as well as HFpEF and HFrEF. Significant aptamer hits were then tested in a separate U.S. cohort of older adults. We subsequently leveraged a recently completed GWAS of HF and its subtypes to investigate the potential causal basis of identified protein associations.

Methods

Requests by qualified researchers to access the datasets supporting this study may be sent to CHS at CHSDATA@uw.edu, to AGES-RS at AGES_data_request@hjarta.is, and to ARIC at aricpub@unc.edu. The research was approved by the institutional review boards of all participating studies and all participants provided written informed consent. Detailed methods for the present work are provided in the Supplemental Materials.

Results

Cohort Characteristics

The baseline characteristics for the primary CHS and AGES-RS cohorts are presented in Table 1. The cohorts were largely comparable in demographic and clinical characteristics. Notable differences included the 18% Black and 1% Hispanic race/ethnic composition of CHS, its higher use of antihypertensive medication, lower lipid concentrations and greater diabetes frequency, as contrasted with AGES-RS. The cumulative incidence of HF was 4-fold higher in CHS, reflecting its longer follow-up time (median 11.3 years, maximum 22.1 years), than in AGES-RS (median follow-up time 5.4 years, maximum 7.9 years). Of the 1,150 incident HF events in CHS, 30% were HFpEF, 26% were HFrEF, and 43% were unclassified. In turn, of the 440 incident HF events in AGES-RS, 43% were HFpEF, 38% were HFrEF, and 19% were

unclassified. As shown in Supplemental Table 7, participants in CHS and/or AGES-RS who went on to experience a HF event were older, less often women, had higher adiposity (CHS only), were less frequently never smokers (AGES-RS), exhibited more hypertension and diabetes but lower HDL cholesterol, had more prevalent and incident MI, and showed lower eGFRcr.

The ARIC replication cohort was of similar age (75.5 ±5.1) and sex distribution (58.5% female) as CHS and AGES-RS, with comparable proportion of Black participants (17.0%) as CHS. CVD risk factors were similar to one or both primary cohorts, but ARIC participants had more diabetes (34.8%) and prevalent MI (14.7%), though incident MI (6.3%) was lower. There were 621 incident HF events during a median follow-up of 9.5 years (maximum, 11.6 years), of which 299 (48%) were HFpEF, 227 (37%) were HFrEF, and 95 (15%) unclassified.

Individual Proteins and Incident HF and Its Subtypes

The multivariable-adjusted associations of individual SomaScan aptamers and incident HF in CHS and AGES-RS are presented separately for each cohort in Supplemental Table 8. Meta-analysis of the associations for these 4,404 aptamers across the two cohorts showed that 128 were significantly associated with incident HF after multiple testing-correction (Figure 1 and Supplemental Table 9). These 128 aptamers correspond to 119 unique proteins, of which 68 are newly linked to incident HF, including 8 proteins not previously associated with prevalent HF, HF predisposing conditions (CHD or AF), or HF-related phenotypes or outcomes in preclinical or clinical studies (Supplemental Table 10). The most significant association was for NPPB (B-type natriuretic peptide), with significant associations also seen for TNNI3 (troponin I) and CST3 (cystatin C), consistent with well-established cardiac and kidney biomarkers of HF risk.

Corresponding cohort-specific associations of individual aptamers with HFpEF and HFrEF following multivariable adjustment are given in Supplemental Tables 11 and 12, respectively. Meta-analysis of the two cohorts revealed that 15 aptamers (15 unique proteins) were significantly associated with incident HFpEF and 12 aptamers (11 unique proteins) with incident HFrEF (Figure 2A and 2B; Supplemental Tables 13 and 14, respectively). All but one aptamer for each HF subtype showed significant associations with overall HF. The exceptions were DEFB135 for HFpEF and ARHGAP1 for HFrEF, the former representing a novel association with HF or related phenotypes or outcomes, the latter a new association with incidence of any form of HF (Supplemental Table 10). Among aptamers significantly associated with a HF subtype, 3 (NPPB, SVEP[11178-21], TREM1) were associated in a concordant direction with both HFpEF and HFrEF (at Bonferroni-corrected significance, Figure 3). In addition, among aptamers associated with overall HF, 8 showed different strengths of association with HFpEF and HFrEF at a nominal level of significance, of which 3 proteins did not meet the Bonferroni-corrected threshold for significance of associations in the subtype-specific analysis (Figure 4).

We next examined whether aptamers significantly associated with HF and its subtypes in the CHS and AGES-RS meta-analysis replicated in ARIC (Supplemental Tables 15-17). Of the 128 significant aptamers (119 proteins) for HF, 115 aptamers (112 proteins) were measured in ARIC; among these, 109 aptamers (106 proteins) showed associations at nominal significance with HF, and 88 aptamers (85 proteins) showed associations at Bonferroni-corrected significance ($p=0.05/115=4.3\times10^{-4}$). For HFpEF, of 15 aptamers/proteins, 14 were measured in ARIC, and all but one replicated, each at Bonferroni-corrected significance ($p=0.05/14=0.0036$). For HFrEF, all 12 aptamers (11 proteins) were measured in ARIC, of which 10 aptamers (9 proteins) replicated, all at Bonferroni-corrected significance ($p=0.05/12=0.0042$). As shown in Supplemental Table

10, among the 68 proteins newly associated with HF in CHS and AGES-RS, 3 did not replicate and 4 were not measured in ARIC. Of the remaining 61 proteins replicated in ARIC, 54 did so at Bonferroni-corrected significance. All 8 proteins not previously associated with prevalent HF, predisposing conditions or HF-related outcomes replicated in ARIC, 7 of them at Bonferroni-corrected significance. Both new proteins associated with HF subtypes replicated, that for HFpEF at Bonferroni-corrected significance, and that for HFrEF at nominal significance.

In sensitivity analyses, replacement of eGFRcr with eGFRcr-cys in the main model in CHS led to meaningful attenuation (≥15% change in the beta coefficient) for 57 of the 128 aptamers shown significantly associated with HF in the CHS and AGES-RS meta-analysis, of which 33 represented novel aptamers/proteins (Supplemental Table 18 and Supplemental Table 10). For HFpEF and HFrEF, using eGFRcr-cys in lieu of eGFRcr resulted in meaningful attenuation of beta coefficients for 3 of 15 aptamers and 3 of 12 aptamers, respectively, though not either of the two novel proteins (Supplemental Tables 19-20). When eGFRcr categories replaced continuous eGFRcr in the main model for both CHS and AGES-RS, 35 of the 128 HF-associated aptamers exhibited meaningful attenuation of their beta coefficients (Supplemental Tables 21 and 10). Of these, 20 aptamers/proteins were new and 10 lost Bonferroni-corrected significance. For HFpEF and HFrEF, eGFR categorization led to meaningful attenuation of associations for 2 of 15 proteins and 2 of 12 aptamers, respectively, all of which became non-significant at the Bonferroni threshold (Supplemental Tables 22-23 and 10). There was no meaningful attenuation of the single novel protein for each HF subtype.

MR Analysis

For MR analysis, after exclusion of NPPB, 84 of the 128 aptamers associated with HF could be instrumented in ARIC. Of these, 7 aptamers (7 unique proteins) showed genetic associations with overall HF at a nominal level of significance ($p<0.05$), though not after Bonferroni correction: LMAN2, CCDC126, APOF, CD14, NPDC1, FSTL3 and ADIPOQ (Figure 5). All replicated in ARIC at a nominal ($p<0.05$) significance level, and 5 at Bonferroni-corrected significance (Supplemental Table 15). Among these 7 proteins, 3 showed directionally concordant observational and genetic associations (APOF, LMAN2 and NPDC1) (Supplemental Table 24).

In the case of HF subtypes, MR analysis was performed for the aptamers showing significant associations with HFpEF (11 aptamers instrumented) and HFrEF (9 aptamers instrumented). MR analysis was also conducted on additional aptamers associated with overall HF that differed significantly in their relations with HFpEF vs. HFrEF (2 and 1 aptamers instrumented, respectively), and for the 7 proteins that showed significant genetic associations with overall HF. These analyses revealed nominally significant genetic associations for 2 proteins with each subtype (Figure 6). For HFpEF, the 2 proteins were ADIPOQ and CD14, also genetically associated with overall HF, of which ADIPOQ retained significance after Bonferroni correction. In the case of HFrEF, the 2 proteins were ARHGAP1 and C9. Both proteins were significantly associated with this subtype in the observational analysis, and both showed significant associations, at least at nominal significance, in ARIC. Of the 4 proteins genetically related to HF subtypes, only C9 showed a concordant direction with the observational association (Supplemental Table 24).

None of the aptamers that emerged as significantly associated with HF or its subtypes in MR analysis could be instrumented with more than 2 variants, so sensitivity analyses for horizontal pleiotropy involving multiple variants could not be conducted. We did perform

colocalization analyses for significant pQTLs identified in MR analysis, the results of which are shown in Supplemental Table 25. Such colocalization analyses did not reveal evidence for a single causal variant for corresponding protein level and either overall HF or its subtypes (all H4 posterior probabilities <75%). There was some evidence, however, that the genetic association for one protein, C9, and HFrEF might reside in a single causal variant, as suggested by an H4 posterior probability of 66.9%. By contrast, there was evidence that one protein, CD14, had distinct causal variants for its level and HF (H3 posterior probability of 91.5%).

In exploratory analyses, we examined whether proteins genetically associated with HF or its subtypes also bore genetic associations with predisposing factors for HF. As shown in Table 2, all proteins except for ADIPOQ and C9 showed genetic associations with at least one predisposing factor at a nominally significant level. APOF showed a genetic association with CHD; CD14, CCDC126 and NPDC1 with diabetes; FSTL3 and LMAN2 with hypertension and diabetes; and ARHGAP1 with AF. With the exception of FSTL3 and LMAN2 with hypertension, and LMAN2 and NPDC1 with diabetes, these associations were also significant upon Bonferroni correction. All but one of the foregoing genetic associations with predisposing conditions were in the same direction as for HF or its subtypes. The exception was CD14, which showed a genetically inverse association with HF, but a genetically positive association instead with diabetes.

Last, we performed pathway enrichment analysis separately in the two cohorts using KEGG canonical pathways. In CHS, only two pathways, the WNT signaling and WNT5A-ROR signaling pathways, showed significant associations with HF after Bonferroni correction (Supplemental Table 26). Meanwhile, AGES-RS showed three pathways, SARS-CoV-2-Spike-to-ANGII-AT1R-NOX2, KSHV-VGPCR-to-GNB-G-ERK, and TRK-Fusion Kinase-to-RAS-ERK, to be associated with overall HF at Bonferroni significance. None the pathways identified in each cohort showed significance in the other cohort. There were no Bonferroni-significant associations of KEGG canonical pathways represented in the data with either HF subtype (Supplemental Tables 27-28).

Discussion

Main Findings

In this large-scale proteomics study of two population-based cohorts of older adults, we identified 128 individual aptamers (119 unique proteins) associated with incident HF after adjustment for clinical covariates at a Bonferroni-corrected level of significance. Of these protein associations, 68 represent new links with incident HF, and 8 are first associations of any kind with HF phenotypes, HF predisposing conditions or HF-related outcomes. We separately found 15 aptamers (15 unique proteins) and 12 aptamers (11 unique proteins) to be independently associated with incident HFpEF and HFrEF, respectively, at Bonferroni-corrected significance, including 1 aptamer/protein for each subtype that was not detected in the overall HF analysis. Each of these represents a new association with any form of incident HF, with the HFpEF-related protein DEFB135 never previously linked to prevalent HF or predisposing conditions. Among the 66 of the 70 novel proteins associated HF or HF subtypes that were measured in ARIC, 55 replicated at Bonferroni-corrected significance, and another 8 at nominal significance. Of the 128 aptamers related to HF, 8 showed different strengths of association with subtypes at a nominal level of significance, including 3 that were not identified in the subtype-specific screen. We pursued exploratory pathway enrichment analysis, which detected only a limited number of canonical pathways associated with HF, though not its subtypes, in each cohort. In MR analyses,

we found genetic associations for 7 HF-associated proteins at a nominal level of significance, including 2 proteins (LMAN2 and NPDC1) not previously linked to HF incidence, 1 (CCDC126) with an earlier genetic association with HF, and several (ADIPOQ, CD14, APOF, FSTL3) with previously reported HF associations. Two of these proteins (ADIPOQ, CD14) newly showed nominally significant genetic associations with HFpEF – which for ADIPOQ was also significant after Bonferroni correction – while two HFrEF-specific proteins (ARHGAP1, C9) newly exhibited nominally significant genetic associations with this subtype. There was no evidence by colocalization analysis, however, that any of the causally implicated pQTLs by MR represented single causal variants for both protein levels and HF or HF subtypes.

Prior Literature

A number of studies have applied proteomics to identify circulating protein markers of incident HF or its subtypes with the goal of improving prediction or illuminating pathobiology.[13,16-20] These have proven successful in advancing predictive accuracy and implicating potentially causal proteins for therapeutic targeting. Most prior studies examined earlier-generation proteomic platforms, but three recent reports – one from AGES-RS, and two ARIC – evaluated the larger SomaScan 5K platform.

The AGES-RS report applied age- and sex-adjusted LASSO to identify partly overlapping protein panels predictive of HF and its subtypes.[20] These included 10 proteins each for HF and HFrEF, the strongest for both being NPPB, MMP12 and TNNI3; and 8 proteins for HFpEF, the strongest being NPPB, MMP12 and TIMP4. These panels improved discrimination over clinical factors, especially early after measurement, findings that were replicated in CHS.

The first ARIC report identified 37 plasma proteins associated with incident HF across participants from two visits (mid-life and late-life) and mid-late life participants from the HUNT study, of which most showed comparable associations with HF subtypes in the ARIC late-life sample.[11] The second screened for proteins associated with both frailty and HF in ARIC, identifying 18 plasma proteins (14 new compared with the earlier report) associated with these disorders. A majority of these proteins were associated with HFpEF and a minority with HFrEF, and all were replicated in CHS.[12] In the first report, MR analysis documented a *trans*-pQTL for SVEP1 associated with HF at Bonferroni-corrected significance, while the second report identified *cis*-pQTLs for EFEMP1, FSTL3 and TREM1 as associated with HF at nominal significance.

The present study extends these previous findings by virtue of its exclusive focus on adults late in the life course; its assessment of individual aptamer associations after extensive adjustment for clinical risk factors, unlike the prior AGES-RS report; use of meta-analysis to amplify the number of incident HF or HF subtype events, which are ~3-fold greater than in the prior ARIC late-life report; involvement of the older ARIC cohort for replication of significant protein associations; and leveraging of HERMES2 to conduct specific MR analysis of HF subtypes and to increase power for MR analysis of overall HF. Specifically, this investigation newly identifies 70 protein markers of incident HF or its subtypes across two separate cohorts, including 1 novel protein marker each for HFpEF and HFrEF, of which 55 showed independent replication at a stringent significance threshold. The current report also newly suggests 5 proteins as potentially causally associated with HF, of which 2 showed a possible causal link with HFpEF, as well as another 2 with HFrEF, although lack of supportive evidence from colocalization analysis makes their causal nature uncertain.

Potential Clinical and Biological Implications

Identification of multiple new biomarkers of incident HF or its subtypes has potential implications for risk prediction, as well as therapeutics. It is notable that multiple biomarkers, new or established, were inversely associated with HF. None were so associated with HFpEF, and only two were inversely associated with HFrEF, although the number of incident events for these subtypes was more limited. Insofar as the identified inverse associations reflect or drive protective processes, they are of particular interest for identifying potential preventive interventions. Of various proteins or peptides that have been previously recognized or validated clinically as HF biomarkers, it is of interest that only a few of their corresponding aptamers achieved Bonferroni-corrected significance, and number did not achieve even nominal significance (Supplemental Table 29). Although SomaLogic's aptamer-based platform has the advantage of achieving higher precision and analytic breath than Olink's antibody-based platform, it has been found to have comparatively lower target specificity and phenotype associations.[21] Our findings point to limitations in using aptamers in place of immunoassays for certain previously identified HF biomarkers in clinical settings.

Findings from our sensitivity analyses adjusting for different measures of CKD, which revealed meaningful attenuation for substantial proportions of aptamer hits, also have implications for understanding relevant pathophysiologic mechanisms. That CST3, a top hit for HF (and HFpEF), remained significantly associated with this outcome after eGFRcr-cys adjustment likely reflects the influence on cystatin C levels of non-GFR sources also associated with HF. These include obesity, diabetes, inflammation and thyroid dysfunction but remain incompletely characterized.[22] There could also be an impact of differential measurement of cystatin C by the aptamer-based and ELISA-based method, although the high correlation (r=0.9) between the two suggests that such impact would be modest.[23] The attenuation of aptamer associations with adjustment by eGFRcr categories suggests the presence of non-linear effects involving CKD, aptamer levels, and HF incidence that need to be considered in such proteomic analyses. However, the observed attenuation, whether by eGFRcr-cys or eGFRcr categories, does not necessarily reflect confounding and could signify the impact of aptamers on HF risk through CKD-related pathways.[24] Further study of the relationship between affected proteins, CKD and HF is necessary to disentangle the pathways involved.

The suggestion of possible causal associations for several proteins may also have implications for understanding disease mechanisms and developing therapeutics. New associations of genetically determined protein levels were documented for LMAN2, APOF, CD14, NPDC1 and ADIPOQ with overall HF, ADIPOQ and CD14 with HFpEF, and ARHGAP1 and C9 with HFrEF. Genetic associations previously reported for FSTL3 and CCDC126 were confirmed,[12,25] with new measurement of circulating CCDC126 level showing that the observational association is directionally discordant (inverse) from the genetic association (positive). Nonetheless, our colocalization analyses failed to detect evidence that corresponding pQTLs for these proteins represent single causal variants for HF or its subtype, such that suggestions of causal associations uncovered here will require additional functional work to determine the true pathophysiologic contributions of these genetically linked proteins and HF outcomes.

Among the newly suggested causal proteins, NPDC1, APOF, and LMAN2 showed concordant positive associations in observational and genetic analyses. NPDC1 (neuronal proliferation, differentiation and control 1 protein) is primarily expressed in neural tissue, where it regulates neuronal proliferation and differentiation, but cardiac and vascular expression also

occur (GTEx).[26] In contrast to the positive association with HF documented here, NPDC1 was previously inversely associated with incident CVD (MI, stroke, and HF),[26] and our exploratory MR analysis linked it inversely to diabetes.[27] The basis for these contrary associations is unclear, as is NPDC1's biological role in HF or glucose dysregulation. Nevertheless, empagliflozin treatment was documented to reduce circulating NPDC1 in HFrEF, illustrating that NPDC1 levels are modifiable in a manner directionally consistent with our findings.[28] Additional investigation is necessary to determine whether and how this protein could be manipulated for effective HF treatment.

APOF (apolipoprotein F), a liver-derived protein predominantly found in HDL particles, plays a role in reverse cholesterol transport and HDL metabolism.[29] Conflicting associations have been reported with lipoprotein particle levels, which may relate to their dependence on lipid composition.[11,29] APOF's functions are complex and remain incompletely characterized. The present findings confirm the observational association with HF previously documented for APOF in ARIC, adding evidence of possible causality. As in ARIC, our MR analysis supports a causal association of APOF with CHD, suggesting that the association is driven by atherosclerosis.[11] Although no drugs capable of modulating APOF were reported in the ChEMBL database, our findings suggest that focused studies on APOF could yield fruitful therapies for CHD and HF.

Produced by the liver, LMAN2 (lectin, mannose binding 2) is involved in regulation of exosome protein trafficking,[30] and closely influences macrophage phagocytotic activity.[31] The protein is shed from the endothelial glycocalyx in sepsis,[32] which may explain its reduced endothelial cell expression in acute MI.[33] Low urinary levels of LMAN2 in acute HF have also been reported,[34] as has an inverse relation of plasma LMAN2 with NT-proBNP in HFpEF.[35] Such associations may reflect endothelial damage or dysfunction, but our findings point to potentially adverse actions of higher LMAN2 expression. This protein, which lacks drug ligands on ChEMBL, will require further study.

All remaining proteins suggested as potentially causal by MR analysis showed discordant observational and genetic associations, indicating that circulating levels are importantly affected by factors beyond their instrumented *cis*-pQTLs. Two proteins, ADIPOQ and CD14, showed inverse genetic associations with HF and HFpEF. Produced by adipose tissue but also by myocardium and skeletal muscle, ADIPOQ (adiponectin) has well-established insulin-sensitizing, anti-inflammatory, and cardioprotective properties.[36] Yet, in contrast to the inverse associations of circulating ADIPOQ with CVD documented in younger, healthy adults, plasma levels of the adipokine have been positively associated with CHD, HF and mortality in older persons or those with comorbidities – in CHS or elsewhere.[37] There is moreover evidence that the latter associations can be U-shaped.[38,39] This has complicated MR analysis, which has failed to find evidence of a causal association with CVD outcomes.[36] The finding that a single pQTL was associated with overall HF and HFpEF, the latter at Bonferroni-corrected significance, is novel. It is also intriguing, because there was no corroborating evidence of this single variant's causal role for these outcomes. Given that an oral adiponectin receptor agonist exists,[36] the present association should motivate study of a potential therapeutic role for such compounds for HF and, particularly, HFpEF.

In turn, CD14 (cluster of differentiation 14) is a membrane glycoprotein expressed on monocytes/macrophages, adipocytes and hepatocytes, as well as cardiomyocytes, among other cell types.[40] Binding of lipopolysaccharide to surface CD14 activates pro-inflammatory pathways, while also stimulating shedding of the membrane glycoprotein as a soluble form

(sCD14).[40] This makes sCD14 a marker of metabolic endotoxemia. Prior work in CHS did not reveal an association of sCD14 with insulin resistance or incident diabetes after adjustment for other inflammatory markers, but did show that sCD14 was associated with incident HF and, especially, HFpEF.[41] The inverse genetic association identified here with HF and HFpEF was directionally opposite not only to the observational association, but also to the positive genetic association detected with diabetes. The explanation for these divergent associations is unclear. It is known, however, that sCD14 can quench circulating lipopopolysaccharide by transferring it to lipoprotein particles, and that sCD14-lipopolysaccharide complexes can deposit on endothelial cells to produce inflammatory activation.[40,41] Our colocalization analysis also showed evidence for distinct causal variants for CD14 level and HF, suggesting that molecular features of the protein separate from its level could be driving its relationships with outcomes. How such molecular features or other factors determine the distinct associations documented here merits additional study.

Of the two proteins that emerged as potentially causally associated with HFrEF, ARHGAP1 (Rho GTPase-Activating Protein 1) is an intracellular protein that is ubiquitously expressed. Circulating levels of the protein have been documented to increase after clinical MI.[42] Experimental data show that cardiomyocyte ischemia induces production of ARHGAP1, which stimulates apoptosis.[43] Consistent with this, cardiomyocyte expression of ARHGAP1 is increased in ischemic cardiomyopathy.[44] These findings would explain the positive observational association documented here with HFrEF, but not the inverse genetic association with this HF subtype or AF. ARHGAP1 appears to play a role in regulation of iron transport across membranes, however, such that higher genetically determined levels could protect against iron dysregulation in dilated cardiomyopathy.[45,46] Although this protein lacks known potential therapeutic ligands, the present findings supporting a possible causal role in HFrEF render it an important target for future study.

The second protein, C9 (complement 9), is a component of the membrane attack complex involved in disruption or lysis of microbial and diseased cells.[47] Such terminal complement activation has been implicated in the pathogenesis of dilated cardiomyopathy as part of the immune response to myocardial injury.[48,49] C9 was previously associated with incident HF and both subtypes in the ARIC and HUNT study,[11] but here we show support for a causal association for HFrEF. This finding strengthens the case that existing[47] or new complement-modulating therapies might have a place in HFrEF prevention or treatment.

Limitations

Several limitations to our study deserve attention. We used fixed-effects meta-analysis to maximize discovery across two separate cohorts of older adults. This approach yields average associations, and is unconcerned with population differences. Future studies will need to investigate how associated proteins vary in distinct populations. Our investigation identified proteins associated with HF, HFpEF and HFrEF in observational and MR analyses, but these findings must be interpreted in the context of substantial differences in the number of cases available for overall HF versus its subtypes, which had more limited power. Hence, while associations documented for a given HF subtype suggest a preferential role in pathophysiology, they do not exclude a consequential role for the other subtype. For MR analysis, all but one of the genetic associations occurred at nominal, but not Bonferroni-corrected, significance. Of these, that of CD14 with HF showed evidence of distinct causal variants in colocalization analysis, which violates MR assumptions for the lead variant and precludes its use for causal

inference. Among the others, only that of C9 with HFrEF had suggestive evidence of colocalization. As such, the associations for these proteins lack corroboration for a potential causal role, and a judgment to that effect will require separate supportive evidence. In our observational analyses, substantial proportions of HF cases could not be subclassified, particularly in CHS. Nor did we have complete characterization of valvular heart disease in CHS or AGES-RS to evaluate its impact on HF here. Nonetheless, our data suggest that the contributions of such primary valvular disease as severe aortic stenosis to our incident HF cases was modest.[50] The current findings come predominantly from older populations of European ancestry, and do not necessarily apply to other groups. Our pathway enrichment analysis method could not be combined across CHS and AGES-RS, and was therefore limited by each cohort's sample size, likely accounting for the method's modest yield in canonical pathways and the pathway differences observed.

Conclusions

In this large-scale proteomic investigation of older adults, we identified 70 novel protein markers of incident HF or its subtypes, 55 of which were externally replicated. We also implicated 5 new possibly causal proteins for HF, 2 of which were specifically linked to HFpEF, as well as another 2 new possibly causal proteins for HFrEF. These findings open the way for additional investigation of these protein markers for risk stratification and biological insight, and support prioritization of a number of suggested causal proteins for investigation as potential candidates for therapeutic testing and development.

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Appendix Authors

The HERMES (Heart Failure Molecular Epidemiology for Therapeutic Targets) Consortium:

Bitten Aagaard[1], Erik Abner[2], Lance Adams[3], Peter Almgren[4], Charlotte Andersson[5,6], Krishna G. Aragam[7,8,9], Johan Ärnlöv[10,11], Folkert W. Asselbergs[12,13,14], Geraldine Asselin[15], Anna Axelsson Raja[16], Joshua D. Backman[17], John Baksi[18], Paul J. R. Barton[19,20,18], Traci M. Bartz[21], Kiran J. Biddinger[7,9], Mary L. Biggs[21,22], Heather L. Bloom[23], Eric Boersma[24], Isabelle Bond[25], Jeffrey Brandimarto[26], Michael R. Brown[27], Søren Brunak[28], Hans-Peter Brunner-La Rocca[29], Mie Topholm Bruun[30], Rachel Buchan[19,20,18], Leonard Buckbinder[31], Henning Bundgaard[16], Douglas Cannie[25,32], Thomas P. Cappola[26], David J. Carey[33], Mark D. Chaffin[9], Philippe Charron[34,35], Daniel I. Chasman[36,37], Olympe Chazara[38], Xing Chen[31], Xu Chen[39], Jonathan H. Chung[17], William Chutkow[40], John G.F. Cleland[41,42], James P. Cook[43,44], Stuart A. Cook[19,20], Tomasz Czuba[45], Trégouët David-Alexandre[46,47], Simon de Denus[15,48], Antonio de Marvao[19,20], Abbas Dehghan[49], Graciela E. Delgado[50], Spiros Denaxas[51,12,52,13], Alexander S. Doney[53], Marcus Dörr[54,55], Joseph Dowsett[56], J. Gustav Smith[57,45,58], Marie-Pierre Dubé[15,59], Samuel C. Dudley[60], Michael E. Dunn[61], Patrick T. Ellinor[62,9], Perry Elliott[25], Gunnar Engström[4], Villard Eric[34,35], Christian Erikstrup[63,64], Tõnu Esko[2,9], Eric H. Farber-Eger[65], Ghazaleh Fatemifar[66,12], Stephan B. Felix[54,55], Chris Finan[25], Sarah Finer[67], Ian Ford[68], Francoise Fougerousse[69], René Fouodjio[15], Catherine Francis[19,18], Sophie Garnier[34,35], Mohsen Ghanbari[70], Sahar Ghasemi[71,55,72], Jonas Ghouse[73], Vilmantas Giedraitis[74], Franco Giulianini[36], John S. Gottdiener[75], Stefan Gross[54,55], Daníel F. Guðbjartsson[76,77], Hongsheng Gui[78], Karl Guo[79], Rebecca Gutmann[80], Sara Hägg[81], Christopher M. Haggerty[33], Brian Halliday[19,18], Åsa K. Hedman[82], Anna Helgadottir[76], Harry Hemingway[12,51], Albert Henry[25,12], Hans Hillege[83], Aroon D. Hingorani[84,25], Hilma Holm[76], Michael V. Holmes[85,86,87], Craig L. Hyde[31], Erik Ingelsson[88,89,90,91], Hanane Issa[12], Jaison Jacob[92], Deleuze Jean-François[93,47,94], J. Wouter Jukema[95,96], Frederick Kamanu[97,98], Isabella Kardys[24], Maryam Kavousi[70], Kay-Tee Khaw[99], Jorge R. Kizer[100], Marcus E. Kleber[50], Lars Køber[101], Andrea Koekemoer[102], Bill Kraus[103], Karoline Kuchenbaecker[104,105], Yi-Pin Lai[31], David Lanfear[78,106], Chim C. Lang[53], Claudia Langenberg[107,108,109], Michael Lee[19], Honghuang Lin[6,110], Lars Lind[111], Cecilia M. Lindgren[112,9,113], Barry London[114], Luca A. Lotta[115], Ruth C. Lovering[25], Brandon D. Lowery[65], Jian'an Luan[109], Steven A. Lubitz[62,9], R. Thomas Lumbers[13,51,12], Patrik Magnusson[81], Anubha Mahajan[113], Anders Malarstig[82,31], Charlotte Manisty[25,116], Douglass Mann[117], Kenneth B. Margulies[26], Nicholas A. Marston[118], Hilary Martin[119], Winfried März[120,121,50], Kathryn McGurk[19,20], John J.V. McMurray[122], Olle Melander[123], Giorgio Melloni[97,98], David Miller[124], James C Moon[125,25], Ify R. Mordi[53], Thomas Morgan[40,126], Michael P. Morley[26], Andrew D. Morris[127], Andrew P. Morris[44,113], Alanna C. Morrison[27], Lori Morton[61], Michael W. Nagle[31], Christopher P. Nelson[102], Christopher Newton-Cheh[7,128,9], Alexander Niessner[129], Teemu Niiranen[130,131], Raymond Noordam[132], Michela Noseda[19], Mahdad Noursadeghi[133], Christoph Nowak[10], Michelle L. O'Donoghue[118], Declan P O'Regan[19,20], Sisse Rye Ostrowski[56,134], Anjali T. Owens[26], Colin N. A. Palmer[135], Antonis Pantazis[18], Guillaume Paré[136,137,138], Helen M. Parry[53], Lavinia Paternoster[139,140], Ole Birger Pedersen[141,134], Markus Perola[131], Louis-Philippe Lemieux Perreault[15], Marie Pigeyre[142,138], Eliana Portilla-Fernandez[70,143], Sanjay K. Prasad[19,18], Bruce M. Psaty[144,145], Kenneth M. Rice[21], Paul M. Ridker[36,37], Simon P. R. Romaine[102], Carolina Roselli[83,9], Jerome I. Rotter[146,147,148],

Christian T. Ruff[118], Mark S. Sabatine[118], Neneh Sallah[12], Perttu Salo[131], Veikko Salomaa[131], Nilesh J. Samani[102], Naveed Sattar[149], Jessica van Setten[150], Sonia Shah[151,25], Svati Shah[152,153,103], Alaa A. Shalaby[154], Akshay Shekhar[61], Diane T. Smelser[33], Nicholas L. Smith[155,145,156], Erik Sørensen[157], Doug Speed[158], Sundararajan Srinivasan[135], Kari Stefansson[76,159], Steen Stender[160], David J. Stott[161], Nicholas P. Sunderland[162], Garðar Sveinbjörnsson[76], Per Svensson[163,164], Daniel I. Swerdlow[165,25], Petros Syrris[25], Mari-Liis Tammesoo[2], Jean-Claude Tardif[15,59], Upasana Tayal[19,18], Kent D. Taylor[166], Maris Teder-Laving[2], Alexander Teumer[55,167,72], Pantazis I Theotokis[20], Guðmundur Thorgeirsson[76,168,159], Unnur Thorsteinsdottir[76,159], Christian Torp-Pedersen[169], Vinicius Tragante[76], Thomas A Treibel[125,25], Stella Trompet[95,132], Danny Tuckwell[92], Benoit Tyl[170], Andre G. Uitterlinden[70,143], Henrik Ullum[171], Ana M Valdes[172], Pim van der Harst[83,150], David van Heel[173], Ramachandran S. Vasan[6,174], Felix Vaura[175,176], Abirami Veluchamy[53,135], Monique Verschuuren[177,178], Niek Verweij[83], Peter M. Visscher[179,180], Christoffer Rasmus Vissing[16], Uwe Völker[55,181], Adriaan A. Voors[83], Marion van Vugt[150], Lars Wallentin[182], Xiaosong Wang[40], Yunzhang Wang[81], James S. Ware[19,183,18,184,9], Nicholas J. Wareham[115], Dawn Waterworth[79], Peter E. Weeke[73], Raul Weiss[185], Quinn Wells[186], Harvey White[187], Kerri L. Wiggins[22], Jemma B. Wilk[31], L. Keoki Williams[78], Heming Xing[40], Xiao Xu[19,20], Jian Yang[179,180], Yifan Yang[26], Laura M. Yerges-Armstrong[79], Bing Yu[27], Faiez Zannad[188], Faye Zhao[92], Jing Hua Zhao[115], Chaoqun Zheng[16], Sean L. Zheng[19,20,18]

Affiliations

1. Department of Clinical Immunology, Aalborg Univeristy Hospital, Aalborg
2. Estonian Genome Center, Institute of Genomics, University of Tartu, Tartu, 51010, Estonia
3. Geisinger Health System, Danville, PA, USA
4. Department of Clinical Sciences, Lund University, Malmö, Sweden
5. Department of Cardiology, Herlev Gentofte Hospital, Herlev Ringvej 57, Herlev, 2650, Denmark
6. National Heart, Lung, and Blood Institute's and Boston University's Framingham Heart Study, Framingham, Massachusetts, USA
7. Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA, USA
8. Center for Genomic Medicine, Massachusetts General Hospital, Boston, MA, USA
9. Program in Medical and Population Genetics, The Broad Institute of MIT and Harvard, Cambridge, MA, USA
10. Department of Neurobiology, Care Sciences and Society/ Section of Family Medicine and Primary Care, Karolinska Institutet, Stockholm, Sweden
11. School of Health and Social Sciences, Dalarna University, Falun, Sweden
12. Institute of Health Informatics, University College London, London, England, UK
13. The National Institute for Health Research University College London Hospitals Biomedical Research Centre, University College London, London, England, UK
14. Department of Cardiology, Amsterdam Cardiovascular Sciences, Amsterdam University Medical Centers, Amsterdam, The Netherlands
15. Montreal Heart Institute, Montreal, Canada
16. Department of Cardiology, The Heart Centre, Copenhagen University Hospital, Rigshospitalet, Ingen Lehmanns vej 7, Copenhagen, 2100, Denmark
17. Regeneron Genetics Center, 777 Old Saw Mill River Road, Tarrytown, NY 10591, USA

18. Royal Brompton & Harefield Hospitals, Guy's and St. Thomas' NHS Foundation Trust, London, England, UK
19. National Heart & Lung Institute, Imperial College London, London, England, UK
20. MRC London Institute of Medical Sciences, London, England, UK
21. Department of Biostatistics, University of Washington, Seattle, WA, USA
22. Department of Medicine, University of Washington, Seattle, WA, USA
23. Department of Medicine, Division of Cardiology, Emory University Medical Center, Atlanta, GA, USA
24. Department of Cardiology, Erasmus University Medical Center, Rotterdam, The Netherlands
25. Institute of Cardiovascular Science, University College London, London, England, UK
26. Penn Cardiovascular Institute, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA
27. Department of Epidemiology, Human Genetics, and Environmental Sciences, The University of Texas School of Public Health, Houston, TX, 77030, USA
28. Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
29. Maastricht University Medical Center, the Netherlands
30. Department of Clinical Immunology, Odense University Hospital, Odense, Denmark
31. Pfizer Worldwide Research & Development, 1 Portland St, Cambridge, MA, USA
32. London, England, UK
33. Department of Molecular and Functional Genomics, Geisinger, Danville, PA, USA
34. UMR-S1166, Research Unit on Cardiovascular Disorders, Metabolism and Nutrition, Team Genomics & Pathophysiology of Cardiovascular Diseases, Sorbonne Université, INSERM, UMR-S1166, Paris, 75013, France
35. ICAN Institute for Cardiometabolism and Nutrition, Paris, 75013, France
36. Division of Preventive Medicine, Brigham and Women's Hospital, Boston, MA, USA
37. Harvard Medical School, Boston, MA, USA
38. Centre for Genomics Research, Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Cambridge, UK
39. Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden.
40. Novartis Institutes for Biomedical Research
41. National Heart and Lung Institute, Imperial College, London, England, UK.
42. Robertson Centre for Biostatistics & Glasgow Clinical Trials Unit, Institute of Health and Wellbeing, University of Glasgow, Glasgow Royal Infirmary, Glasgow, UK
43. Department of Health Data Science, University of Liverpool, Liverpool, England, UK
44. Department of Biostatistics, University of Liverpool, Liverpool, England, UK
45. Department of Molecular and Clinical Medicine, Institute of Medicine, Gothenburg University and Sahlgrenska University Hospital, Gothenburg, Sweden
46. U1219, Univ. Bordeaux, INSERM, BPH, Bordeaux, 33000, France
47. Laboratory of Excellence GENMED (Medical Genomics), France
48. Faculty of Pharmacy, Université de Montréal, Montreal, Canada

49. MRC-PHE Centre for Environment and Health, Department of Epidemiology and Biostatistics, Imperial College London, London, England, UK
50. Vth Department of Medicine (Nephrology, Hypertensiology, Endocrinology, Diabetology, Rheumatology), Medical Faculty of Mannheim, University of Heidelberg, Heidelberg, Germany
51. Health Data Research UK, London, England, UK
52. British Heart Foundation Data Science Centre, London, England, UK
53. Division of Molecular & Clinical Medicine, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD1 9SY, Scotland, UK
54. Department of Internal Medicine B, University Medicine Greifswald, Greifswald, Germany
55. DZHK (German Center for Cardiovascular Research), partner site Greifswald, Greifswald, Germany
56. Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark
57. Department of Cardiology, Clinical Sciences, Lund University and Skåne University Hospital, Lund, Sweden
58. Wallenberg Center for Molecular Medicine and Lund University Diabetes Center, Lund University, Lund, Sweden
59. Faculty of Medicine, Université de Montréal, Montreal, Canada
60. Department of Medicine, Cardiovascular Division, University of Minnesota, Minneapolis, MN, USA
61. Cardiovascular Research, Regeneron Pharmaceuticals, Tarrytown, NY, USA
62. Cardiac Arrhythmia Service and Cardiovascular Research Center, Massachusetts General Hospital, Boston, MA, USA
63. Department of Clinical Immunology, Aarhus University Hospital, Aarhus, Denmark
64. Deparment of Clinical Medicine, Health, Aarhus University, Aarhus, Denmark
65. Vanderbilt Institute for Clinical and Translational Research, Vanderbilt University Medical Center, Nashville, TN, USA
66. Health Data Research UK London, University College London, England, UK
67. Centre for Primary Care and Public Health, Wolfson Institute of Population Health, Queen Mary University of London, London, E1 4NS, England, UK
68. Robertson Center for Biostatistics, Institute of Health and Wellbeing, University of Glasgow, Glasgow, Scotland, UK
69. Translational and Clinical Research, Servier Cardiovascular Center for Therapeutic Innovation, Suresnes, France
70. Department of Epidemiology, Erasmus University Medical Center, Rotterdam, The Netherlands
71. Institute of Genetic Epidemiology, Faculty of Medicine and Medical Center, University of Freiburg, Freiburg, Germany
72. Department of Psychiatry and Psychotherapy, University Medicine Greifswald, Greifswald, Germany
73. Department of Cardiology, Rigshospitalet, Copenhagen University Hospital, Denmark

74. Department of Public Health and Caring Sciences, Geriatrics, Uppsala, Sweden
75. Department of Medicine, Division of Cardiology, University of Maryland School of Medicine, Baltimore, MD, USA
76. deCODE genetics/Amgen Inc., Sturlugata 8, Reykjavik, 101, Iceland
77. School of Engineering and Natural Sciences, University of Iceland, Sæmundargata 2, Reykjavik, 101, Iceland
78. Center for Individualized and Genomic Medicine Research, Department of Internal Medicine, Henry Ford Hospital, Detroit, MI, USA
79. Human Genetics, GlaxoSmithKline, Collegeville, PA, USA
80. Division of Cardiovascular Medicine, University of Iowa Carver College of Medicine, Iowa City, IA, USA
81. Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden
82. Cardiovascular Medicine unit, Department of Medicine Solna, Karolinska Institute, Stockholm, Sweden
83. Department of Cardiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands
84. BHF Research Accelerator, University College London, England, UK
85. Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health, Big Data Institute, University of Oxford, Oxford, UK
86. Medical Research Council Population Health Research Unit at the University of Oxford, Oxford, UK
87. National Institute for Health Research Oxford Biomedical Research Centre, Oxford University Hospital, Oxford, UK
88. Department of Medical Sciences, Molecular Epidemiology and Science for Life Laboratory, Uppsala University, Uppsala, Sweden.
89. Department of Medicine, Division of Cardiovascular Medicine, Stanford University School of Medicine, Stanford, CA 94305
90. Stanford Cardiovascular Institute, Stanford University, Stanford, CA 94305
91. Stanford Diabetes Research Center, Stanford University, Stanford, CA 94305
92. Novartis Institutes for Biomedical Research, Cambridge, MA, USA
93. Centre National de Recherche en Génomique Humaine (CNRGH), Institut de Biologie François Jacob, CEA, Université Paris-Saclay, Evry, 91057, France
94. Centre d'Etude du Polymorphisme Humain, Fondation Jean Dausset, Paris, France
95. Department of Cardiology, Leiden University Medical Center, Leiden, The Netherlands
96. Einthoven Laboratory for Experimental Vascular Medicine, LUMC, Leiden, The Netherlands
97. Cardiovascular Disease Initiative, The Broad Institute of MIT and Harvard, Cambridge, MA, USA
98. TIMI Study Group, Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA
99. Department of Public Health and Primary Care, University of Cambridge, Cambridge, CB2 0QQ, UK

100. Cardiology Section, San Francisco Veterans Affairs Health System, and Departments of Medicine, Epidemiology and Biostatistics, University of California San Francisco, CA, USA
101. Department of Cardiology, Nordsjaellands Hospital, Hilleroed, Copenhagen, Denmark
102. Department of Cardiovascular Sciences, University of Leicester and NIHR Leicester Biomedical Research Centre, Glenfield Hospital, Leicester, England, UK
103. Duke Molecular Physiology Institute, Durham, NC, USA
104. Division of Psychiatry, University College London, London, W1T 7NF, England, UK
105. UCL Genetics Institute, University College London, London, WC1E 6BT, England, UK
106. Heart and Vascular Institute, Henry Ford Hospital, Detroit, MI, USA
107. Precision Healthcare University Research Institute, Queen Mary University of London, London, England, UK
108. Computational Medicine, Berlin Institute of Health (BIH) at Charité - Universitätsmedizin Berlin, Berlin, Germany
109. MRC Epidemiology Unit, Institute of Metabolic Science, University of Cambridge, Cambridge, CB2 0QQ, England, UK
110. Department of Medicine, University of Massachusetts Chan Medical School, Worcester, Massachusetts, USA
111. Department of Medical Sciences, Uppsala University, Uppsala, Sweden
112. Big Data Institute at the Li Ka Shing Centre for Health Information and Discovery, University of Oxford, Oxford, England, UK
113. Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, England, UK
114. Division of Cardiovascular Medicine and Abboud Cardiovascular Research Center, University of Iowa, Iowa City, IA, USA
115. MRC Epidemiology Unit, Institute of Metabolic Science, University of Cambridge School of Clinical Medicine, Cambridge, CB2 0QQ, UK
116. St Bartholomew's Hospital, Barts Heart Centre, UK
117. Center for Cardiovascular Research, Division of Cardiology, Department of Medicine, Washington University School of Medicine, St. Louis, MO, USA
118. TIMI Study Group, Division of Cardiovascular Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA, USA
119. Wellcome Sanger Institute, Wellcome Genome Campus, Hinxton, CB10 1RQ, England, UK
120. Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University of Graz, Graz, Austria
121. Synlab Academy, Synlab Holding Deutschland GmbH, Mannheim, Germany
122. BHF Cardiovascular Research Centre, University of Glasgow, Scotland, United Kingdom
123. Department of Internal Medicine, Clinical Sciences, Lund University and Skåne University Hospital, Malmö, Sweden
124. Division of Biosciences, University College London, London, England, UK
125. St Bartholomew's Hospital, Barts Heart Centre
126. Vanderbilt University School of Medicine

127. Usher Institute of Population Health Sciences and Informatics, University of Edinburgh, Edinburgh, Scotland, UK
128. Center for Human Genetic Research, Massachusetts General Hospital, Boston, MA, USA
129. Department of Internal Medicine II, Division of Cardiology, Medical University of Vienna, Vienna, Austria
130. Department of Medicine, Turku University Hospital and University of Turku, Turku, Finland
131. National Institute for Health and Welfare, Helsinki, Finland
132. Section of Gerontology and Geriatrics, Department of Internal Medicine, Leiden University Medical Center, Leiden, The Netherlands
133. National Heart and Lung Institute, Imperial College, London, England, UK
134. Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark
135. Division of Population Health and Genomics, University of Dundee, Ninewells Hospital and Medical School, Dundee, DD1 9SY, Scotland, UK
136. Department of Pathology and Molecular Medicine, McMaster University, Hamilton, ON, Canada
137. Thrombosis and Atherosclerosis Research Institute, Hamilton, ON, Canada
138. Population Health Research Institute, Hamilton, ON, Canada
139. Medical Research Council Integrative Epidemiology Unit, Bristol Medical School, University of Bristol, Bristol, England, UK
140. Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, England, UK
141. Department of Clinical Immunology, Zealand University Hospital, Køge, Denmark
142. Department of Medicine, McMaster University, Hamilton, ON, Canada
143. Department of Internal Medicine, Erasmus University Medical Center, Rotterdam, The Netherlands
144. Cardiovascular Health Research Unit, University of Washington, Seattle, WA, USA
145. Kaiser Permanente Washington Health Research Institute, Kaiser Permanente Washington, Seattle, WA, USA
146. The Institute for Translational Genomics and Population Sciences, Harbor-UCLA Medical Center, Torrance, CA, USA
147. Departments of Pediatrics and Medicine, Harbor-UCLA Medical Center, Torrance, CA, USA
148. Los Angeles Biomedical Research Institute, Harbor-UCLA Medical Center, Torrance, CA, USA
149. BHF Cardiovascular Research Centre, University of Glasgow, Glasgow, Scotland, UK
150. Department of Cardiology, Division of Heart and Lungs, University Medical Center Utrecht, Utrecht, 3584 CX, The Netherlands
151. Institute for Molecular Bioscience, The University of Queensland, Brisbane, Australia
152. Division of Cardiology, Department of Medicine, Duke University Medical Center, Durham, NC, USA
153. Duke Clinical Research Institute, Durham, NC, USA

154. Department of Medicine, Division of Cardiology, University of Pittsburgh Medical Center and VA Pittsburgh HCS, Pittsburgh, PA, USA
155. Department of Epidemiology, University of Washington, Seattle, WA, USA
156. Seattle Epidemiologic Research and Information Center, Department of Veterans Affairs Office of Research & Development, Seattle, WA, USA
157. Department of Clinical Immunology, Copenhagen University Hospital, Rigshospitalet, Blegdamsvej 9, Copenhagen, Denmark
158. Quantitative Genetics and Genomics, Aarhus University, Aarhus, Denmark
159. Department of Medicine, University of Iceland, Sæmundargata 2, Reykjavik, 101, Iceland
160. Department of Clinical Biochemistry, Copenhagen University Hospital, Herlev and Gentofte, Denmark
161. Institute of Cardiovascular and Medical Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, United Kingdom
162. Bristol Heart Institute / University Hospitals Bristol & Weston, University of Bristol, Bristol, England, UK
163. Department of Cardiology, Söderjukhuset, Stockholm, Sweden
164. Department of Clinical Science and Education-Södersjukhuset, Karolinska Institutet, Stockholm, Sweden
165. BenevolentAI, London, England, UK
166. Institute for Translational Genomics and Population Sciences, LABiomed and Departments of Pediatrics at Harbor-UCLA Medical Center, Torrance, Calif., USA 90502
167. Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany
168. Department of Internal Medicine, Division of Cardiology, National University Hospital of Iceland, Eiríksgata 5, Reykjavik, 101, Iceland
169. Department of Epidemiology and Biostatistics, Aalborg University Hospital, Aalborg, Denmark
170. Translational and Clinical Research, Servier Cardiovascular Center for Therapeutic Innovation, 50 rue Carnot 92284 Suresnes, France
171. Statens Serum Institut, Artillerivej 5, Copenhagen, 2300, Denmark
172. Injury, Recovery and Inflammation Sciences, School of Medicine, Nottingham, UK
173. Centre for Genomics and Child Health, Blizard Institute, Queen Mary University of London, London, E1 4AT, England, UK
174. Sections of Cardiology, Preventive Medicine and Epidemiology, Department of Medicine, Boston University Schools of Medicine and Public Health, Boston, Massachusetts, USA
175. Department of Clinical Medicine, University of Turku, Turku, Finland
176. Finnish Institute for Health and Welfare, Helsinki, Finland
177. Department Life Course and Health, Centre for Nutrition, Prevention and Health Services, National Institute for Public Health and the Environment, Bilthoven, The Netherlands

178. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands
179. Institute for Molecular Bioscience, The University of Queensland, Brisbane, Queensland 4072, Australia
180. Queensland Brain Institute, The University of Queensland, Brisbane, Queensland 4072, Australia
181. Interfaculty Institute for Genetics and Functional Genomics, University Medicine Greifswald, Greifswald, Germany
182. Uppsala Clinical Research Center, Uppsala University, Uppsala, Sweden
183. MRC London Institute of Medical Sciences, Imperial College London, London, England, UK
184. Hammersmith Hospital, Imperial College Hospitals NHS Trust, London, England, UK
185. Department of Internal Medicine, Division of Cardiovascular Medicine, The Ohio State University Medical Center, Columbus, OH, USA
186. Division of Cardiovascular Medicine, Vanderbilt University, Nashville, TN, USA
187. Green Lane Cardiovascular Service, Auckland City Hospital, USA
188. Université de Lorraine, CHU de Nancy, Inserm and INI-CRCT (F-CRIN), Institut Lorrain du Coeur et des Vaisseaux, Vandoeuvre Lès Nancy, 54500, France

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Supplemental Materials

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Figure Legends:

Figure 1. Associations of Individual Aptamers with Incidence of Overall Heart Failure.
Volcano plot of individual aptamer associations with incidence of overall heart failure after multivariable adjustment in CHS and AGES-RS. Red dots denote significance at the Bonferroni-corrected level.
AGES-RS = Aging Gene/Environment Susceptibility – Reykjavik Study; CHS = Cardiovascular Health Study; HF = Heart failure.

Figure 2. Associations of Individual Aptamers and Incidence of HFpEF and HFrEF.
Volcano plots of individual aptamer associations with incidence of HFpEF (Panel A) and HFrEF (Panel B) after multivariable adjustment in CHS and AGES-RS.
AGES-RS = Aging Gene/Environment Susceptibility – Reykjavik Study, CHS = Cardiovascular Health Study; HFpEF = Heart failure with preserved ejection fraction; HFrEF = Heart failure with reduced ejection fraction.

Figure 3. Relations of Aptamer Hits for Each HF Subtype with the Alternate Subtype and Overall HF. Forest plot of aptamers associated with HFpEF and/or HFrEF at Bonferroni-corrected significance after multivariable adjustment, showing corresponding associations with the alternate subtype and overall heart failure.
*Significant at the Bonferroni-corrected threshold.
HF = Heart failure; HFpEF = Heart failure with preserved ejection fraction; HFrEF = Heart failure with reduced ejection fraction.

Figure 4. Differential Strengths of Associations with HFpEF and HFrEF for Aptamer Hits for Overall HF. Aptamers associated with overall HF showing differential strengths of association between HFpEF and HFrEF at a nominal level of significance.
HF = Heart failure; HFpEF = Heart failure with preserved ejection fraction; HFrEF = Heart failure with reduced ejection fraction.

Figure 5. Mendelian Randomization Analysis of Aptamer Hits for Overall HF. Genetic associations with incident heart failure for aptamers instrumented in ARIC and HERMES2 that met a nominal level of significance.
ARIC = Atherosclerotic Risk in Communities; HERMES2 = Heart Failure Molecular Epidemiology for Therapeutic Targets Consortium 2; HF = Heart failure; MR = Mendelian randomization.

Figure 6. Mendelian Randomization Analysis of Aptamer Hits for HF Subtypes. Genetic associations with HFpEF (Panel A) or HFrEF (Panel B) for aptamers observationally associated with either subtype at Bonferroni significance, differentially observationally associated with the subtypes at nominal significance, or showing nominally significant genetic associations with overall heart failure.
HF = Heart failure; HFpEF = Heart failure with preserved ejection fraction; HFrEF = Heart failure with reduced ejection fraction; MR = Mendelian randomization.

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