ESC European Society of Cardiology

European Journal of Heart Failure (2024)
doi:10.1002/ejhf.3502

RESEARCH ARTICLE

Proteomic biomarkers and pathway analysis for progression to heart failure in three epidemiological representative cohorts

Anna Dieden[1,2,3], Nicolas Girerd[4,5], Filip Ottosson[6], John Molvin[1,7], Manan Pareek[8,9], Olle Melander[1,10], Erasmus Bachus[1], Lennart Råstam[1], Ulf Lindblad[11], Bledar Daka[11], Margrét Leósdóttir[1,7], Peter M. Nilsson[1,10], Michael H. Olsen[12,13], Andrew L. Clark[14], John G.F. Cleland[15], Christian Delles[15], Arantxa González[16,17,18], Zohra Lamiral[4,5], Kevin Duarte[4,5], Patrick Rossignol[4,5,19], Faiez Zannad[4,5], Petri Gudmundsson[2,3], Amra Jujić[1,7], and Martin Magnusson[1,7,20,21]*

[1]Department of Clinical Sciences Malmö, Lund University, Malmö, Sweden; [2]Department of Biomedical Science, Malmö University, Malmö, Sweden; [3]Biofilms Research Centre for Biointerfaces, Malmö University, Malmö, Sweden; [4]Université de Lorraine, Centre d'Investigations Cliniques Plurithématique 1433, Institut National de la Santé et de la Recherche Médicale 1116, Centre Hospitalier Universitaire Régional de Nancy, Nancy, France; [5]French Clinical Research Infrastructure Network 'Investigation Network Initiative-Cardiovascular and Renal Clinical Trialists' Cardiovascular and Renal Clinical Trialists Network, Nancy, France; [6]Section for Clinical Mass Spectrometry, Danish Center for Neonatal Screening, Department of Congenital Disorders, Statens Serum Institut, Copenhagen, Denmark; [7]Department of Cardiology, Skåne University Hospital, Malmö, Sweden; [8]Center for Translational Cardiology and Pragmatic Randomized Trials, Department of Biomedical Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark; [9]Department of Cardiology, Copenhagen University Hospital, Rigshospitalet, Copenhagen, Denmark; [10]Department of Internal Medicine, Skåne University Hospital, Malmö, Sweden; [11]Institute of Medicine, School of Public Health and Community Medicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden; [12]Department of Regional Health Research, University of Southern Denmark, Odense, Denmark; [13]Department of Internal Medicine 1 and Steno Diabetes Center Zealand, Holbaek Hospital, Holbaek, Denmark; [14]Hull York Medical School, Castle Hill Hospital, Cottingham, UK; [15]British Heart Foundation Cardiovascular Research Centre, School of Cardiovascular and Metabolic Health, University of Glasgow, Glasgow, UK; [16]Program of Cardiovascular Diseases, Centre for Applied Medical Research (CIMA), and Department of Cardiology and Cardiac Surgery, Clínica Universidad de Navarra, University of Navarra, Pamplona, Spain; [17]CIBERCV, Carlos III Institute of Health, Madrid, Spain; [18]Instituto de Investigación Sanitaria de Navarra (IdiSNA), Pamplona, Spain; [19]Medical Specialties and Nephrology Departments, Princess Grace Hospital and Monaco Private Hemodialysis Center, Monaco, Monaco; [20]Hypertension in Africa Research Team (HART), North West University, Potchefstroom, South Africa; and [21]Wallenberg Center for Molecular Medicine, Lund University, Lund, Sweden

Received 2 July 2024; revised 16 September 2024; accepted 6 October 2024

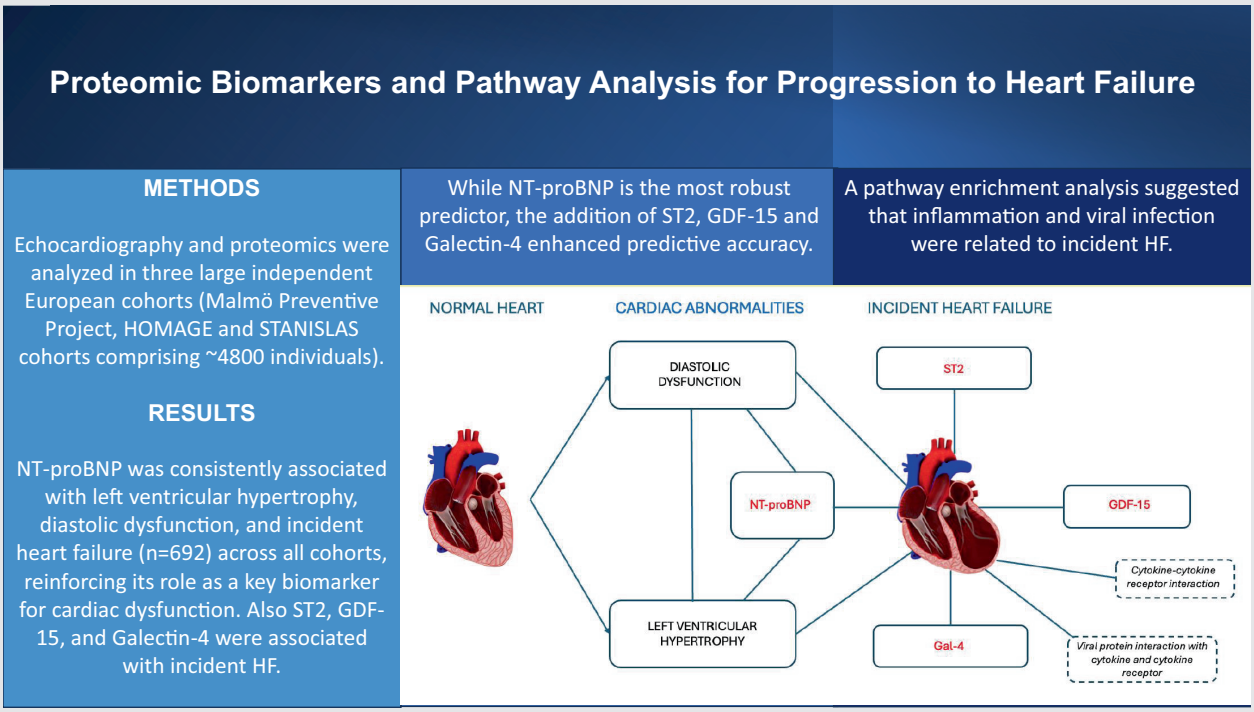
Aims Biomarkers associated with asymptomatic ventricular dysfunction might improve risk stratification and identify pathways leading to heart failure (HF). We explored the association between proteomic biomarkers and left ventricular hypertrophy (LVH), diastolic dysfunction (DD) and incident HF in three population-based cohorts.

Methods and results A chip was used to measure 92 protein biomarkers in blood samples from >1500 Malmö Preventive Project (MPP) participants, of whom 514 had LVH (34%), 462 had DD (32.4%) and, over a median follow-up of 13 (11–14) years, 130 developed HF (7.7%). Findings were confirmed in the STANISLAS ($n > 1500$, 238 participants with LVH, 76 with DD) and HOMAGE case-control (562 cases of incident HF, 871 controls) cohorts. In multivariable logistic or Cox regression analyses adjusted for age, sex and cardiovascular risk factors, N-terminal pro-B-type natriuretic peptide (NT-proBNP) was associated with LVH, DD and incident HF in all cohorts: MPP (LVH odds ratio [OR] [95% confidence interval] 1.48 [1.28–1.71]; DD OR 1.71 [1.53–1.92]; HF HR 1.98 [1.66–2.36]); STANISLAS (LVH OR 1.20 [1.02–1.41]; DD OR 1.46 [1.12–1.90]); HOMAGE (HF HR 1.85 [1.62–2.12]). Galectin-4, growth differentiation factor 15 and suppression of tumorigenicity-2 were associated with incident HF in MPP and HOMAGE. A pathway enrichment analysis suggested that inflammation and viral infection were related to incident HF.

*Corresponding author. Malmö University Hospital, Lund University, 205 02 Malmö, Sweden. Email: martin.magnusson@med.lu.se

Conclusion In conclusion, our study reinforces the role of NT-proBNP as a key biomarker for asymptomatic cardiac dysfunction and incident HF, consistent with its established use in clinical practice. This underscores the value of NT-proBNP for identifying patients at high risk for HF, and provides insights into pathways leading to HF and potential therapeutic targets.

Graphical Abstract



Markers of progression to heart failure (HF). Gal-4, galectin-4; GDF-15, growth differentiation factor 15; NT-proBNP, N-terminal pro-B-type natriuretic peptide; ST2, suppression of tumorigenicity-2.

Keywords Heart failure • Proteomics • Diastolic dysfunction • Left ventricular hypertrophy

Introduction

The progression from a healthy to a failing heart involves complex molecular, cellular, and physiological adaptations to cardiac stressors. Prolonged exposure to various cardiovascular risk factors may lead to structural and functional cardiac problems, such as left ventricular hypertrophy (LVH) and diastolic dysfunction (DD), culminating in the development of symptoms and signs, fulfilling the current diagnostic criteria for heart failure (HF).[1] Understanding these stages is vital for the early identification, intervention, and treatment of HF.[2–4] Recognizing and treating cardiac dysfunction could potentially delay or prevent the onset of symptoms of HF. Identifying biomarkers associated with asymptomatic cardiac dysfunction and incident HF could improve understanding of pathophysiological pathways leading to HF, detect 'silent' disease progression and trigger renewed efforts to optimize cardiovascular risk factor control to delay the onset of HF.

Numerous studies have studied the proteome in various stages of HF, such as LVH, DD, and the onset of HF itself,[5–9] but few have covered all HF stages. Therefore, we investigated the associations between plasma biomarkers and asymptomatic cardiac dysfunction, specifically LVH and DD, as well as incident HF, using samples from three population-based cohorts.

Methods

Cohorts

Discovery cohort

The Malmö Preventive Project (MPP), conducted in Malmö, Sweden from 1974 to 1992, screened 33 346 residents for cardiovascular risk factors, alcohol abuse, and impaired glucose tolerance. Residents of Malmö, born 1921–1949, were invited. A re-examination (MPP-RES) of surviving participants took place 2002–2006, with 17 284 individuals attending. A sub-sample of 1792 participants, randomly selected and categorized by fasting plasma glucose and diabetes status[10] underwent echocardiography (MPP-RES-Echo).

The replication cohort for left ventricular hypertrophy and diastolic dysfunction

The STANISLAS cohort is a longitudinal cohort comprising 4295 participants from the Nancy region of France between 1993 and 1995. Follow-up visits occurred 5 and 10 years later. For a fourth visit (2011–2016; follow-up at 18–23 years), 1705 individuals from the original cohort were re-examined, and used in the present study.[11]

The replication cohort for incident heart failure

The HOMAGE database enrolled over 40 000 healthy individuals, patients with HF and patients at high risk of cardiovascular disease in 21 studies.[12] Within HOMAGE, we selected cohorts with individuals free from HF, with follow-up data until first HF hospitalization, from two suitable cohorts and one clinical trial; PREDICTOR, PROSPER and HEALTH-ABC.[12] A nested matched case-control design was used, excluding individuals with a history of HF at baseline. Individuals who developed HF were considered to be at risk (eligible as controls until they became a case). We identified 852 incident HF cases (44 from PREDICTOR, 234 from PROSPER, 574 from HEALTH-ABC), with controls matched for age, sex, and follow-up time (time from study entry to incident HF).

The HOMAGE study had two phases, discovery and replication, though in the present study, it functioned as a replication cohort. For discovery, 300 cases and 599 controls were randomly selected. The final match consisted of 286 cases and 591 controls due to missing or poor-quality samples. For replication, 315 cases and 315 controls were randomly selected. Merging both phases (replication and discovery), the final study group had 562 cases and 871 controls, after excluding missing or poor-quality samples.

The study was conducted in accordance with the Declaration of Helsinki and approved by the ethical review board at each site. Written informed consent was obtained from all participants.

Echocardiography

Discovery cohort

Images were obtained and analysed by experienced sonographers, using a 3V2c transducer (Acuson Sequoia, Mountain View, CA, USA) or an S3 transducer (Sonos 5500 Philips, Andover, MA, USA), while measurements were made offline using Xcelera.

Left ventricular ejection fraction (LVEF) was quantified visually.

The replication cohort for diastolic function and left ventricular hypertrophy

Experienced sonographers obtained and analysed the images with an M5S transducer (Vivid 9, General Electric Medical Systems, Horten, Norway). Measurements were made using EchoPAC (version 110.1.0, GE Healthcare). LVEF was calculated using Simpson's biplane method.

For discovery, as well as replication cohort, left ventricular (LV) filling patterns were obtained with the transducer in the apical position and with the pulsed-wave Doppler sample volume placed at the tip of the mitral leaflets. Peak velocity of the early diastolic wave (E) was identified. With the sample volume placed within 1 cm of the septal and lateral borders of the mitral annulus, pulsed-wave tissue Doppler imaging was used to obtain the septal and lateral tissue velocity in diastole (e'). LV mass was determined in the parasternal long-axis view, from end-diastolic measurements at the tip of the mitral leaflets, using the formula recommended by the American Society of Echocardiography,[13] and indexed for body surface area to obtain LV mass index (LVMI). The left atrium was traced in the 4-chamber view.

Laboratory

In MPP-RES-Echo, fasting blood samples were centrifuged and stored at −80°C until the time of analysis. Plasma samples from 1737 participants who had echocardiography performed were successfully analysed for proteomic profiling. Participants were followed from re-examination to event or censoring (31 December 2018), with a median follow-up time of 13 (11–14) years. For analyses of plasma cystatin C and creatinine, standardized methods were used at each site. In MPP-RES-Echo, N-terminal pro-B-type natriuretic peptide (NT-proBNP) was measured at the Department of Clinical Chemistry, Akershus University Hospital, Lorenskog, Norway (electrochemiluminescence immunoassay, Elecsys, Roche Diagnostics, Basel, Switzerland). In HOMAGE and STANISLAS, NT-proBNP was measured using a proximity extension assay.

Proteomic assay

In all three cohorts, plasma samples were analysed by the proximity extension assay technique, using the Proseek Multiplex CVD III 96 × 96 reagents kit (Olink Bioscience, Uppsala, Sweden).[14] In MPP-RES-Echo, one protein had concentrations below detectable limits in >15% samples (NT-proBNP) and was thus excluded. Further information about the assays is available on the Olink homepage (http://www.olink.com).

Definitions and outcomes

In all cohorts, standardized methods were used to measure anthropometrics, heart rate, and blood pressure (BP).[10–12] DD was considered present for participants with both (i) average E/e' ratio ≥8 and (ii) left atrial (LA) volume index >28 ml/m^2.[15,16] LVH was defined as LVMI ≥115 g/m^2 for men and ≥95 g/m^2 for women.[17] Medications, medical history, and smoking habits were self-reported.

Discovery cohort

In MPP-RES-Echo, diabetes was defined as a self-reported diagnosis, a prior diabetes diagnosis, use of diabetes medication, two separate fasting plasma glucose (FPG) measurements of ≥7.0 mmol/L, or a single measurement of ≥11.1 mmol/L. LA volume was derived using the following formula: LA volume = LA area (cm^2) * 3.075 − 4.420[18] and thereafter indexed for body surface area. HF diagnosis was retrieved through national and regional Swedish registers for inpatients and outpatients, administered by the Swedish National Board of Health and Welfare.

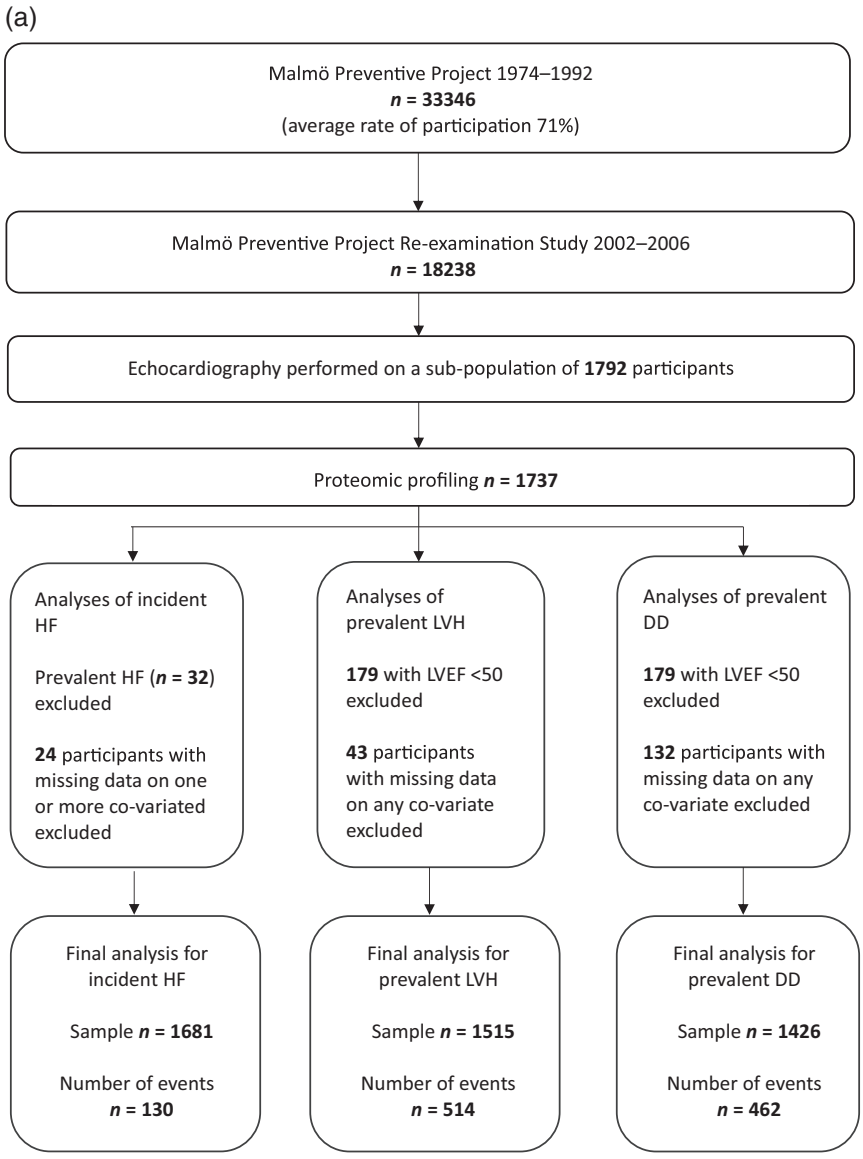


Figure 1 (*A*) Flowchart of the discovery cohort population (MPP-RES-Echo). (*B*) Flowchart of the replication cohort population (HOMAGE). (*C*) Flowchart of the replication cohort population (STANISLAS). DD, diastolic dysfunction; HF, heart failure; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy.

The replication cohort for diastolic function and left ventricular hypertrophy

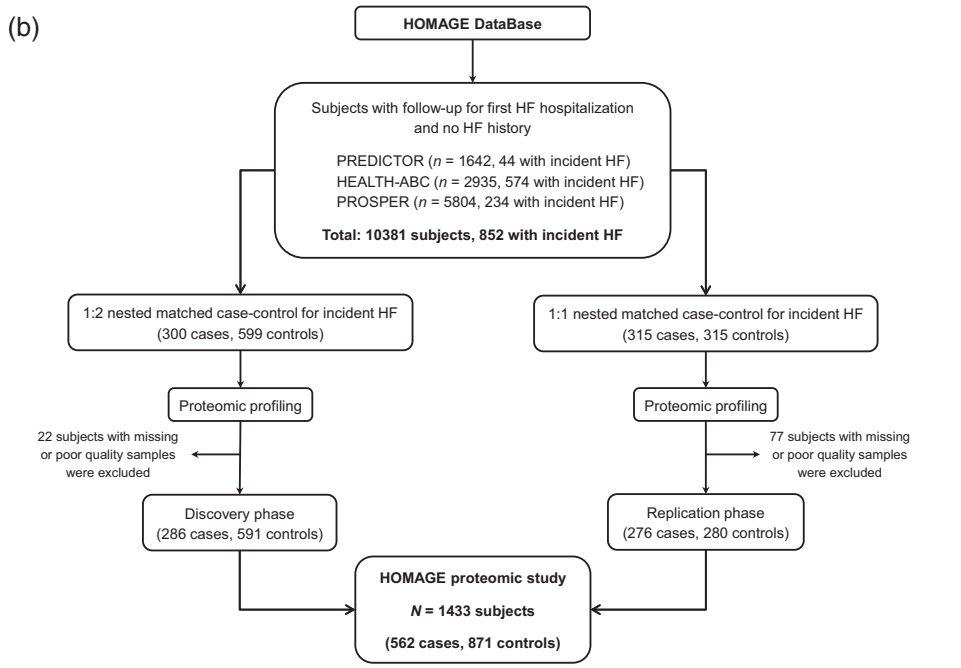
In STANISLAS, the definition of diabetes was based on FPG and/or glycated haemoglobin and/or use of diabetes medication.

The replication cohort for incident heart failure

In HOMAGE, diabetes was defined as a self-reported diagnosis only. Incident HF was defined as first hospitalization for HF as primary diagnosis (adjudicated by the investigators of the respective cohorts).

Final study populations

Participants with missing data on any covariate were excluded. For DD and LVH analysis, those with LVEF <50% were excluded ($n = 96$). For incident HF analysis in MPP-Res-Echo, individuals with a history of HF (ICD-10 I50) were excluded ($n = 32$). The final MPP-RES-Echo sample included 1681 individuals for incident HF analysis ($n = 130$), 1426 individuals for prevalent DD ($n = 462$; 32.4%) and 1515 individuals for prevalent LVH ($n = 514$; 33.9%) analysis (*Figure 1A*). In HOMAGE, the final study sample was 1433 individuals (*Figure 1B*). In STANISLAS, the final study sample was 1584 individuals for DD analysis and 1630 for LVH analysis (*Figure 1C*).



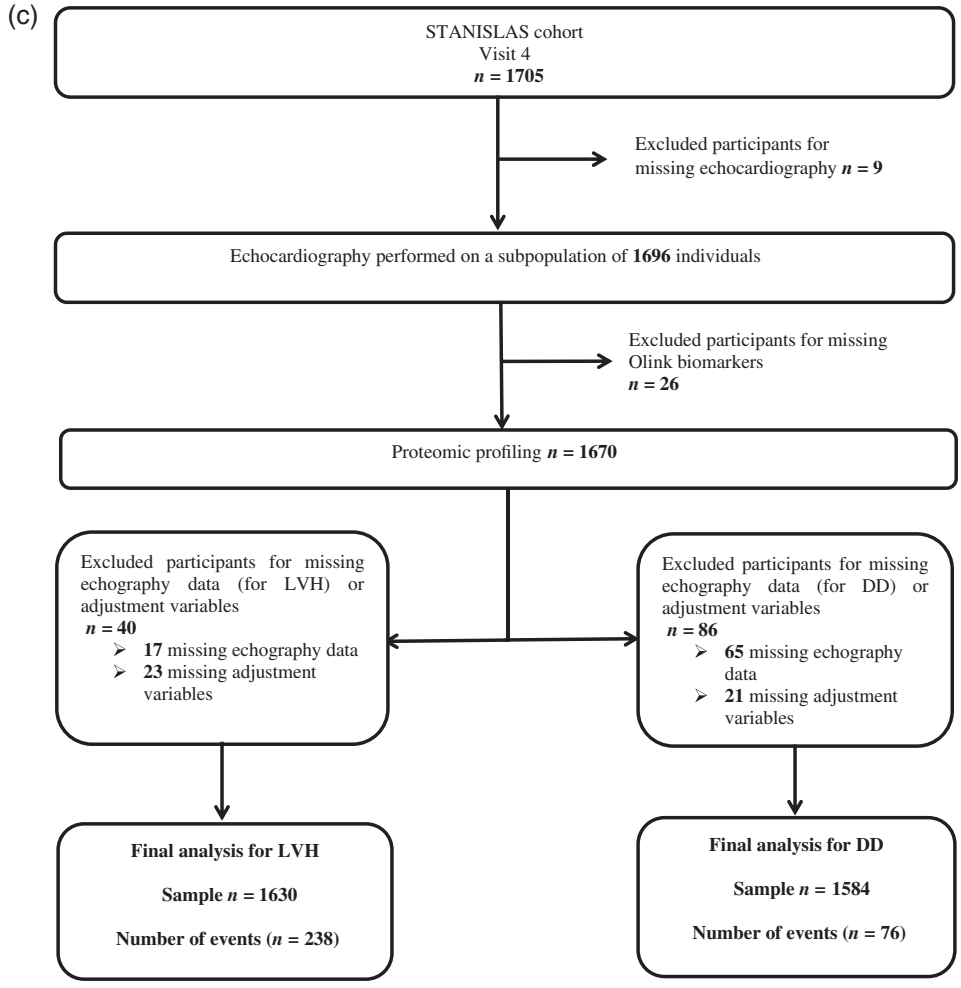


Figure 1 Continued.

Statistical analysis

The variables are presented as means (± standard deviation) or median (25th−75th interquartile range). Comparisons between individuals without and with incident HF used independent samples *t*-test, the Mann−Whitney U test, or Pearson's χ^2 test.

Unadjusted logistic regressions for associations of 92 markers with prevalent LVH and DD were performed, with adjusted *p*-values calculated using the Benjamini−Hochberg method. A 5% false discovery rate (FDR) identified proteins for further analysis in Model 1 (adjusted for age and sex). Significant associations were analysed in Model 2 (adjusted for body mass index, systolic BP, prevalent diabetes, antihypertensive treatment, cystatin C, smoking, heart rate and history of coronary artery disease). Significant findings from MPP-RES-Echo were examined in the STANISLAS replication cohort using Model 2, substituting creatinine for cystatin C.

In MPP-RES-Echo, unadjusted Cox regressions analysed the 92 proteins associations with incident HF. Significant associations (FDR <5%) were further analysed in Model 1. Subsequently, significant associations were further adjusted according to Model 2.[12] Significantly associated proteins in MPP-RES-Echo were further analysed in HOMAGE. As the proteins were not measured at the same time in the two phases in HOMAGE, multivariable logistic regressions adjusted for follow-up time and phase were added to Model 2, and estimated glomerular filtration rate replaced cystatin C. The proportional hazard assumption was violated for suppression of tumorigenicity-2 (ST2) and incident HF ($p = 0.006$) in the discovery cohort. An interaction with time, both continuous and with a cut-off >2.5 years, was included. No other violations of the proportional hazard assumption were found. Given that the MPP-Res-Echo cohort was enriched for individuals with glycaemic disturbances, we conducted an interaction analysis specifically focusing on interactions with diabetes for the proteins that demonstrated an association with incident HF in the MPP-Res-Echo cohort.

In MPP-RES-Echo, unadjusted Cox regressions analysed associations between DD, LVH, and incident HF. Significant associations were subsequently analysed in Model 1 and, if significant, further analysed in Model 2. Multivariable logistic regression analyses, adjusted for age, sex, systolic BP and antihypertensive treatment, were used to explore the association between DD and prevalent LVH, respectively.

There may be a multicollinearity issue in the models, making it difficult to determine individual effects of the proteins and masking true associations. In MPP-RES-Echo, we therefore identified proteins significantly associated with incident HF in crude analysis. Employing unadjusted linear regression models, we explored which of these proteins had at least moderate ($R^2 > 0.3$) and significant ($p < 0.001$) explanatory power for the variance of proteins associated with incident HF in fully adjusted models. Using Enrichr (https://maayanlab.cloud/Enrichr/) and the KEGG pathway database, we conducted pathway enrichment analysis on the proteins meeting this threshold.

For incident HF, the added predictive value associated with the addition of the significant biomarkers in discovery and replication cohorts, on top of NT-proBNP, was assessed using the increase in c-index and continuous net reclassification improvement (cNRI).[19]

Analyses were carried out using R (version 3.6.1), SPSS (version 25.0) and SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

Results

In MPP-RES-Echo, those who subsequently developed HF had a worse metabolic and cardiovascular risk factor profile compared to those who did not (*Table 1*) and differed in all aspects except systolic and diastolic BP, smoking, and heart rate. Characteristics of individuals in the replication cohorts HOMAGE and STANISLAS can be found in *Table 1* and online supplementary *Table S6*. A complete list of all 92 proteins is presented in online supplementary *Table Appendix S1*.

Left ventricular hypertrophy

In MPP-RES-Echo, 12 proteins showed FDR-adjusted associations with LVH (online supplementary *Table S2*). After adjusting according to Model 1, five markers were still associated with LVH (online supplementary *Table S3*). After further adjustment according to Model 2, two markers remained associated with LVH: azurocidin-1 (AZU1) and NT-proBNP, which was confirmed only for NT-proBNP in the replication cohort (STANISLAS) (*Table 2*).

Prevalent diastolic dysfunction

In MPP-RES-Echo, 47 markers had FDR-adjusted associations with DD (online supplementary *Table S4*). After Model 1 adjustments, six markers were still associated with DD (online supplementary *Table S5*). After Model 2 adjustments, NT-proBNP, matrix metalloprotinease-9, osteopontin, interleukin-2 receptor subunit alpha, metalloprotinease inhibitor 4 (*Table 2*) remained associated. In STANISLAS, an association was confirmed only for NT-proBNP (*Table 2*). The characteristics of the STANISLAS cohort are presented in online supplementary *Table S6*.

Incident heart failure

In unadjusted analyses, 29 markers were associated with incident HF ($n = 130$) (online supplementary *Table S7*). Seventeen markers remained associated when adjusted per Model 1 (online supplementary *Table S8*). In Model 2, ST2, growth differentiation factor 15 (GDF-15), AZU1, myeloperoxidase (MPO), galectin-4 (Gal-4) and NT-proBNP were associated with incident HF (*Table 2*). In HOMAGE, the Model 2-adjusted analysis confirmed associations between ST2, GDF-15, Gal-4 and NT-proBNP and incident HF (*Table 2*). In analyses of ST2 and incident HF, each doubling in concentration of ST2 was associated with a higher risk of incident HF (hazard ratio [HR] 3.13; $p = 0.003$) for the first 2.5 years, but after that, no such association was seen (HR 1.22; $p = 0.239$; online supplementary *Figure Appendix S1*). There were no significant interactions between diabetes and the associations of MPO ($p = 0.301$) and AZU1 ($p = 0.525$) with incident HF. *Figure 2* provides a schematic representation elucidating the key findings of the study.

Pathway enrichment analysis

Exploring the 29 proteins associated with incident HF in unadjusted analysis that showed at least moderate explanatory power ($R^2 > 0.3$) with respect to GDF-15, ST2, Gal-4, and NT-proBNP, respectively, 15 proteins met the threshold for GDF-15; one protein (insulin-like growth factor binding protein-7) for ST2; 11

Table 1 Characteristics of individuals included in analyses of incident heart failure in the discovery cohort

	All individuals ($n = 1681$)	Individuals without incident HF ($n = 1551$)	Individuals with incident HF ($n = 130$)	*p*-value	All individuals ($n = 1433$)	Individuals without incident HF ($n = 871$)	Individuals with incident HF ($n = 562$)	*p*-value
	The Malmö Preventive Project cohort (discovery)				**HOMAGE cohort (replication)**			
Clinical								
Age (years)	67.6 (± 6.0)	67.1 (± 6.0)	70.3 (± 4.8)	**<0.001**	74.7 ± 3.5	74.7 ± 3.5	74.8 ± 3.5	0.64
Female sex, *n* (%)	490 (29.1)	462 (29.8)	28 (21.5)	**0.047**	644 (44.9)	393 (45.1)	251 (44.7)	0.87
BMI (kg/m^2)	28.3 (± 4.3)	28.2 (± 4.3)	29.5 (± 5.0)	**0.001**	27.3 ± 4.5	26.9 ± 4.3	27.9 ± 4.8	**0.0001**
SBP (mmHg)	147 (± 20)	147 (± 20)	148 (± 21)	0.418	144 ± 23	143 ± 23	145 ± 23	0.054
DBP (mmHg)	85 (± 11)	85 (± 11)	84 (± 12)	0.188	76 ± 13	76 ± 12	77 ± 14	0.11
Smoking, *n* (%)	297 (17.7)	274 (17.7)	23 (17.7)	0.994	206 (14.4)	119 (13.7)	87 (15.5)	0.36
Heart rate (bpm)	72 (± 13)	72 (± 13)	70 (± 13)	0.138	66 ± 11	65 ± 11	67 ± 12	**0.0002**
Diabetes, *n* (%)	666 (39.6)	590 (38.0)	76 (58.5)	**<0.001**	205 (14.3)	100 (11.5)	105 (18.7)	**0.0002**
CAD, *n* (%)	112 (6.7)	81 (5.2)	31 (23.8)	**<0.001**	422 (29.5)	204 (23.4)	218 (38.9)	**<0.0001**
AHT, *n* (%)	777 (46.2)	691 (44.6)	86 (66.2)	**<0.001**	884 (61.7)	494 (56.7)	390 (69.4)	**<0.0001**
Laboratory								
Cystatin C (mg/L)	1.1 (1.0–1.2)	1.1 (1.0–1.2)	1.2 (1.0–1.3)	**<0.001**	–	–	–	
Creatinine (μmol/L)	–	–	–	–	97 ± 24	94 ± 21	101 ± 28	**<0.0001**
eGFR (ml/min/1.73 m^2)					62 ± 15	63 ± 14	60 ± 16	**<0.0001**
NT-proBNP[a]	11 (6–24)	11 (6–22)	32 (15–69)	**<0.001**	5.1 ± 1.0	4.9 ± 1.0	5.4 ± 0.9	**<0.0001**
	The Malmö Preventive Project cohort (discovery)				**STANISLAS cohort (replication)**			
					All individuals ($n = 1584$)			
Echocardiography								
LVEF (%)	61 (57–65)	61 (57–64)	58 (53–65)	**0.001**	65.1 ± 6.7			
LVMI (g/m^2)	87 (74–102)	86 (74–101)	102 (80–121)	**<0.001**	76.3 ± 19.3			
E/é	8 (6–10)	8 (6–10)	10 (8–14)	**<0.001**	6.47 ± 1.86			
E/e' ratio ≥8, *n* (%)	803 (50.4)	713 (48.4)	90 (75.0)	**<0.001**	252 (16.0)			
LVH, *n* (%)	582 (34.6)	507 (32.7)	75 (57.7)	**<0.001**	238 (14.6)			
LAVI (ml/m^2)	29 (25–32)	29 (25–32)	33 (27–38)	**<0.001**	22 (18–27)			
LAVI >28 ml/m^2, *n* (%)	869 (55.1)	786 (54.0)	83 (68.0)	**0.003**	331 53 (20.9)			
Diastolic dysfunction, *n* (%)	462/1426 (32.4)	409/1327 (30.8)	53/99 (53.5)	**<0.001**	288 (17.8)			

Values are means and (± standard deviations), median (25th–75th interquartile range), or *n* (%).

'All individuals' refers to all individuals included in analyses of incident HF; individuals with prevalent HF excluded.

AHT, antihypertensive treatment; BMI, body mass index; CAD, coronary artery disease (prevalent coronary event); DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate (Chronic Kidney Disease Epidemiology Collaboration formula); HF, heart failure; LAVI, left atrial volume index; LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; LVMI, left ventricular mass index; NT-proBNP, N-terminal pro B-type natriuretic peptide; SBP, systolic blood pressure.

[a]In the discovery cohort, the unit for NT-proBNP is ng/L. In the replication cohort, the unit is an arbitrary unit (normalized protein expression).

Table 2 Significant associations between biomarkers and incident heart failure, diastolic dysfunction, and hypertrophy in discovery and replication cohorts

	Diastolic dysfunction			
	Malmö Preventive Project (462 cases, 964 controls)		STANISLAS (76 cases, 1508 controls)	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
NT-proBNP	1.71 (1.53–1.92)	**<0.001**	1.46 (1.12–1.90)	**0.006**
MMP9	1.20 (1.02–1.41)	**0.025**	0.91 (0.66–1.25)	0.56
IL2-RA	1.44 (1.13–1.83)	**0.003**	1.16 (0.64–2.11)	0.63
OPN	1.29 (1.05–1.58)	**0.015**	0.90 (0.50–1.63)	0.72
TIMP4	1.35 (1.07–1.69)	**0.010**	1.18 (0.65–2.16)	0.58
Gal-4	1.17 (0.94–1.44)	0.16	–	–
	Left ventricular hypertrophy			
	Malmö Preventive Project (514 cases, 1001 controls)		STANISLAS (238 cases, 1392 controls)	
	OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
AZU1	1.26 (1.04–1.53)	**0.019**	1.04 (0.91–1.19)	0.56
NT-proBNP	1.48 (1.28–1.71)	**<0.001**	1.20 (1.02–1.41)	**0.0025**
	Incident heart failure			
	Malmö Preventive Project (130 cases, 1551 controls)		HOMAGE (562 cases, 871 controls)	
	HR (95% CI)	*p*-value	OR (95% CI)	*p*-value
ST2[a]	1.40 (1.03–1.91)	**0.031**	1.31 (1.09–1.58)	**0.004**
GDF-15	1.33 (1.00–1.77)	**0.048**	1.95 (1.58–2.39)	**2.8×10^{-10}**
AZU1	1.42 (1.10–1.82)	**0.006**	1.06 (0.93–1.20)	0.41
MPO	1.48 (1.02–2.15)	**0.041**	1.19 (0.98–1.43)	0.078
Gal-4	1.37 (1.02–1.85)	**0.038**	1.42 (1.17–1.72)	**4.0×10^{-4}**
NT-proBNP	1.98 (1.66–2.36)	**2.1×10^{-14}**	1.85 (1.62–2.12)	**8.9×10^{-20}**

Values are HR or OR and 95% CI for proteins associations with incident heart failure, diastolic dysfunction and left ventricular hypertrophy.
AZU1, azurocidin-1; Gal-4, galectin-4; CI, confidence interval; GDF-15, growth differentiation factor 15; HR, hazard ratio; IL2-RA, interleukin-2 receptor subunit alpha; MMP9, matrix metalloproteinase-9; MPO, myeloperoxidase; NT-proBNP, N-terminal pro-B-type natriuretic peptide; OPN, osteopontin; OR, odds ratio; ST2, suppression of tumorigenicity-2; TIMP4, metalloproteinase inhibitor 4.
Analyses are adjusted for age, sex, body mass index, smoking, heart rate, systolic blood pressure, diabetes mellitus, history of coronary artery disease, cystatin C (discovery) or estimated glomerular filtration rate (Chronic Kidney Disease Epidemiology Collaboration formula; HOMAGE cohort), or creatinine (STANISLAS cohort), and antihypertensive treatment. The HOMAGE cohort was additionally adjusted for 'phase' and follow-up time.
[a]In the discovery cohort, ST2 showed time-varying effects, where each doubling in concentration of ST2 was associated with higher risk of incident heart failure (HR 3.13; $p = 0.003$) for the first 2.5 years, but after that no such association was observed (HR 1.22; $p = 0.239$).

proteins for Gal-4; and none for NT-proBNP (online supplementary *Table S9*).

Of the proteins showing at least moderate explanatory power for GDF-15 and Gal-4, respectively, pathway enrichment analysis found links to viral protein interaction with cytokine and cytokine receptor and cytokine–cytokine receptor interaction (adjusted $p < 0.0001$).

Diastolic dysfunction and incident heart failure

Prevalent DD was associated with incident HF in an unadjusted model (HR 2.60; 95% confidence interval [CI] 1.80–3.75; $p < 0.001$) when adjusted per Model 1 (HR 1.86; 95% CI 1.26–2.74; $p = 0.002$), and when further adjusted for body mass index, systolic BP, smoking, diabetes status, history of coronary artery disease, antihypertensive treatment, heart rate and cystatin C (HR 1.69; 95% CI 1.13–2.52; $p = 0.011$). LVH was also associated with incident HF in an unadjusted model (HR 3.17; 95% CI 2.21–4.55; $p = 3.6 \times 10^{-10}$), after adjusting per Model 1 (HR 2.40; 95% CI 1.65–3.48; $p = 4.0 \times 10^{-6}$) and following further adjustment per Model 2 (HR 2.05; 95% CI 1.38–3.03; $p = 3.4 \times 10^{-4}$). Finally, DD was associated with LVH (odds ratio 1.76; 95% CI 1.34–2.28), when adjusted for age, sex, systolic BP and antihypertensive treatment.

Added predictive value

The addition of GDF-15, ST2, and Gal-4 on top of NT-proBNP modestly improved prediction of incident HF, as evidenced by significant increases in cNRI in MPP (delta c 0.014 [−0.002 to 0.030]; $p = 0.088$; cNRI 0.272 [0.073 to 0.540], $p = 0.012$) and delta

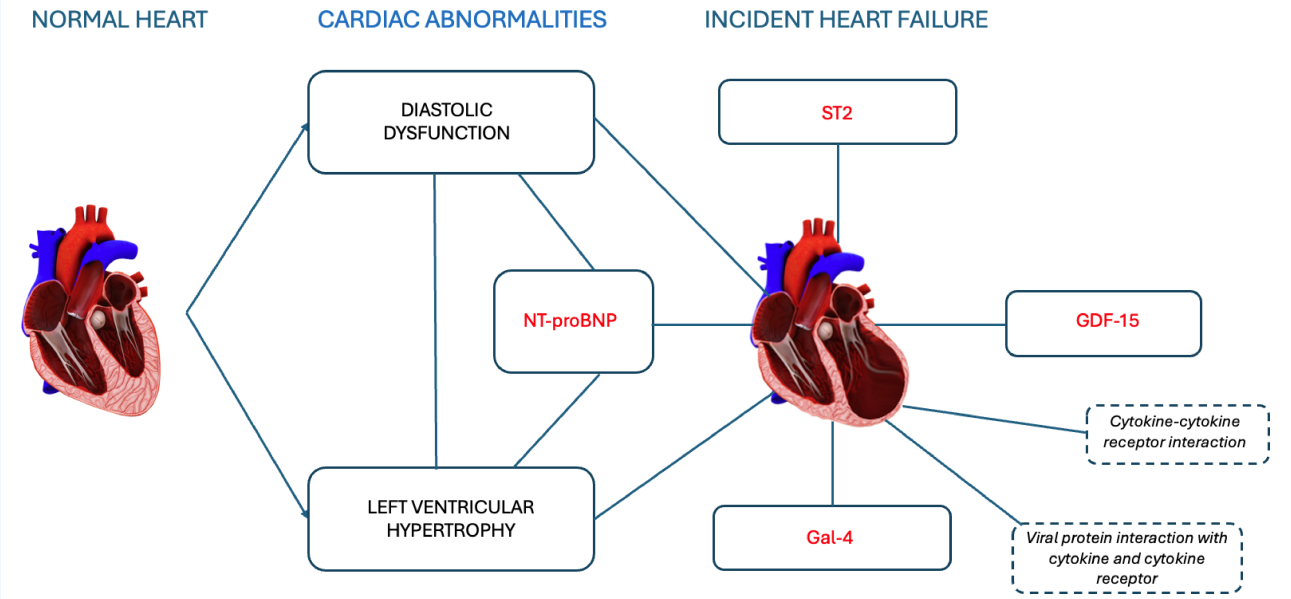


Figure 2 A schematic representation of the key findings of the study. Prevalent diastolic dysfunction and left ventricular hypertrophy at baseline were both associated with increased risk of incident heart failure. Suppression of tumorigenicity-2 (ST2), growth differentiation factor 15 (GDF-15) and galectin-4 (Gal-4) were all associated with increased risk of incident heart failure, whereas N-terminal pro-B-type natriuretic peptide (NT-proBNP) was the only biomarker associated with left ventricular hypertrophy and diastolic dysfunction at baseline as well as with incident heart failure. The boxes with the dotted lines indicate pathways of identified and related proteins.

c and cNRI in HOMAGE (delta c 0.028 [0.010 to 0.046], $p = 0.003$, cNRI 0.301 [0.196 to 0.405], $p < 0.0001$) cohorts.

Discussion

We found that (i) NT-proBNP was associated with prevalent LVH and DD, (ii) increased plasma concentrations of ST2, GDF-15, Gal-4 and NT-proBNP were associated with incident HF, irrespective of established risk factors, and (iii) DD and LVH commonly co-existed and were both associated with incident HF (*Graphical Abstract*). These findings are consistent with the view that hypertension is an important risk factor for HF and that either NT-proBNP or echocardiography may identify cardiac end-organ damage indicating an increased risk of developing HF. Reasonably, NT-proBNP should be used routinely to identify people at increased risk of cardiovascular events for inclusion in clinical trials and for intensified management of cardiovascular risk in clinical practice.[20] While NT-proBNP is the most robust predictor, the addition of ST2, GDF-15 and Gal-4 enhances predictive accuracy, as evidenced by improvements in the c-index and cNRI beyond NT-proBNP.

NT-proBNP is a well-established biomarker for assessing risk of developing HF and its subsequent prognosis,[21,22] and has already been used to identify and treat patients to reduce the risk of HF.[20,23] NT-proBNP was the only protein associated with LVH and DD in both cohorts. Although six biomarkers were associated with either LVH or DD in the MPP-RES-Echo, this association was confirmed only for NT-proBNP in STANISLAS. Variations in demographics, participant selection, genetics, culture, environment, or echocardiographic technique could have led to divergent results.

NT-proBNP was associated with all three outcomes. Traditionally, the definition of HF has been based on symptoms and signs, accompanied by abnormal cardiac structure or function.[2] Current guidelines also acknowledge the evolving role of biomarkers for the definition of HF.[24] A recently proposed global definition of HF includes elevated natriuretic peptides into the definition, making the diagnosis more precise.[1] Moreover, guidelines recommend considering natriuretic peptide biomarker-based screening in patients at risk of HF, to help prevent the onset of LV dysfunction.[24] In a randomized controlled trial, participants with increased risk of HF and preselected based on NT-proBNP levels, up-titration of medication was an effective way of preventing cardiovascular events.[23] This study confirms that NT-proBNP is an important marker of asymptomatic cardiac dysfunction as well as incident HF. Likely, many patients with an increased NT-proBNP and dilated left atrium have impaired exercise capacity but either take little exercise or attribute their symptoms to age rather than disease.[1] In other words, they would have symptoms of HF if asked.

The finding that ST2, GDF-15, and Gal-4 are associated with incident HF, but not consistently with DD or LVH, suggests the possibility of alternative pathways leading to HF. However, it is important to acknowledge that the majority of incident HF cases in our study likely represent HF with preserved ejection fraction (HFpEF), a condition where DD and/or LVH are typically present. The notion that HFpEF could develop without any evidence of DD or LVH is indeed difficult to reconcile with current

pathophysiological understanding. Thus, while our results indicate potential non-DD and non-LVH pathways to HF, this interpretation must be approached with caution, and further replication of these findings is necessary. ST2 plays a role in inflammation, cardiac remodelling, and fibrosis, and although it is not cardiac specific, it is expressed by cardiomyocytes and cardiac fibroblasts in response to mechanical stress. Soluble ST2 serves as a decoy receptor for interleukin-33, resulting in reduced activation of the anti-inflammatory pathway and an increase in pro-inflammatory cytokine production. An association between ST2 and incident HF has been shown in other population cohorts.[8,25]

Growth differentiation factor-15 has antioxidative, anti-inflammatory, and antiapoptotic properties and is expressed in tissues due to mechanical stress or injury. There is a robust association between GDF-15 and incident HF.[6,8,26] Analysing the association of 84 proteins with incident HF, myocardial infarction and ischaemia separately, Lind *et al.*[26] found GDF-15 to be associated with all three conditions.

Galectin-4 is a lectin mostly expressed in enterocytes. In patients with chronic HF, high plasma concentrations of Gal-4, and increase in Gal-4 over time, were associated with adverse outcomes.[27] We previously reported an association between Gal-4 and incident HF in a single population cohort study.[28] To the best of our knowledge, the present study is the first to replicate these findings.

Most proteomic markers were not significantly associated with cardiac remodelling or incident HF. Proteins highly correlated with one another can make it difficult to determine individual effects of proteins which is why we performed an enrichment analysis for proteins with at least moderate explanatory power for the variance of GDF-15, Gal-4, ST2 and NT-proBNP. These proteins potentially represent 'head of clusters' of proteins correlated to one another. Pathway analysis revealed two significantly enriched pathways; viral protein interaction with cytokine and cytokine receptor and cytokine–cytokine receptor interaction. It is well known that inflammation[29] and viral infection[30] can contribute to the exacerbation or development of HF, which is also what these pathways suggest. These findings may highlight potential targets for risk stratification or therapeutic intervention, although further mechanistic studies are needed.

Limitations

Since the discovery cohort consisted of mainly older, white men, our findings are not generalizable to other populations. However, the replication cohorts were more representative, at least for people of European descent. The MPP-RES-Echo cohort oversampled for individuals with diabetes and impaired FPG, which was not the case for other cohorts. Participants in STANISLAS were also younger. Blood samples were obtained under fasting conditions only for the MPP cohort. The impact of fasting versus non-fasting states on protein concentrations remains uncertain. A significant limitation of our study is the lack of data on HF phenotype, specifically the classification of LVEF, which precludes us from distinguishing between HFpEF and HF with reduced ejection fraction in the incident HF cases. Any conclusions about causality based on observational studies should be extremely cautious.

Conclusions

In conclusion, our study reinforces the role of NT-proBNP as a key biomarker for asymptomatic cardiac dysfunction and incident HF, consistent with its established use in clinical practice. This underscores the value of NT-proBNP for identifying patients at high risk for HF, and provides insights into pathways leading to HF and potential therapeutic targets.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Acknowledgements

The Knut and Alice Wallenberg foundation is acknowledged for generous support.

Funding

The MPP was funded by the Heart and Lung Foundation of Sweden (2004045806), and Region Skåne (PMN), and by Merck, Sharp & Dohme, Hulda and E Conrad Mossfelts Foundation and Ernhold Lundströms Foundation. M.P. was supported by from Aase and Ejnar Danielsens Fond. A.J. was funded by Region Skåne and Lund University. M.M. was supported by Medical Faculty of Lund University; Skane University Hospital; Crafoord Foundation; Region Skåne; Swedish Heart and Lung Foundation, the Wallenberg Center for Molecular Medicine, Swedish Research Council and Lund University.

Conflict of interest: N.G. reports fees from AstraZeneca, Novartis, Boehringer, Bayer, Lilly and Vifor. M.P. is on the advisory board for AstraZeneca and Janssen-Cilag and reports grant support from Danish Cardiovascular Academy, funded by the Novo Nordisk Foundation and the Danish Heart Foundation (grant number CPD5Y-2 022 004-HF) and speaker honorarium from AstraZeneca, Bayer, Boehringer Ingelheim, Bristol Myers Squibb, Janssen-Cilag. E.B. is employed by AstraZeneca. M.L. has received speaker honoraria from AstraZeneca, Novo Nordisk, Amarin, Sanofi, Amgen and investigator-initiated grant support from Pfizer, AstraZeneca and Novo Nordisk (unrelated to the current study). M.H.O. has received speaker honoraria from Novo Nordisk A/S, Teva A/S and AstraZeneca. J.G.F.C. reports honoraria from Abbot, Amgen, Bayer, Bristol Myers Squibb, Novartis, Medtronic, Idorsia, Vifor, Pharmacosmos, Cytokinetics, Servier, AstraZeneca, Innolife, Torrent, Johnson & Johnson, Myokardia, Respicardia, Stealth Biopharmaceuticals, Viscardia and grants from Bayer, Vifor, Pharmacosmos, Cytokinetics, and support from Boehringer Ingelheim. P.R. reports fees from Idorsia, G3P, honoraria from AstraZeneca, Bayer, CinCor, CVRx, Fresenius, Grunenthal, Novartis, NovoNordisk, Relypsa, Roche, Servier, Stealth Peptides, and Vifor Fresenius Medical Care Renal Pharma; and travel grants from AstraZeneca, Bayer, CVRx, Novartis, and Vifor Fresenius Medical Care Renal Pharma; Cofounder: CardioRenal. F.Z. reports steering committee personal fees from Applied Therapeutics, Amgen, Bayer, Boehringer, CVRx, Novartis, Merck, advisory board and consultancy personal fees from Cardior, Cereno pharmaceutical, Cellprothera, Owkin, NovoNordisk, Vifor Fresenius, stock options at Cereno pharmaceutical, Cardior and G3Pharmaceutical, Owner and founder of Global Cardiovascular Clinical Trialist Forum. All other authors have nothing to disclose.

References

1. Cleland JGF, Pfeffer MA, Clark AL, Januzzi JL, McMurray JJV, Mueller C, *et al.* The struggle towards a universal definition of heart failure-how to proceed? *Eur Heart J* 2021;**42**:2331–2343. https://doi.org/10.1093/eurheartj/ehab082
2. McDonagh TA, Metra M, Adamo M, Gardner RS, Baumbach A, Böhm M, *et al.* 2021 ESC guidelines for the diagnosis and treatment of acute and chronic heart failure: Developed by the Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). With the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur J Heart Fail* 2022;**24**:4–131. https://doi.org/10.1002/ejhf.2333
3. Echouffo-Tcheugui JB, Erqou S, Butler J, Yancy CW, Fonarow GC. Assessing the risk of progression from asymptomatic left ventricular dysfunction to overt heart failure: A systematic overview and meta-analysis. *JACC Heart Fail* 2016;**4**:237–248. https://doi.org/10.1016/j.jchf.2015.09.015
4. Bluemke DA, Kronmal RA, Lima JA, Liu K, Olson J, Burke GL, *et al.* The relationship of left ventricular mass and geometry to incident cardiovascular events: The MESA (Multi-Ethnic Study of Atherosclerosis) study. *J Am Coll Cardiol* 2008;**52**:2148–2155. https://doi.org/10.1016/j.jacc.2008.09.014
5. Shimada YJ, Raita Y, Liang LW, Maurer MS, Hasegawa K, Fifer MA, *et al.* Comprehensive proteomics profiling reveals circulating biomarkers of hypertrophic cardiomyopathy. *Circ Heart Fail* 2021;**14**:e007849. https://doi.org/10.1161/CIRCHEARTFAILURE.120.007849
6. Ferreira JP, Verdonschot J, Collier T, Wang P, Pizard A, Bär C, *et al.* Proteomic bioprofiles and mechanistic pathways of progression to heart failure. *Circ Heart Fail* 2019;**12**:e005897. https://doi.org/10.1161/CIRCHEARTFAILURE.118.005897
7. Girerd N, Levy D, Duarte K, Ferreira JP, Ballantyne C, Collier T, *et al.* Protein biomarkers of new-onset heart failure: Insights from the Heart Omics and Ageing cohort, the Atherosclerosis Risk in Communities study, and the Framingham Heart Study. *Circ Heart Fail* 2023;**16**:e009694. https://doi.org/10.1161/CIRCHEARTFAILURE.122.009694
8. Stenemo M, Nowak C, Byberg L, Sundström J, Giedraitis V, Lind L, *et al.* Circulating proteins as predictors of incident heart failure in the elderly. *Eur J Heart Fail* 2018;**20**:55–62. https://doi.org/10.1002/ejhf.980
9. Egerstedt A, Berntsson J, Smith ML, Gidlöf O, Nilsson R, Benson M, *et al.* Profiling of the plasma proteome across different stages of human heart failure. *Nat Commun* 2019;**10**:5830. https://doi.org/10.1038/s41467-019-13306-y
10. Leosdottir M, Willenheimer R, Plehn J, Borgquist R, Gudmundsson P, Harris TB, *et al.* Myocardial structure and function by echocardiography in relation to glucometabolic status in elderly subjects from 2 population-based cohorts: A cross-sectional study. *Am Heart J* 2010;**159**:414–420 e414. https://doi.org/10.1016/j.ahj.2009.12.028
11. Ferreira JP, Girerd N, Bozec E, Mercklé L, Pizard A, Bouali S, *et al.* Cohort profile: Rationale and design of the fourth visit of the STANISLAS cohort: A familial longitudinal population-based cohort from the Nancy region of France. *Int J Epidemiol* 2018;**47**:395–395j. https://doi.org/10.1093/ije/dyx240
12. Jacobs L, Efremov L, Ferreira JP, Thijs L, Yang WY, Zhang ZY, *et al.*; Heart 'Omics' in AGEing (HOMAGE) Investigators. Risk for incident heart failure: A subject-level meta-analysis from the Heart 'Omics' in AGEing (HOMAGE) study. *J Am Heart Assoc* 2017;**6**:e005231. https://doi.org/10.1161/JAHA.116.005231
13. Lang RM, Bierig M, Devereux RB, Flachskampf FA, Foster E, Pellikka PA, *et al.* Recommendations for chamber quantification. *Eur J Echocardiogr* 2006;**7**:79–108. https://doi.org/10.1016/j.euje.2005.12.014
14. Assarsson E, Lundberg M, Holmquist G, Björkesten J, Bucht Thorsen S, Ekman D, *et al.* Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLoS One* 2014;**9**:e95192. https://doi.org/10.1371/journal.pone.0095192
15. Nagueh SF, Smiseth OA, Appleton CP, Byrd BF III, Dokainish H, Edvardsen T, *et al.* Recommendations for the evaluation of left ventricular diastolic function by echocardiography: An update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. *J Am Soc Echocardiogr* 2016;**29**:277–314. https://doi.org/10.1016/j.echo.2016.01.011
16. Pellicori P, Ferreira JP, Mariottoni B, Brunner-la Rocca HP, Ahmed FZ, Verdonschot J, *et al.* Effects of spironolactone on serum markers of fibrosis in people at high risk of developing heart failure: Rationale, design and baseline characteristics of a proof-of-concept, randomised, precision-medicine, prevention trial. The Heart OMics in AGing (HOMAGE) trial. *Eur J Heart Fail* 2020;**22**:1711–1723. https://doi.org/10.1002/ejhf.1716
17. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, *et al.*; ESC Scientific Document Group. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC). Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J* 2016;**37**:2129–2200. https://doi.org/10.1093/eurheartj/ehw128
18. Kobayashi M, Huttin O, Magnusson M, Ferreira JP, Bozec E, Huby AC, *et al.*; STANISLAS Study Investigators. Machine learning-derived echocardiographic phenotypes predict heart failure incidence in asymptomatic individuals. *JACC Cardiovasc Imaging* 2022;**15**:193–208. https://doi.org/10.1016/j.jcmg.2021.07.004
19. Pencina MJ, D'Agostino RB Sr, Steyerberg EW. Extensions of net reclassification improvement calculations to measure usefulness of new biomarkers. *Stat Med* 2011;**30**:11–21. https://doi.org/10.1002/sim.4085
20. Cleland JGF, Butler J, Januzzi JL Jr, Pellicori P, McDonagh T. Only people with increased plasma concentrations of natriuretic peptides should be included in outcome trials of diabetes, cardiovascular and kidney disease: Implications for clinical practice. *Eur J Heart Fail* 2022;**24**:678–680. https://doi.org/10.1002/ejhf.2488
21. Bansal N, Zelnick L, Go A, Anderson A, Christenson R, Deo R, *et al.* Cardiac biomarkers and risk of incident heart failure in chronic kidney disease: The CRIC (Chronic Renal Insufficiency Cohort) study. *J Am Heart Assoc* 2019;**8**:e012336. https://doi.org/10.1161/JAHA.119.012336
22. Natriuretic Peptides Studies Collaboration;Willeit P, Kaptoge S, Welsh P, Butterworth AS, Chowdhury R, Spackman SA, *et al.* Natriuretic peptides and integrated risk assessment for cardiovascular disease: An individual-participant-data meta-analysis. *Lancet Diabetes Endocrinol* 2016;**4**:840–849. https://doi.org/10.1016/S2213-8587(16)30196-6
23. Huelsmann M, Neuhold S, Resl M, Strunk G, Brath H, Francesconi C, *et al.* PONTIAC (NT-proBNP selected prevention of cardiac events in a population of diabetic patients without a history of cardiac disease): A prospective randomized controlled trial. *J Am Coll Cardiol* 2013;**62**:1365–1372. https://doi.org/10.1016/j.jacc.2013.05.069
24. Heidenreich PA, Bozkurt B, Aguilar D, Allen LA, Byun JJ, Colvin MM, *et al.* 2022 AHA/ACC/HFSA Guideline for the management of heart failure: Executive summary: A report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *J Am Coll Cardiol* 2022;**79**:1757–1780. https://doi.org/10.1016/j.jacc.2021.12.011
25. Parikh RH, Seliger SL, Christenson R, Gottdiener JS, Psaty BM, deFilippi C. Soluble ST2 for prediction of heart failure and cardiovascular death in an elderly, community-dwelling population. *J Am Heart Assoc* 2016;**5**:e003188. https://doi.org/10.1161/JAHA.115.003188
26. Lind L, Arnlov J, Sundstrom J. Plasma protein profile of incident myocardial infarction, ischemic stroke, and heart failure in 2 cohorts. *J Am Heart Assoc* 2021;**10**:e017900. https://doi.org/10.1161/JAHA.120.017900
27. Bouwens E, Brankovic M, Mouthaan H, Baart S, Rizopoulos D, van Boven N, *et al.* Temporal patterns of 14 blood biomarker candidates of cardiac remodeling in relation to prognosis of patients with chronic heart failure – The Bio-SHiFT study. *J Am Heart Assoc* 2019;**8**:e009555. https://doi.org/10.1161/JAHA.118.009555
28. Molvin J, Jujic A, Melander O, Pareek M, Råstam L, Lindblad U, *et al.* Proteomic exploration of common pathophysiological pathways in diabetes and cardiovascular disease. *ESC Heart Fail* 2020;**7**:4151–4158. https://doi.org/10.1002/ehf2.13036
29. Murphy SP, Kakkar R, McCarthy CP, Januzzi JL Jr. Inflammation in heart failure: JACC state-of-the-art review. *J Am Coll Cardiol* 2020;**75**:1324–1340. https://doi.org/10.1016/j.jacc.2020.01.014
30. Tschope C, Ammirati E, Bozkurt B, Caforio ALP, Cooper LT, Felix SB, *et al.* Myocarditis and inflammatory cardiomyopathy: Current evidence and future directions. *Nat Rev Cardiol* 2021;**18**:169–193. https://doi.org/10.1038/s41569-020-00435-x