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Proteomic Bioprofiles and Mechanistic Pathways of Progression to Heart Failure:

The HOMAGE Study

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Abstract

BACKGROUND: Identifying the mechanistic pathways potentially associated with incident heart failure (HF) may provide a basis for novel preventive strategies.

METHODS AND RESULTS: To identify proteomic biomarkers and the potential underlying mechanistic pathways that may be associated with incident HF defined as the first hospitalization for HF, a nested-matched case-control design was used with cases (incident HF) and controls (without HF) selected from 3 cohorts (>20 000 individuals). Controls were matched on cohort, follow-up time, age, and sex. Two independent sample sets (a discovery set with 286 cases and 591 controls and a replication set with 276 cases and 280 controls) were used to discover and replicate the findings. Two hundred fifty-two circulating proteins in the plasma were studied. Adjusting for the matching variables age, sex, and follow-up time (and correcting for multiplicity of tests), 89 proteins were found to be associated with incident HF in the discovery phase, of which 38 were also associated with incident HF in the replication phase. These 38 proteins pointed to 4 main network clusters underlying incident HF: (1) inflammation and apoptosis, indicated by the expression of the TNF (tumor necrosis factor)-family members; (2) extracellular matrix remodeling, angiogenesis and growth, indicated by the expression of proteins associated with collagen metabolism, endothelial function, and vascular homeostasis; (3) blood pressure regulation, indicated by the expression of natriuretic peptides and proteins related to the renin-angiotensin-aldosterone system; and (4) metabolism, associated with cholesterol and atherosclerosis.

CONCLUSIONS: Clusters of biomarkers associated with mechanistic pathways leading to HF were identified linking inflammation, apoptosis, vascular function, matrix remodeling, blood pressure control, and metabolism. These findings provide important insight on the pathophysiological mechanisms leading to HF.

CLINICAL TRIAL REGISTRATION: URL: https://www.clinicaltrials.gov. Unique identifier: NCT02556450.

Keywords

apoptosis; atherosclerosis; blood pressure; heart failure; proteomics

Heart failure (HF) is a major cause of morbidity and mortality worldwide and the most frequent cause of hospitalization for patients over 65 years of age.[1–3] The incidence and prevalence of HF are increasing because of the aging of the population as well as rising rates of HF risk factors such as diabetes mellitus, obesity, and hypertension.[3–6] Identifying mechanistic pathways leading to HF may help improve preventive strategies.[7]

In the last decade, circulating biomarkers, such as NT-proBNP (N-terminal pro-B-type natriuretic peptide), have been studied for prediction of incident HF.[7–10] A recently published study,[10] also investigated the association of multiple proteins with incident HF for prediction purposes; these proteins (n=80) added little gain to the prognostic model, including natriuretic peptides. However, above and beyond prediction, biomarkers may reflect pathophysiological processes and thus may help in assessing the underlying pathways that contribute to the progression towards HF. Investigating the pathophysiological processes

may provide potential targets for future therapies. For this purpose, knowledge-based network analysis with induced network approach may help identify the links among the identified protein bio-markers, providing the basis for the identification of the underlying pathways leading to HF.[11]

The HOMAGE (Heart Omics in Ageing consortium; URL: https://www.clinicaltrials.gov. Unique identifier: NCT02556450) is an EU funded program that aims to identify and validate omics biomarkers associated with incident HF to potentially develop new and personalized preventive strategies. We report proteomics results, based on assays of 252 plasma proteins related to cardiovascular disease (CVD) and inflammation, testing the associations of these proteins with incident HF, applying knowledge-based network analysis to identify mechanistic pathways underlying the progression to HF.

METHODS

Study Population

The HOMAGE consortium included 20 completed and ongoing studies conducted in 8 European countries that enrolled healthy subjects, patients with HF and patients at high risk of CVD, all of which were pooled in a common database.[12] From the HOMAGE population with >20 000 patients, we identified cohorts in whom individuals had been followed-up until first hospitalization for HF. Patients from 2 suitable cohorts and one clinical trial population were identified: PREDICTOR,[13] HEALTH-ABC,[14] and PROSPER.[12,15] Patients with a history of HF at baseline were excluded. We then used a nested matched case-control design were individuals who developed HF were considered to be at risk, that is, eligible to be selected as controls up until the time they became a case[16]: a total of 852 incident HF cases were identified (574 in HEALTH-ABC, 234 in PROSPER, and 44 in PREDICTOR); within the respective cohorts controls were selected, matched age, sex, and follow-up time (defined as time of incident HF from entry to the cohort). The final numbers after the matching procedures are provided below.

The data that support the findings of this study are available from the corresponding author on reasonable request.

Discovery and Replication

The HOMAGE study had 2 independent phases: discovery and replication. For the discovery phase, we selected 300 cases and 599 controls (1 case only had 1 match) randomly selected without replacement in a 1:2 proportion[17–19]; because of 22 missing or poor-quality samples, the final match was 286 cases to 591 controls. For replication, we selected 315 cases and 315 controls randomly selected without replacement in a 1:1 proportion; because of 74 missing or poor-quality samples, the final match was 276 cases to 280 controls.

The study was conducted in accordance with the Declaration of Helsinki and approved by each site ethics committees. All participants provided written informed consent.

Outcome

The outcome was incident HF which was defined as first hospitalization for HF as primary admission diagnosis (adjudicated by the investigators of the respective cohorts).

Sample Handling

All sample shipments and sample data acquisition within the HOMAGE consortium are according to predefined standard operating procedures and material transfer agreements to maintain uniformity. Figure I in the Data Supplement shows the sample handling and storage per cohort and the sample flow until protein measurement at the TATAA Biocenter (Gothenburg, Sweden). Aliquoting of the samples at Biobank Maastricht was performed using a multipipette in 1 run to reduce freeze/thaw cycles and batch effects. The entire sampling handling/protein measurement was performed fully blind to case-control status. The cases and controls were separately identified and selected by the study statistician. All patient information was then removed and a randomly sorted list of patient/sample IDs for each cohort was sent to Maastricht University Medical Center.

Assays and Studied Biomarkers

Baseline plasma samples were analyzed for protein biomarkers by the TATAA biocenter using the Olink Proseek Multiplex cardiovascular (CVD) II, CVD III, and inflammation panels. These panels were selected by the well-balanced inclusion of proteins with already established associations with CVD and HF (eg, BNP, ST2, and GDF [growth differentiation factor]15) and others with less well-established associations (eg, TWEAK [tumor necrosis factor ligand superfamily member 12] and PON3 [paraoxonase]). The assay uses a proximity extension assay technology,[20] where 92 oligonucleotide-labeled antibody probe pairs per panel are allowed to bind to their respective targets in the sample in 96-well plate format. When binding to their correct targets, they give rise to new DNA amplicons with each ID-barcoding their respective antigens. The amplicons are subsequently quantified using a Fluidigm BioMark HD real-time polymerase chain reaction platform. The platform provides $\log_2$-NPX (normalized protein expression) data. A detailed description of the Olink technology is depicted in Addenda I in the Data Supplement. For 9 proteins measured in both the inflammation panel and CVD panels, the one from CVD panels was used for further data analyses (the results for these proteins were strongly correlated ≥0.9). In addition, 15 proteins that were below the limit of detection, were not included in the analysis. The Olink quality control samples are considered as flagged if they deviate >0.3 NPX from the median of all samples in one of 2 control assays for incubation and detection. The limit of detection is defined by the 3 negative controls run on each plate and set to 3 SDs above the measured background. Patients with missing or unusable samples (22 samples in the discovery phase and 74 samples in the replication phase) were not considered for the analyses. Where the assay results were partially missing, that is, results were missing for 1 or 2 of the 3 plates (83 patients in the discovery phase and 4 patients in the replication phase) then multiple imputation using chained equations was used.[21]

The abbreviations, full names, and respective Olink multiplex panels of the studied proteins are described in Table I in the Data Supplement.

The assays were performed blinded to case/control status with cases and controls randomly distributed across plates. The proteomic results were then merged with the baseline data, which included the case-control status, matching variables, and the clinical risk factors.

Statistical and Bioinformatics Considerations

For the baseline clinical characteristics, continuous variables are expressed as means and respective SD. Categorical variables are presented as frequencies and percentages. Patient baseline characteristics were compared between cases and controls using χ^2 tests for categorical variables and *t* tests for continuous variables.

The main aim of this study was to test multiple proteins with regards to their association with incident HF and the respective underlying mechanistic pathways. Logistic regression models adjusting for the matching variables (age, sex, cohort, and follow-up time) were used to identify protein biomarkers associated with incident HF in the discovery and replication phases[22] (Table II in the Data Supplement). Only those proteins which were found to be statistically significant (after correction for false discoveries) in the discovery phase (n=89) were taken forward for consideration in the replication phase. In both phases, we corrected for multiple testing using a false discovery rate of 1%.[23] Additional adjustment for the prespecified clinical risk factors previously found to represent the best clinical prognostic model for incident HF in the HOMAGE population[24] (smoking, diabetes mellitus, history of coronary artery disease, serum creatinine, body mass index, systolic blood pressure, use of antihypertensive medication, and heart rate) was also performed, providing similar results (Table III in the Data Supplement). Since proteins were measured using NPX values on a $\log_2$ scale, the odds ratio for each protein estimates the increase in the odds of HF associated with a doubling in the protein concentration. After the identification of the top proteins, common to the derivation and replication phases, we performed a multivariable a stepwise forward model adjusted on age, sex, cohort, phase, follow-up time, smoking, diabetes mellitus, history of coronary artery disease, serum creatinine, body mass index, systolic blood pressure, use of antihypertensive medication, and heart rate forced into the model with a *P* value for inclusion set at 0.05. This set of analyses was performed using STATA version 15 software (StataCorp 2017, Stata Statistical Software: Release 15; College Station, TX: StataCorp LP).

We used knowledge-based network analysis with induced network approach by consensusPathDB online server (accessed on January 29, 2019) from Max Planck Institute for Molecular Genetics to identify the links among the protein biomarkers selected in the previous step (discovery and replication with adjustment on the matching variables), based on known knowledge of interaction networks (protein interactions, genetic interactions, biochemical interactions, and gene regulatory interactions).[11] The network analysis also identifies additional proteins (intermediate nodes) based on knowledge-based interactions (with the exclusion of low-confidence interactions quantified by a *Z* score ≤20 calculated for each intermediate node). As a validation step, network analysis was repeated using ClueGO network analysis (version 2.5.3), using implemented biological GO processes.[25] Extra known connection between BNP and angiotensin was added to the network manually because of their well-described interplay on blood pressure and hydroelectrolytic

regulation.[26,27] The generated network was reorganized in Cytoscape (version 3.5) to merge genes with their expressed proteins and visualize the results. An additional overrepresentation analysis was performed using only the GO-biological processes and molecular function enriched by selected proteins against proteins on the OLINK panels, introducing an adjustment for the clustering of proteins on the network and consolidating the strength of true enrichment.

RESULTS

Study Population

The baseline characteristics of the studied population for both discovery (IA) and replication phases (IB) is depicted in Table 1. Cases and controls were well matched for age, sex, cohort, and follow-up time (ie, the matching variables) in both phases. Cases had higher body mass index, creatinine, were more often hypertensive (with antihypertensive medications), diabetic, and had more often coronary artery disease. All these variables were included in the HOMAGE prognostic model[24] and were used for further adjustment in the models (please see below).

Biomarkers Associated With Incident HF

Of the 252 proteins studied, adjusting for the matching variables age, sex, and follow-up time, 89 proteins were found to be associated with incident HF in the discovery phase, of which 38 were also associated with incident HF in the replication phase, Table 2. All 38 proteins were positively associated with incident HF, except for TWEAK and PON3, where patients with higher concentrations of these proteins were less likely to develop HF. Further adjusting for the clinical risk factors (smoking, diabetes mellitus, history of coronary artery disease, serum creatinine, body mass index, systolic blood pressure, use of antihypertensive medication, and heart rate) previously determined in the well-calibrated HOMAGE clinical risk model,[24] provides similar associations to those presented in Table 2, suggesting that these associations were independent of the patients' clinical risk (also supported by the weak correlation between the study proteins and the clinical risk factors), Tables III and IV in the Data Supplement.

The multivariable stepwise model including the matching variables and the clinical risk factors forced into the model, plus the 38 proteins independently identified in both the discovery and replication phases, retained BNP, TWEAK, NT-proBNP, REN (renin), TRAILR2 (trail receptor 2), PON3, CCL16 (C-C motif chemokine 16), and SLAMF1 (signalling lymphocytic activation molecule family member 1) as the biomarkers with stronger association with incident HF, Table V in the Data Supplement.

Induced Network Results

The 38 incident HF-associated protein biomarkers were linked with each other by known protein interactions, biochemical interactions, and gene regulatory interactions (Figure), directly or via intermediate nodes (Table VI in the Data Supplement). Our results pointed to 4 clusters with clearly defined functions: (1) inflammatory/apoptosis of mainly TNF-family members, (2) extracellular matrix remodeling, angiogenesis, and cell growth, (3)

a renin-angiotensin system associated with blood pressure regulation and one minor cluster including metabolic proteins, and (4) metabolism, associated with cholesterol and atherosclerosis. The 2 major clusters inflammatory/apoptosis and blood pressure regulation were also detected as the main groups using the ClueGO network analysis (Figure II in the Data Supplement). The TNF-family members, their representative pathways and blood pressure regulation remained significantly enriched after adjustment for the preselection of proteins (Table VII in the Data Supplement).

In addition, this analysis revealed multiple intracellular transcription factors: TP53 (tumor protein 53), HNF1B (hepatocyte nuclear factor-1-β), HIF1A/ARNT (hypoxia-inducible factor α/aryl hydrocarbon receptor nuclear translocator), and STAT6 (signal transducer and activator of transcription 6), which are not detected with our plasma protein panels. However, these transcription factors may supplement the biomarker profile of patients at high risk for incident HF, providing additional perspective on the interpretation of the pathophysiological processes driving HF. The role of each biomarker linked to the identified network clusters is furtherly detailed in the discussion section.

DISCUSSION

In the present study, we identified 38 plasma proteins associated with incident HF (in both the discovery and replication phases). The selected proteins allowed the identification of 4 main network clusters underpinning incident HF: (1) inflammation and apoptosis; (2) extracellular matrix remodeling, angiogenesis, and growth; (3) blood pressure regulation; and (4) metabolism. These findings are original and provide important insight on the pathophysiological mechanisms leading to HF, potentially creating the basis for the development of new HF prevention strategies, personalized to each individual patient underlying mechanism.

A recently published study[10] investigated the longitudinal association between high-throughput proteomics (also using OLINK technology) and HF risk in 2 community-based prospective cohorts of elderly individuals without HF at baseline. To some extent, the proteins identified in that study overlapped with ours. Specifically, TRAILR2, GDF-15, and MMP-12 (matrix metalloproteinase 12) were identified in both studies across all discovery steps. However, the study by Stenemo et al[10] studied 80 proteins, whereas ours analyzed 252. Moreover, our study was aimed to identify the biological signatures leading to HF.

Inflammation and Apoptosis Cluster

Inflammation and apoptosis, as pointed by the expression of TNF-family members, may be an important pathway leading to HF that may be identified by the expression of circulation proteins such as TRAILR2, IL-16 (interleukin 16), IL4RA (interleukin 4 receptor α), CD4 (T-cell surface glycoprotein CD4), TNFRSF (tumor necrosis factor receptor superfamily member) 10A, TNFRSF11A, TNFR1, TNFR2 (tumor necrosis factor receptor 2), TNFRS-F13B, TNFRSF14, CCL16, SLAMF1, and TWEAK. Elevated TNF signaling restrains cardiomyocyte differentiation of resident cardiac stem cells and enhances adrenergic activation, promoting adverse cardiac remodeling (also reflected by the elevated remodeling markers).[28] The TRAILR2 protein (otherwise known as death receptor 5) is encoded by

the *TNFSF10* gene and is a receptor belonging to the TNF superfamily that preferentially induces apoptosis after binding of its ligand TRAIL.[29,30] Increased levels of TRAILR2 have been associated with adverse cardiovascular events in patients with myocardial infarction, probably because of intensified apoptotic activity.[31] ILs, as upstream biomarkers of inflammation converge on the central TNF signaling pathway, having major infl ence on atherosclerosis, and consequently on the risk of CVD.[32] Another TNF superfamily member —TWEAK—activates the NF-κB (nuclear factor κB) and regulates several cell functions, such as proliferation, migration, differentiation, cell death, inflammation, angiogenesis, and collagen synthesis of cardiac fibroblasts.[33–35] Low TWEAK has been associated with increased risk of death in patients with overt HF[36] and patients with HF and reduced ejection fraction had lower TWEAK levels compared with controls.[34] The TWEAK-induced proliferation of cardiomyocytes and its immunomodulatory effects may provide a basis to these findings.[36] Apart from binding to its active receptor Fn14, TWEAK can also bind to a scavenger receptor CD163, which was shown to be upregulated in HF, explaining the decreased levels and activity of TWEAK in HF.[34]

The inflammation/apoptosis cluster grouped many proteins with a strong and independent association with HF: TWEAK, TRAILR2, CCL16, and SLAMF1.

Extracellular Matrix Remodeling, Angiogenesis, and Growth Cluster

Another major pathway identified as leading to HF was, extracellular matrix remodeling, angiogenesis, and growth supported by the expression of ADM (adreno-medullin), IGFBP7 (insulin-like growth factor-binding protein 7), PGF (placenta growth factor), PLC (perlecan), GAL9 (galectin 9), MMP-12, UPAR (urokinase plasminogen activator surface receptor), SLAMF1, CEACAM8 (carcinoembryonic antigen-related cell adhesion molecule 8), GDF-15, FGF23 (fibroblast growth factor 23), and OPN (osteopontin). ADM is a vasodilator peptide predominantly produced by the vascular endothelium and smooth muscle that increases in response to hemodynamic stress.[37] IGFBP7 participates in the regulation of the availability of insulin growth factor in body fluids and tissues. IGFBP7 has been found to be associated with diastolic dysfunction and is also a strong prognosticator in HF.[38] PGF is increased by pressure overload in the heart where it is expressed in both myocytes and other cells infiltrating the heart.[39] PLC is constituent of the extracellular matrix that regulates angiogenesis and cell autophagy.[40] PLC proangiogenic effects may be used for the treatment of ischemic diseases.[41] GAL9 is produced by the extracellular matrix and may be increased in patients with ischemic stroke, its role in HF requires further study.[42] MMPs degrade extracellular matrix proteins and play important roles in development and tissue repair. MMP-12 contributes to plaque growth and destabilization and increased levels of this proteins have been associated with higher atherosclerotic disease burden.[43] SLAMF1 is expressed in the surface of lymphocytes and is involved in the control of infectious and neoplastic processes.[44] CEACAM8 is released by granulocytes and is also involved in immune regulation.[45] The role of SLAMF1 and CEACAM8 in HF requires further investigation. However, they are both related to UPAR that induces cardiac fibrosis and macrophage accumulation and is associated with worse prognosis in HF.[46,47] GDF-15 regulates inflammation and apoptosis, both key mechanisms in cardiac remodeling that are potentially associated with incident HF.[48,49] FGF23 is released by the osteocytes and

is essential for the regulation of the metabolism of phosphate, calcium, and vitamin D. Importantly, FGF23 promotes myocardial fibrosis and has been associated with coronary heart disease and HF.[50,51] OPN is a member of the extracellular matrix protein family. OPN expression increases under a variety of pathophysiological conditions affecting the heart and has been associated with an increased incidence of CVDs, including HF.[52]

Blood Pressure Regulation Cluster

Another major pathway identified as leading to HF was associated with blood pressure regulation, supported by the increased expression of renin, angiotensin-converting enzyme, and BNP/NT-proBNP. The renin-angiotensin-aldosterone and the natriuretic peptide systems have been thoroughly associated with CVD, including hypertension and HF.[53] Natriuretic peptides (BNP and NT-proBNP) are produced by the cardiomyocytes, endothelial cells, T cells, and macrophages infiltrating the heart in response to cardiac overload.[54] BNP/NT-proBNP are recommended in the current guidelines for diagnostic and prognostic assessment in HF.[55,56] Natriuretic peptides have been associated with incident HF,[57] and a natriuretic peptide-based strategies for preventing HF have reduced the rates of both systolic and diastolic dysfunction.[58] The renin-angiotensin-aldosterone and BNP are closely related by inhibition on to each other by having counterbalanced regulatory functions on blood pressure.[26] Moreover, angiotensin can upregulate cardiac BNP gene expression.[27] Enhanced renin-angiotensin-aldosterone under high BNP may reflect a dysregulation on blood pressure leading to HF development. Natriuretic peptides and renin were strongly and independently associated with incident HF.

Metabolism Cluster

The other pathway identified as leading to HF was associated with metabolism, supported by the identification of PON3, FABP4 (fatty acid-binding protein 4), and RARRES2 (retinoic acid receptor responder protein 2). PON3 has been associated with HDL (high-density lipoprotein) increase and with the inhibition of LDL (low-density lipoprotein) oxidation, thus PON3 expression might be protective in the cardiovascular setting.[59] In our study, PON3 was negatively associated with incident HF risk, suggesting that antioxidation may play a role in the mechanisms associated with HF development. In line with TWEAK, preclinical evidence supports a cardioprotective role for PON3 (which was also independently associated with incident HF in the multivariable model). FABP4 is predominantly expressed in macrophages and adipose tissue where it regulates fatty acids storage and lipolysis; FABP4 is also an important mediator of inflammation that has been associated with a higher risk of cardiovascular events.[60,61] RARRES2 (or chemerin) is an adipose-derived signaling molecule that regulates adipogenesis and adipocyte metabolism, and it has also been associated with a higher risk of cardiovascular events.[62,63]

Overall, the results of our proteomic biomarker assessments in patients at risk of developing HF suggest that progression towards HF is likely to involve the interplay of several pathophysiological mechanisms, such as heart stress, blood pressure regulation, apoptosis, inflammation, and metabolism-related mechanisms. A previous report identified cytokine response, extracellular matrix organization, and inflammation as major pathways underlining HF with preserved ejection fraction.[64] The next step of this important data is to determine

the activity of these processes in different HF stages and eventually per individual. This will provide the basis for further development strategies in preventing HF and focusing on these specific pathways at early stages of the disease for an individual treatment approach. The intracellular transcription factors TP53, HNF1B, HIF1A/ARNT, and STAT6, which are not measured in our plasma protein panels, may complement the biomarker profile of patients at high risk for incident HF,[65] suggesting a combined multi-omics approach currently being investigated within the HOMAGE consortium.

Limitations

Several limitations should be highlighted in the present study. First, this is an observational case-control study, hence causality cannot be ascertained. The bioinformatics approach also does not allow causality assessment but allows for the generation of hypothesis on the underlying pathways associated with this proteomic expression. Also, we must be aware of the biases and oversimplification in network topology. For example, most network databases are overrepresented by well-studied proteins and their interactions. This will lead to overrepresentation of these interactions in the analysis as there are more interactions known for these proteins.[66] Second, incident HF was defined as first HF hospitalization, which does not exclude patients that might already have HF but without previous hospitalizations. Also, for the avoidance of competing risk, we excluded patients who died during follow-up. Therefore, it is possible that we missed patients where death was the first (and last) manifestation of HF. Third, we did not have access to the reported ejection fraction at the time of hospitalization, therefore, we cannot assess the potential value of these biomarkers in distinguishing progression to HF with reduced ejection fraction from HF with preserved ejection fraction and the HF cause. Fourth, clinical detail (signs, symptoms, ECG, and other complementary exams), troponin, and natriuretic peptides at the time of hospitalization are not available in the dataset. This information would help in further phenotyping these patients and in differentiating the cases from the controls. Fifth, the proteomics assay does not provide standard concentration units, making comparisons with clinically applied cutoffs difficult, however, the Olink standard procedures reassure a good correlation with the standard measurement methodologies. In addition, we did not use large unbiased screens but rather selected protein biomarkers based on mechanistic hypotheses. The 3 studied Olink panels (CVD II, III, and inflammation) contain circulating proteins previously found to be associated with cardiovascular and inflammatory diseases. Many other pathways are missing, for example, metabolism(omics), that could enrich our networks. Therefore, we cannot exclude the role of other mechanisms not targeted with our proteomics screen. Finally, prospective validation of these biomarkers in other populations is required to improve the external validation of these results.

Conclusions

After adjustment for the matching variables age, sex, follow-up time, and correction for multiplicity of tests, we identified 38 proteins in 2 independent sets associated with incident HF. Cluster of the selected proteins allowed the identification of 4 main networks leading to HF: (1) inflammation and apoptosis; (2) extracellular matrix remodeling, angiogenesis, and growth; (3) blood pressure regulation; and (4) metabolism. These findings provide important insight on the pathophysiological mechanisms leading to HF.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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WHAT IS NEW?

- We present a nested case:control study (with derivation and replication cohorts) to study 252 circulation proteins and their association with new-onset heart failure, to assess the mechanistic pathways that may lead to the development of heart failure.

WHAT ARE THE CLINICAL IMPLICATIONS?

- We identified 4 main networks that may lead to heart failure. These include inflammation and apoptosis; extracellular matrix remodeling, angio-genesis and growth; blood pressure regulation; and metabolism.
- These findings provide important insight on the mechanisms leading to heart failure and may help in the development of future personalized therapies.

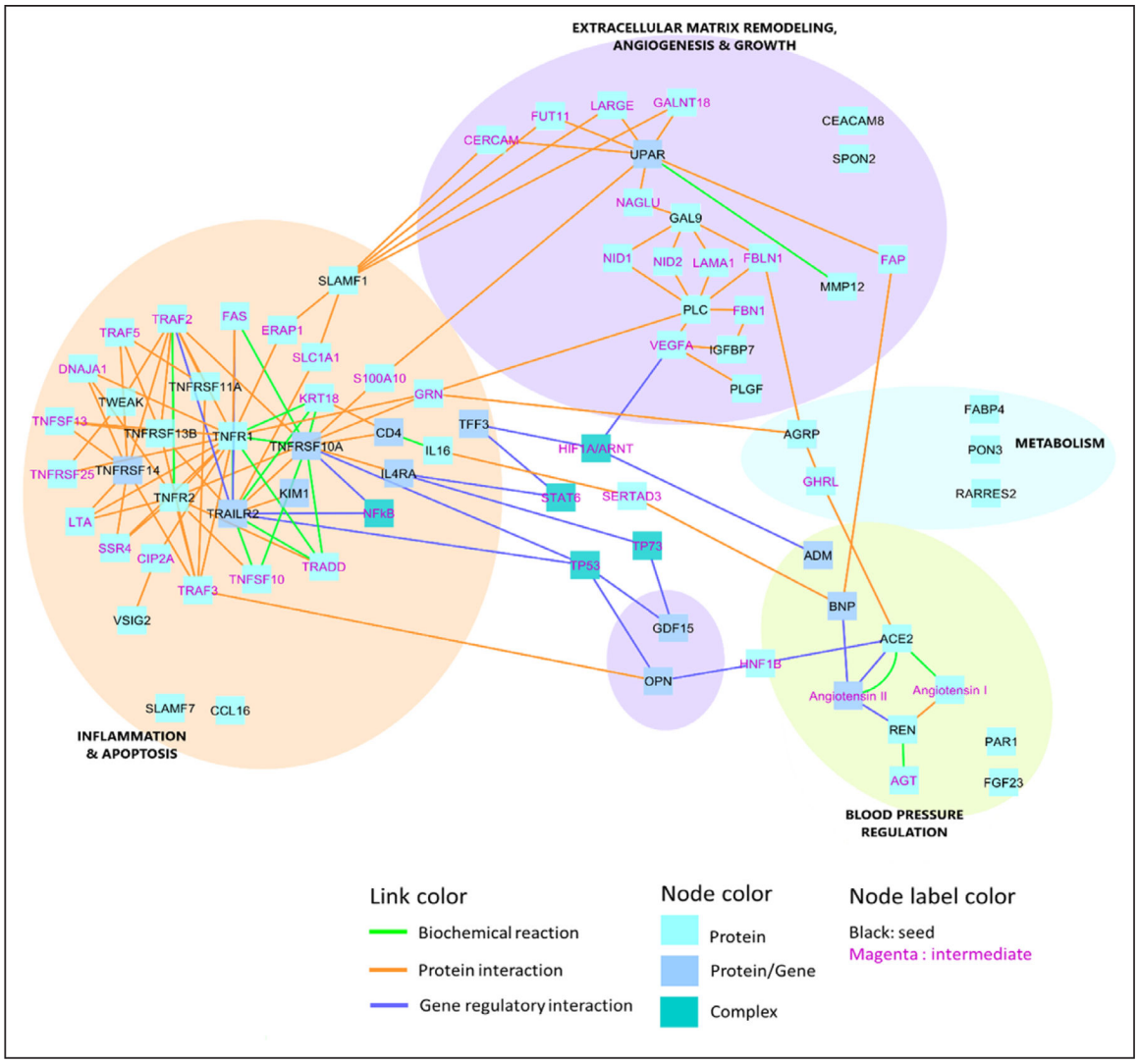

Figure.

Induced network analysis: protein interactions, biochemical interactions, and gene regulatory interactions. Please see Table IV in the Data Supplement for the full names of the biomarkers and intermediates. ACE indicates angiotensin-converting enzyme; ADM, adrenomedullin; AGRP, agouti-related protein; BNP, B-type natriuretic peptide; AGT, angiotensinogen; CCL16, C-C motif chemokine 16; CEACAM8, carcinoembryonic antigen-related cell adhesion molecule 8; CERCAM, cerebral endothelial cell adhesion molecule; FAP, fibroblast activation protein alpha; FBLN1, fibulin-1; FGF23, fibroblast growth factor 23; GAL9, galectin 9; GALNT18, polypeptide N-acetylgalactosaminyltransferase 18; GDF-15, growth differentiation factor 15; GHRL, ghrelin; HNF1B, hepatocyte nuclear factor-1-β; IGFBP7, insulin-like growth factor-binding protein 7; LAMA1, laminin subunit alpha 1; LARGE, LARGE xylosyl- and glucuronyltransferase 1; LTA, lymphotoxin alpha; MMP-12, matrix metalloproteinase 12; NAGLU, N-acetyl-alpha-glucosaminidase; NF-κB, nuclear factor κB; OPN, osteopontin; PAR1, proteinase-activated receptor 1; PGF, placenta growth factor; PLC, perlecan; PLGF, placenta growth factor; RARRES2, retinoic acid

receptor responder protein 2; SLAMF1, signalling lymphocytic activation molecule family member 1; SPON2, spondin-2; TNFRSF, tumor necrosis factor receptor superfamily member; TRAF, TNF receptor associated factor 1; TRAILR2, trail receptor 2; TWEAK, tumor necrosis factor ligand superfamily member 12; UPAR, urokinase plasminogen activator surface receptor; VEGFA, vascular endothelial growth factor A; and VSIG2, v-set and immunoglobulin domain-containing protein 2.

Table 1.

Characteristics of the Study Population for Both Discovery and Replication Phases

	Discovery			Replication		
	Cases	Controls		Cases	Controls	
Characteristics	**N=286**	**N=591**	***P* Value**	**N=276**	**N=280**	***P* Value**
Age, y	74.4 (3.5)	74.4 (3.5)	0.94	75.1 (3.5)	75.2 (3.6)	0.81
Male sex, n (%)	154 (53.8)	320 (54.1)	0.93	157 (56.9)	158 (56.4)	0.91
Cohort, n (%)			0.74			0.57
HEALTH-ABC	215 (75.2)	433 (73.3)		109 (39.5)	99 (35.4)	
PREDICTOR	15 (5.2)	29 (4.9)		29 (10.5)	29 (10.4)	
PROSPER	56 (19.6)	129 (21.8)		138 (50.0)	152 (54.3)	
Smoking status, n (%)			0.29			0.50
Never	139 (48.6)	309 (52.5)		157 (56.9)	169 (60.4)	
Current	30 (10.5)	71 (12.1)		57 (20.7)	47 (16.8)	
Past	117 (40.9)	209 (35.5)		62 (22.5)	64 (22.9)	
Body mass index, kg/m^2	28.1 (4.8)	27.0 (4.4)	<0.001	27.6 (4.7)	26.7 (4.2)	0.02
Systolic blood pressure, mm Hg	142.4 (23.4)	141.2 (23.0)	0.48	148.2 (22.9)	145.9 (22.7)	0.25
Diastolic blood pressure, mm Hg	75.2 (13.7)	74.6 (12.1)	0.45	78.5 (13.7)	78.2 (12.6)	0.76
Heart rate, bpm	67 (11.8)	64 (11.1)	0.004	68 (11.9)	66 (10.9)	0.09
Serum creatinine, μmol/L	98.8 (27.7)	93.5 (21.3)	0.002	102.8 (28.2)	95.5 (21.5)	<0.001
Total cholesterol, mmol/L	5.33 (1.00)	5.31 (0.99)	0.88	5.34 (0.97)	5.49 (0.95)	0.08
HDL-cholesterol, mmol/L	1.28 (0.39)	1.34 (0.41)	0.04	1.29 (0.37)	1.36 (0.42)	0.02
LDL-cholesterol, mmol/L	3.32 (0.89)	3.29 (0.92)	0.64	3.41 (0.91)	3.48 (0.85)	0.35
Triglycerides, mmol/L	1.61 (0.83)	1.55 (0.96)	0.33	1.53 (0.77)	1.45 (0.66)	0.19
Blood glucose, mmol/L	5.99 (2.18)	5.60 (1.56)	0.002	5.92 (1.99)	5.49 (1.34)	0.003
Comorbidities, n (%)						
Hypertension	219 (76.8)	408 (69.2)	0.02	184 (66.9)	177 (63.4)	0.39
Diabetes mellitus	55 (19.2)	68 (11.5)	0.002	49 (17.8)	32 (11.4)	0.03
CVD	124 (44.4)	168 (28.9)	<0.001	149 (54.0)	105 (37.5)	<0.001
CAD	103 (36.5)	126 (21.6)	<0.001	114 (41.3)	78 (27.9)	<0.001

Characteristics	Discovery			Replication		
	Cases	Controls		Cases	Controls	
	N=286	N=591	*P* Value	N=276	N=280	*P* Value
PAD	14 (5.0)	22 (3.8)	0.40	14 (5.1)	4 (1.4)	0.01
Cerebrovascular disease	25 (8.9)	49 (8.3)	0.76	34 (12.3)	25 (8.9)	0.19
Medications, n (%)						
Antihypertensives	192 (67.1)	318 (54.0)	<0.001	198 (71.7)	174 (62.1)	0.02
ACE inhibitor	64 (22.4)	74 (12.6)	<0.001	63 (22.8)	47 (16.8)	0.07
CCB	86 (30.1)	114 (19.4)	<0.001	77 (27.9)	53 (18.9)	0.01
Diuretics	88 (30.8)	141 (23.9)	0.03	102 (37.0)	89 (31.8)	0.20
β-blockers	51 (17.8)	97 (16.5)	0.61	62 (22.5)	54 (19.3)	0.36
ARBs	13 (4.5)	14 (2.4)	0.08	17 (6.2)	13 (4.6)	0.43
Antiplatelet	128 (44.8)	228 (38.7)	0.09	126 (45.7)	102 (36.4)	0.03

Numbers are mean (SD) unless otherwise specified. ACE indicates angiotensin-converting enzyme; ARBs, angiotensin receptors blockers; CAD, coronary artery diseases; CCB, calcium channel blockers; CVD, cardiovascular diseases; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and PAD, peripheral arterial diseases.

Table 2.

Odds Ratios (OR) and 95% Confidence Intervals for the Selected Proteins With Regards to Their Association With Incident Heart Failure After Adjustment for the Matching Variables and Correction for Multiple Comparisons in Both Discovery and Replication Sets

	Discovery			Replication		
Protein Name	**OR**	**(95% CI)**	***P* Value**	**OR**	**(95% CI)**	***P* Value**
BNP	1.62	(1.42–1.86)	<0.0001	1.73	(1.50–2.01)	<0.0001
NT-proBNP	1.74	(1.46–2.06)	<0.0001	2.00	(1.64–2.44)	<0.0001
TRAILR2	3.26	(2.32–4.60)	<0.0001	3.22	(2.21–4.69)	<0.0001
TNFRSF13B	1.74	(1.34–2.24)	<0.0001	2.61	(1.82–3.74)	<0.0001
GAL9	2.96	(1.95–4.49)	<0.0001	3.14	(2.01–4.91)	<0.0001
FGF23	1.70	(1.40–2.07)	<0.0001	1.84	(1.44–2.36)	<0.0001
TNFRSF10A	2.63	(1.88–3.69)	<0.0001	2.56	(1.74–3.77)	<0.0001
REN	1.31	(1.11–1.55)	0.0018	1.54	(1.29–1.85)	<0.0001
TNFRSF11A	2.09	(1.61–2.72)	<0.0001	1.95	(1.47–2.59)	<0.0001
GDF-15	2.74	(2.08–3.61)	<0.0001	1.89	(1.44–2.47)	<0.0001
FABP4	1.82	(1.51–2.19)	<0.0001	1.57	(1.29–1.92)	<0.0001
SLAMF7	1.55	(1.26–1.91)	<0.0001	2.16	(1.52–3.06)	<0.0001
CCL16	1.47	(1.21–1.79)	<0.0001	1.72	(1.34–2.22)	<0.0001
TWEAK	0.51	(0.36–0.73)	0.0003	0.53	(0.39–0.71)	<0.0001
KIM1	1.41	(1.19–1.68)	<0.0001	1.51	(1.23–1.84)	<0.0001
CD4	2.06	(1.49–2.85)	<0.0001	2.17	(1.49–3.16)	<0.0001
VSIG2	1.43	(1.14–1.81)	0.0025	1.76	(1.33–2.32)	<0.0001
PON3	0.71	(0.58–0.87)	0.0011	0.64	(0.51–0.80)	<0.0001
PLGF	2.17	(1.54–3.06)	<0.0001	1.95	(1.38–2.75)	0.0002
MMP-12	1.64	(1.32–2.03)	<0.0001	1.54	(1.23–1.92)	0.0002
ADM	2.56	(1.82–3.59)	<0.0001	1.80	(1.32–2.45)	0.0002
RARRES2	3.33	(2.02–5.51)	<0.0001	2.05	(1.38–3.05)	0.0004
CEACAM8	1.51	(1.22–1.86)	0.0001	1.49	(1.19–1.86)	0.0005
SLAMF1	1.44	(1.17–1.76)	0.0005	1.62	(1.23–2.13)	0.0005
TNFR1	2.40	(1.75–3.28)	<0.0001	1.63	(1.23–2.16)	0.0007

Protein Name	Discovery			Replication		
	OR	(95% CI)	*P* Value	OR	(95% CI)	*P* Value
AGRP	1.82	(1.38–2.40)	<0.0001	1.90	(1.31–2.76)	0.0007
TNFR2	2.11	(1.58–2.82)	<0.0001	1.55	(1.20–2.00)	0.0008
IGFBP7	1.66	(1.24–2.23)	0.0006	1.60	(1.21–2.11)	0.0010
UPAR	2.70	(1.94–3.75)	<0.0001	1.67	(1.23–2.28)	0.0011
PAR1	1.86	(1.33–2.60)	0.0003	1.74	(1.24–2.45)	0.0014
PLC	2.26	(1.59–3.21)	<0.0001	1.76	(1.24–2.49)	0.0015
ACE2	1.60	(1.27–2.02)	<0.0001	1.52	(1.17–1.98)	0.0016
IL-16	1.54	(1.20–1.97)	0.0007	1.46	(1.15–1.86)	0.0019
TFF3	1.61	(1.32–1.95)	<0.0001	1.45	(1.15–1.84)	0.0019
OPN	2.00	(1.52–2.63)	<0.0001	1.50	(1.16–1.95)	0.0021
SPON2	2.86	(1.50–5.48)	0.0015	2.40	(1.36–4.25)	0.0026
IL4RA	1.52	(1.15–2.02)	0.0033	1.62	(1.18–2.22)	0.0029
TNFRSF14	2.09	(1.55–2.82)	<0.0001	1.52	(1.15–2.01)	0.0034

ACE2 indicates angiotensin-converting enzyme 2; ADM, adrenomedullin; AGRP, agouti-related protein; BNP, brain natriuretic peptide; CCL16, C-C motif chemokine 16; CD4, T-cell surface glycoprotein CD4; CEACAM8, carcinoembryonic antigen-related cell adhesion molecule 8; FABP4, fatty acid-binding protein; FGF23, fibroblast growth factor 23; GAL9, galectin 9; GDF-15, growth/differentiation factor 15; IGFBP7, insulin-like growth factor-binding protein 7; IL-16, pro-interleukin-16; IL4RA, interleukin 4 receptor subunit alpha; KIM1, kidney injury molecule 1; MMP-12, matrix metalloproteinase 12; NT-proBNP, N-terminal pro-B-type natriuretic peptide; OPN, osteopontin; PAR1, proteinase-activated receptor 1; PLC, perlecan; PLGF, placenta growth factor; PON3, paraoxonase; RARRES2, retinoic acid receptor responder protein 2; REN, renin; SLAMF1, signaling lymphocytic activation molecule; SLAMF7, SLAM family member 7; SPON2, spondin-2; TFF3, trefoil factor 3; TNFR1, tumor necrosis factor receptor 1; TNFR2, tumor necrosis factor receptor 2; TNFRSF10A, tumor necrosis factor receptor superfamily member 10A; TNFRSF11A, tumor necrosis factor receptor superfamily member 11A; TNFRSF13B, tumor necrosis factor receptor superfamily member 13B; TNFRSF14, tumor necrosis factor receptor superfamily member 14; TRAILR2, TNF-related apoptosis-inducing ligand receptor 2; TWEAK, tumor necrosis factor (ligand) superfamily, member 12; UPAR, urokinase plasminogen activator surface receptor; and VSIG2, v-set and immunoglobulin domain-containing protein 2.