Proteomic Bioprofiles and Mechanistic Pathways of Progression to Heart Failure: the HOMAGE (Heart OMics in AGEing) study

João Pedro Ferreira, MD, PhD[1,2]* & Job Verdonschot, MD[3,4]*; Timothy Collier, PhD[5]; Ping Wang, PhD[4]; Anne Pizard, PhD[1,6]; Christian Bär, MD, PhD[7]; Jens Björkman, PhD[8]; Alessandro Boccanelli, MD[9]; Javed Butler, MD, PhD[10]; Andrew Clark, MD, PhD[11]; John G. Cleland, MD, PhD[12,13]; Christian Delles, MD, PhD[14]; Javier Diez, MD, PhD[15,16,17,18]; Nicolas Girerd, MD, PhD[1]; Arantxa González, MD, PhD[15,16,17]; Mark Hazebroek, MD, PhD[3]; Anne-Cécile Huby, PhD[1]; Wouter Jukema, MD, PhD[19]; Roberto Latini, MD, PhD[20]; Joost Leenders, MD, PhD[21]; Daniel Levy, MD, PhD[22,23]; Alexandre Mebazaa, MD, PhD[24]; Harald Mischak, MD, PhD[25]; Florence Pinet, MD, PhD[26]; Patrick Rossignol, MD, PhD[1]; Naveed Sattar, MD, PhD[27]; Peter Sever, MD, PhD[28]; Jan A. Staessen, MD, PhD[29,30]; Thomas Thum, MD, PhD[7,31]; Nicolas Vodovar, PhD[24]; Zhen-Yu Zhang, MD[29]; Stephane Heymans, MD, PhD[3,32,33]** & Faiez Zannad, MD, PhD[1]**

*co-first authors
**co-last authors

[1] Université de Lorraine, Inserm, Centre d'Investigations Cliniques- Plurithématique 14-33, and Inserm U1116, CHRU, F-CRIN INI-CRCT (Cardiovascular and Renal Clinical Trialists), Nancy, France.
[2] Department of Physiology and Cardiothoracic Surgery, Cardiovascular Research and Development Unit, Faculty of Medicine, University of Porto, Porto, Portugal.
[3] Department of Cardiology, Maastricht University Medical Centre, Center for Heart Failure Research, Cardiovascular Research Institute Maastricht (CARIM), University Hospital Maastricht, P. Debyelaan 25, 6229 HX Maastricht, The Netherlands.
[4] Department of Clinical Genetics, Maastricht University Medical Center, 6202 AZ Maastricht, The Netherlands.
[5] London School of Hygiene and Tropical Medicine, London, UK.
[6] Inserm 1024, Institut de Biologie de l'École Normale Supérieure (IBENS), PSL University of Paris, Paris, France.
[7] Institute of Molecular and Translational Therapeutic Strategies (IMTTS), Hannover Medical School, Hannover, Germany.
[8] TATAA Biocenter AB, Gothenburg, Sweden.
[9] Casa di cura Quisisana, Rome, Italy.
[10] Department of Medicine, University of Mississippi School of Medicine, Jackson, MI.
[10] Excellence Cluster REBIRTH, Hannover Medical School, Hannover, Germany.
[11] Hull York Medical School, Castle Hill Hospital, Cottingham, UK.

[12] Robertson Centre for Biostatistics and Clinical Trials, Institute of Health and Wellbeing, Glasgow, UK.
[13] University of Glasgow, United Kingdom. National Heart and Lung Institute, Royal Brompton and Harefield Hospitals, Imperial College, London, UK.
[14] Institute of Cardiovascular and Medical Sciences, University of Glasgow, Scotland, UK.
[15] Program of Cardiovascular Diseases, Centre for Applied Medical Research, University of Navarra, Pamplona, Spain.
[16] CIBERCV, Carlos III Institute of Health, Madrid, Spain.
[17] Instituto de Investigación Sanitaria de Navarra (IdiSNA), Spain.
[18] Departments of Nephrology, and Cardiology and Cardiac Surgery, University of Navarra Clinic, Pamplona, Spain.
[19] Department of Cardiology, Leiden University Medical Center, Leiden, Netherlands
[20] IRCCS-Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy.
[21] ACS Biomarker BV, Amsterdam, The Netherlands.
[22] National Heart, Lung, and Blood Institute's and Boston University's Framingham Heart Study, Framingham, MA, United States.
[23] Population Sciences Branch, Division of Intramural Research, National Heart, Lung, and Blood Institute, Bethesda, MD, United States.
[24] UMRS 942; University Paris Diderot; APHP, University Hospitals Saint Louis Lariboisière, Paris, France.
[25] Mosaiques-diagnostics, Rotenburger Str. 20, D-30659 Hannover, Germany.
[26] Inserm U1167, Institut Pasteur de Lille, Université de Lille, FHU-REMOD-VHF, Lille, France.
[27] Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, UK.
[28] International Centre for Circulatory Health, National Heart and Lung Institute, Imperial College London, London, England.
[29] Studies Coordinating Centre, Research Unit Hypertension and Cardiovascular Epidemiology, KU Leuven Department of Cardiovascular Sciences, University of Leuven, Leuven, Belgium.
[30] Cardiovascular Research Institute Maastricht (CARIM), Maastricht University, Maastricht, Netherlands.
[31] Imperial College London, National Heart and Lung Institute.
[32] Department of Cardiovascular Research, University of Leuven, UZ Herestraat 49, 3000 Leuven, Belgium.
[33] Netherlands Heart Institute (ICIN), Moreelsepark 1, 3511 EP Utrecht, The Netherlands.

Word-count: 3322 words (excluding abstract and references).

Contact to:
Professor Faiez Zannad
Centre d'Investigation Clinique 1433 module Plurithématique, CHRU Nancy - Hopitaux de Brabois,
Institut Lorrain du Coeur et des Vaisseaux Louis Mathieu
4 rue du Morvan, 54500 Vandoeuvre les Nancy
Tel : 03 83 15 73 15
Fax : 03 83 15 73 24
Mail: f.zannad@chru-nancy.fr

Abstract

Background: Identifying the mechanistic pathways potentially associated with incident 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 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. 252 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-family members; 2) extracellular matrix remodelling, 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.

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

Key-words: incident heart failure; proteomics; ageing; prognostic; mechanistic pathways

Introduction

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, 2, 3]. The incidence and prevalence of HF are increasing due to the ageing of the population as well as rising rates of HF risk factors such as diabetes, 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 N-terminal pro brain natriuretic peptide (NT-proBNP), 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 biomarkers, providing the basis for the identification of the underlying pathways leading to HF[11].

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

Methods

Study Population

The HOMAGE-consortium included 20 completed and ongoing studies conducted in eight European countries that enrolled healthy subjects, patients with HF and patients at high risk of CV disease, 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 hospitalisation for heart failure (HF). Patients from two 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 employed a nested matched case-control design were individuals who developed HF were considered to be at-risk *i.e*. 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, 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 upon reasonable request.

Discovery and replication

The HOMAGE study had two 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]; due to 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; due to 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 hospitalisation 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. **Supplemental Figure 1** 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 multi-pipette in 1 run to reduce freeze/thaw cycles and batch effects. The entire sampling handling/protein measurement was carried out 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 (MUMC).

Assays and studied biomarkers

Baseline plasma samples were analysed 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 CV disease and HF (*e.g*. BNP, ST2, GDF15) and others with less well-established associations (*e.g*. TWEAK, PON3). The assay use a proximity extension assay (PEA) technology[20], where 92 oligonucleotide-labelled 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 PCR platform. The platform provides log_2-normalized protein expression (NPX) data. A detailed description of the Olink® technology is depicted in the **Supplemental Addenda 1**. 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 (LOD), were not included in the analysis. The Olink® quality control samples are considered as "flagged" if they deviate more than 0.3 NPX from the median of all samples in one of two control assays for incubation and detection. The LOD is defined by the three negative controls run on each plate and set to three standard deviations 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 *i.e*. 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 the **Supplemental Table 1**.

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 standard deviation (SD). Categorical variables are presented as frequencies and percentages. Patient baseline characteristics were compared between cases and controls using Chi^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, follow-up time) were used to identify protein biomarkers associated with incident HF in the discovery and replication phases[22] (**Supplemental Table 2**). 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 (FDR) of 1%[23]. Additional adjustment for the pre-specified clinical risk factors previously found to represent the best clinical prognostic model for incident HF in the HOMAGE population[24] (smoking, diabetes, 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 (**Supplemental Table 3**). Since proteins were measured using NPX (Normalized Protein eXpression) 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, 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 (CPDB) online server (accessed on 29 January 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 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 (brain natriuretic peptide) and angiotensin was added to the network manually due to their well described interplay on blood pressure and hydro-electrolytic 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 (i.e. the matching variables) in both phases. Cases had higher BMI, creatinine, were more often hypertensive (with anti-hypertensive 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 heart failure

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 (tumor necrosis factor ligand superfamily member 12) and PON3 (paraoxonase), where patients with higher concentrations of these proteins were less likely to develop HF. Further adjusting for the clinical risk factors (smoking, diabetes, 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). **Supplemental Table 3 & 4.**

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, NTproBNP, REN, TRAILR2, PON3, CCL16, and SLAMF1 as the biomarkers with stronger association with incident HF. **Supplemental Table 5**.

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 1**), directly or via intermediate nodes (**Supplemental Table 6**). Our results pointed to 4 clusters with clearly defined functions: 1) inflammatory/apoptosis of mainly TNF-family members, 2) extracellular matrix remodelling, 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 two major clusters inflammatory/apoptosis and blood pressure regulation were also detected as the main groups using the ClueGO network analysis (**Supplemental Figure 2).** The TNF-family members, their representative pathways and blood pressure regulation remained significantly enriched after adjustment for the preselection of proteins (**Supplemental Table 7)**.

In addition, this analysis revealed multiple intracellular transcription factors: TP53 (tumor protein 53), HNF1B (hepatocyte nuclear factor-1-beta), HIF1A/ARNT (hypoxia inducible factor alfa/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 remodelling, 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 two 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 (trail receptor 2), GDF-15 (growth differentiation factor 15), and MMP-12 (matrix metalloproteinase 12) were identified in both studies across all discovery steps. However, the study by Stenemo *et al*. studied 80 proteins, whereas ours analysed 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, IL16 (interleukin 16), IL4RA (interleukin 4 receptor alfa), CD4 (T-cell surface glycoprotein CD4), TNFRSF10A (tumor necrosis factor receptor superfamily member 10A), TNFRSF11A (tumor necrosis factor receptor superfamily member 11A), TNFR1 (tumor necrosis factor receptor 1), TNFR2 (tumor necrosis factor receptor 2), TNFRSF13B (tumor necrosis factor receptor superfamily member 13B), TNFRSF14 (tumor necrosis factor receptor superfamily member 14), CCL16 (C-C motif chemokine 16), SLAMF1 (signalling lymphocytic activation molecule family member 1), and TWEAK. Elevated TNF signalling restrains cardiomyocyte differentiation of resident cardiac stem cells and enhance adrenergic activation, promoting adverse cardiac remodelling (also reflected by the elevated remodelling 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 due to intensified apoptotic activity[31]. Interleukins, as "upstream" biomarkers of inflammation converge on the central TNF signalling pathway, having major influence on atherosclerosis, and consequently on the risk of cardiovascular disease[32]. Another TNF superfamily member – TWEAK - activates the NF-κB (nuclear factor kappa B) and regulates several cell functions such as proliferation, migration, differentiation, cell death, inflammation, angiogenesis, and collagen synthesis of cardiac fibroblasts[33,34, 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 to "controls"[34]. The TWEAK-induced proliferation of cardiomyocytes and its immunomodulatory effects may provide 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 strong and independent association with HF: TWEAK, TRAILR2, CCL16 and SLAMF1.

Extracellular matrix remodelling, angiogenesis and growth cluster

Another major pathway identified as leading to HF was, *extracellular matrix remodelling, angiogenesis and growth* supported by the expression of ADM (adrenomedullin), IGFBP7 (insulin-

like growth factor-binding protein 7), PGF (placenta growth factor), PLC (perlecan), GAL9 (galectin 9), MMP12 (matrix metalloproteinase 12), UPAR (urokinase plasminogen activator surface receptor), SLAMF1 (signalling lymphocytic activation molecule), CEACAM8 (carcinoembryonic antigen related cell adhesion molecule 8), GDF15 (growth differentiation factor 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 extra-cellular matrix that regulates angiogenesis and cell autophagy[40]. PLC pro-angiogenic effects may be used for the treatment of ischemic diseases[41]. GAL9 is produced by the extracellular matrix and may be increased inpatients with ischemic stroke, its role in HF requires further study[42]. Matrix metalloproteinases 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]. GDF15 regulates inflammation and apoptosis, both key mechanisms in cardiac remodelling 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 increased incidence of cardiovascular diseases, 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/NTproBNP. The renin-angiotensin-aldosterone (RAAS) and the natriuretic peptide systems have been thoroughly associated with cardiovascular disease, including hypertension and HF[53]. Natriuretic peptides (BNP and NTproBNP) are produced by the cardiomyocytes, endothelial cells, T cells, and macrophages infiltrating the heart in response to cardiac overload[54]. BNP/NTproBNP 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 RAAS 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 RAAS 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 increase and with the inhibition of LDL 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 anti-oxidation 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 higher risk of cardiovascular events[60, 61]. RARRES2 (or chemerin) is an adipose-derived signalling molecule that regulates adipogenesis and adipocyte metabolism, and it has also been associated with 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 allow 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/or 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 cut-offs 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/or 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 two 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 remodelling, angiogenesis and growth; 3) blood pressure regulation; and 4) metabolism. These findings provide important insight on the pathophysiological mechanisms leading to HF.

Funding

The research leading to these results has received funding from the European Union Commission's Seventh Framework programme under grant agreement N° 305507 (HOMAGE). We acknowledge the support from the Netherlands Cardiovascular Research Initiative, an initiative with support of the Dutch Heart FoundationCVON2016-Early HFPEF, and CVON 2017, ShePREDICTS.

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 four main networks that may lead to heart failure. These include inflammation and apoptosis; extracellular matrix remodelling, angiogenesis 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.

Disclosures

JF, PR and FZ are supported by the French National Research Agency Fighting Heart Failure (ANR-15-RHU-0004), by the French PIA project «Lorraine Université d'Excellence » GEENAGE (ANR-15-IDEX-04-LUE) programs, and the Contrat de Plan Etat Région Lorraine and FEDER IT2MP; HM is the co-founder and co-owner of Mosaiques Diagnostics; AM received honoraria for lectures from Roche and Abbott, consultation fees from Sanofi and Servier and research grants from Adrenomed and Sphyngotec. All other authors have no conflicts of interest to report with regards to the present study.

Bibliography

1. Zannad F. Rising incidence of heart failure demands action. *Lancet*. 2017. Feb 10;391(10120):518-519.
2. McMurray JJ, Petrie MC, Murdoch DR and Davie AP. Clinical epidemiology of heart failure: public and private health burden. *Eur Heart J*. 1998;19 Suppl P:P9-16.
3. McMurray JJ and Stewart S. Epidemiology, aetiology, and prognosis of heart failure. *Heart*. 2000;83:596-602.
4. Petrie M and McMurray J. Changes in notions about heart failure. *Lancet*. 2001;358:432-4.
5. Conrad N, Judge A, Tran J, Mohseni H, Hedgecott D, Crespillo AP, Allison M, Hemingway H, Cleland JG, McMurray JJV and Rahimi K. Temporal trends and patterns in heart failure incidence: a population-based study of 4 million individuals. *Lancet*. 2017. Feb 10;391(10120):572-580.
6. Manzano L, Babalis D, Roughton M, Shibata M, Anker SD, Ghio S, van Veldhuisen DJ, Cohen-Solal A, Coats AJ, Poole-Wilson PP and Flather MD. Predictors of clinical outcomes in elderly patients with heart failure. *Eur J Heart Fail*. 2011;13:528-36.
7. Echouffo-Tcheugui JB, Greene SJ, Papadimitriou L, Zannad F, Yancy CW, Gheorghiade M and Butler J. Population risk prediction models for incident heart failure: a systematic review. *Circ Heart Fail*. 2015;8:438-47.
8. Wang TJ, Wollert KC, Larson MG, Coglianese E, McCabe EL, Cheng S, Ho JE, Fradley MG, Ghorbani A, Xanthakis V, Kempf T, Benjamin EJ, Levy D, Vasan RS and Januzzi JL. Prognostic utility of novel biomarkers of cardiovascular stress: the Framingham Heart Study. *Circulation*. 2012;126:1596-604.
9. Borne Y, Persson M, Melander O, Smith JG and Engstrom G. Increased plasma level of soluble urokinase plasminogen activator receptor is associated with incidence of heart failure but not atrial fibrillation. *Eur J Heart Fail*. 2014;16:377-83.

10. Stenemo M, Nowak C, Byberg L, Sundstrom J, Giedraitis V, Lind L, Ingelsson E, Fall T and Arnlov J. Circulating proteins as predictors of incident heart failure in the elderly. *Eur J Heart Fail*. 2017. Jan;20(1):55-62.
11. Herwig R, Hardt C, Lienhard M and Kamburov A. Analyzing and interpreting genome data at the network level with ConsensusPathDB. *Nat Protoc*. 2016;11:1889-907.
12. Jacobs L, Thijs L, Jin Y, Zannad F, Mebazaa A, Rouet P, Pinet F, Bauters C, Pieske B, Tomaschitz A, Mamas M, Diez J, McDonald K, Cleland JG, Rocca HP, Heymans S, Latini R, Masson S, Sever P, Delles C, Pocock S, Collier T, Kuznetsova T and Staessen JA. Heart 'omics' in AGEing (HOMAGE): design, research objectives and characteristics of the common database. *J Biomed Res*. 2014;28:349-59.
13. Mureddu GF, Agabiti N, Rizzello V, Forastiere F, Latini R, Cesaroni G, Masson S, Cacciatore G, Colivicchi F, Uguccioni M, Perucci CA and Boccanelli A. Prevalence of preclinical and clinical heart failure in the elderly. A population-based study in Central Italy. *Eur J Heart Fail*. 2012;14:718-29.
14. Beavers KM, Hsu FC, Houston DK, Beavers DP, Harris TB, Hue TF, Kim LJ, Koster A, Penninx BW, Simonsick EM, Strotmeyer ES, Kritchevsky SB and Nicklas BJ. The role of metabolic syndrome, adiposity, and inflammation in physical performance in the Health ABC Study. *J Gerontol A Biol Sci Med Sci*. 2013;68:617-23.
15. Shepherd J, Blauw GJ, Murphy MB, Cobbe SM, Bollen EL, Buckley BM, Ford I, Jukema JW, Hyland M, Gaw A, Lagaay AM, Perry IJ, Macfarlane PW, Meinders AE, Sweeney BJ, Packard CJ, Westendorp RG, Twomey C and Stott DJ. The design of a prospective study of Pravastatin in the Elderly at Risk (PROSPER). PROSPER Study Group. PROspective Study of Pravastatin in the Elderly at Risk. *Am J Cardiol*. 1999;84:1192-7.
16. Essebag V, Genest J, Jr., Suissa S and Pilote L. The nested case-control study in cardiology. *Am Heart J*. 2003;146:581-90.
17. Wacholder S, McLaughlin JK, Silverman DT and Mandel JS. Selection of controls in case-control studies. I. Principles. *Am J Epidemiol*. 1992;135:1019-28.
18. Wacholder S, Silverman DT, McLaughlin JK and Mandel JS. Selection of controls in case-control studies. II. Types of controls. *Am J Epidemiol*. 1992;135:1029-41.
19. Wacholder S, Silverman DT, McLaughlin JK and Mandel JS. Selection of controls in case-control studies. III. Design options. *Am J Epidemiol*. 1992;135:1042-50.
20. Lundberg M, Eriksson A, Tran B, Assarsson E and Fredriksson S. Homogeneous antibody-based proximity extension assays provide sensitive and specific detection of low-abundant proteins in human blood. *Nucleic Acids Res*. 2011;39:e102.
21. Resche-Rigon M and White IR. Multiple imputation by chained equations for systematically and sporadically missing multilevel data. *Stat Methods Med Res*. 2018;27:1634-1649.
22. Pearce N. Analysis of matched case-control studies. *Bmj*. 2016;352:i969.
23. Green GH and Diggle PJ. On the operational characteristics of the Benjamini and Hochberg False Discovery Rate procedure. *Stat Appl Genet Mol Biol*. 2007;6:Article27.
24. Jacobs L, Efremov L, Ferreira JP, Thijs L, Yang WY, Zhang ZY, Latini R, Masson S, Agabiti N, Sever P, Delles C, Sattar N, Butler J, Cleland JGF, Kuznetsova T, Staessen JA and Zannad F. Risk for Incident Heart Failure: A Subject-Level Meta-Analysis From the Heart "OMics" in AGEing (HOMAGE) Study. *J Am Heart Assoc*. 2017;6.
25. Bindea G, Mlecnik B, Hackl H, Charoentong P, Tosolini M, Kirilovsky A, Fridman WH, Pages F, Trajanoski Z and Galon J. ClueGO: a Cytoscape plug-in to decipher functionally grouped gene ontology and pathway annotation networks. *Bioinformatics*. 2009;25:1091-3.
26. Richards AM. The renin-angiotensin-aldosterone system and the cardiac natriuretic peptides. *Heart*. 1996;76:36-44.
27. Majalahti T, Suo-Palosaari M, Sarman B, Hautala N, Pikkarainen S, Tokola H, Vuolteenaho O, Wang J, Paradis P, Nemer M and Ruskoaho H. Cardiac BNP gene activation by angiotensin II in vivo. *Mol Cell Endocrinol*. 2007;273:59-67.

28. Hamid T, Xu Y, Ismahil MA, Li Q, Jones SP, Bhatnagar A, Bolli R and Prabhu SD. TNF receptor signaling inhibits cardiomyogenic differentiation of cardiac stem cells and promotes a neuroadrenergic-like fate. *Am J Physiol Heart Circ Physiol*. 2016;311:H1189-h1201.
29. Ramamurthy V, Yamniuk AP, Lawrence EJ, Yong W, Schneeweis LA, Cheng L, Murdock M, Corbett MJ, Doyle ML and Sheriff S. The structure of the death receptor 4-TNF-related apoptosis-inducing ligand (DR4-TRAIL) complex. *Acta Crystallogr F Struct Biol Commun*. 2015;71:1273-81.
30. Hymowitz SG, Christinger HW, Fuh G, Ultsch M, O'Connell M, Kelley RF, Ashkenazi A and de Vos AM. Triggering cell death: the crystal structure of Apo2L/TRAIL in a complex with death receptor 5. *Mol Cell*. 1999;4:563-71.
31. Skau E, Henriksen E, Wagner P, Hedberg P, Siegbahn A and Leppert J. GDF-15 and TRAIL-R2 are powerful predictors of long-term mortality in patients with acute myocardial infarction. *Eur J Prev Cardiol*. 2017;24:1576-1583.
32. Ridker PM and Luscher TF. Anti-inflammatory therapies for cardiovascular disease. *Eur Heart J*. 2014;35:1782-91.
33. Blanco-Colio LM. TWEAK/Fn14 Axis: A Promising Target for the Treatment of Cardiovascular Diseases. *Front Immunol*. 2014;5:3.
34. Ptaszynska-Kopczynska K, Marcinkiewicz-Siemion M, Lisowska A, Waszkiewicz E, Witkowski M, Jasiewicz M, Miklasz P, Jakim P, Galar B, Musial WJ and Kaminski KA. Alterations of soluble TWEAK and CD163 concentrations in patients with chronic heart failure. *Cytokine*. 2016;80:7-12.
35. Chen HN, Wang DJ, Ren MY, Wang QL and Sui SJ. TWEAK/Fn14 promotes the proliferation and collagen synthesis of rat cardiac fibroblasts via the NF-small ka, CyrillicB pathway. *Mol Biol Rep*. 2012;39:8231-41.
36. Richter B, Rychli K, Hohensinner PJ, Berger R, Mortl D, Neuhold S, Zorn G, Huber K, Maurer G, Wojta J, Pacher R, Hulsmann M and Niessner A. Differences in the predictive value of tumor necrosis factor-like weak inducer of apoptosis (TWEAK) in advanced ischemic and non-ischemic heart failure. *Atherosclerosis*. 2010;213:545-8.
37. Nishikimi T and Nakagawa Y. Adrenomedullin as a Biomarker of Heart Failure. *Heart Fail Clin*. 2018;14:49-55.
38. Gandhi PU, Gaggin HK, Redfield MM, Chen HH, Stevens SR, Anstrom KJ, Semigran MJ, Liu P and Januzzi JL, Jr. Insulin-Like Growth Factor-Binding Protein-7 as a Biomarker of Diastolic Dysfunction and Functional Capacity in Heart Failure With Preserved Ejection Fraction: Results From the RELAX Trial. *JACC Heart Fail*. 2016;4:860-869.
39. Accornero F, van Berlo JH, Benard MJ, Lorenz JN, Carmeliet P and Molkentin JD. Placental growth factor regulates cardiac adaptation and hypertrophy through a paracrine mechanism. *Circ Res*. 2011;109:272-80.
40. Gubbiotti MA, Neill T and Iozzo RV. A current view of perlecan in physiology and pathology: A mosaic of functions. *Matrix Biol*. 2017;57-58:285-298.
41. Segev A, Nili N and Strauss BH. The role of perlecan in arterial injury and angiogenesis. *Cardiovasc Res*. 2004;63:603-10.
42. He XW, Li WL, Li C, Liu P, Shen YG, Zhu M and Jin XP. Serum levels of galectin-1, galectin-3, and galectin-9 are associated with large artery atherosclerotic stroke. *Sci Rep*. 2017;7:40994.
43. Goncalves I, Bengtsson E, Colhoun HM, Shore AC, Palombo C, Natali A, Edsfeldt A, Duner P, Fredrikson GN, Bjorkbacka H, Ostling G, Aizawa K, Casanova F, Persson M, Gooding K, Strain D, Khan F, Looker HC, Adams F, Belch J, Pinnoli S, Venturi E, Kozakova M, Gan LM, Schnecke V and Nilsson J. Elevated Plasma Levels of MMP-12 Are Associated With Atherosclerotic Burden and Symptomatic Cardiovascular Disease in Subjects With Type 2 Diabetes. *Arterioscler Thromb Vasc Biol*. 2015;35:1723-31.
44. Wang N, Halibozek PJ, Yigit B, Zhao H, O'Keeffe MS, Sage P, Sharpe A and Terhorst C. Negative Regulation of Humoral Immunity Due to Interplay between the SLAMF1, SLAMF5, and SLAMF6 Receptors. *Front Immunol*. 2015;6:158.

45. Singer BB, Opp L, Heinrich A, Schreiber F, Binding-Liermann R, Berrocal-Almanza LC, Heyl KA, Muller MM, Weimann A, Zweigner J and Slevogt H. Soluble CEACAM8 interacts with CEACAM1 inhibiting TLR2-triggered immune responses. *PLoS One*. 2014;9:e94106.
46. Stempien-Otero A, Plawman A, Meznarich J, Dyamenahalli T, Otsuka G and Dichek DA. Mechanisms of cardiac fibrosis induced by urokinase plasminogen activator. *J Biol Chem*. 2006;281:15345-51.
47. Lichtenauer M, Jirak P, Wernly B, Paar V, Rohm I, Jung C, Schernthaner C, Kraus J, Motloch LJ, Yilmaz A, Hoppe UC, Christian Schulze P, Kretzschmar D and Pistulli R. A comparative analysis of novel cardiovascular biomarkers in patients with chronic heart failure. *Eur J Intern Med*. 2017;44:31-38.
48. Wollert KC, Kempf T and Wallentin L. Growth Differentiation Factor 15 as a Biomarker in Cardiovascular Disease. *Clin Chem*. 2017;63:140-151.
49. Seropian IM, Toldo S, Van Tassell BW and Abbate A. Anti-inflammatory strategies for ventricular remodeling following ST-segment elevation acute myocardial infarction. *J Am Coll Cardiol*. 2014;63:1593-603.
50. Panwar B, Judd SE, Wadley VG, Jenny NS, Howard VJ, Safford MM and Gutierrez OM. Association of Fibroblast Growth Factor 23 With Risk of Incident Coronary Heart Disease in Community-Living Adults. *JAMA Cardiol*. 2018;3:318-325.
51. Hao H, Li X, Li Q, Lin H, Chen Z, Xie J, Xuan W, Liao W, Bin J, Huang X, Kitakaze M and Liao Y. FGF23 promotes myocardial fibrosis in mice through activation of beta-catenin. *Oncotarget*. 2016;7:64649-64664.
52. Singh M, Dalal S and Singh K. Osteopontin: At the cross-roads of myocyte survival and myocardial function. *Life Sci*. 2014;118:1-6.
53. Sayer G and Bhat G. The renin-angiotensin-aldosterone system and heart failure. *Cardiol Clin*. 2014;32:21-32, vii.
54. Bruggink AH, de Jonge N, van Oosterhout MF, Van Wichen DF, de Koning E, Lahpor JR, Kemperman H, Gmelig-Meyling FH and de Weger RA. Brain natriuretic peptide is produced both by cardiomyocytes and cells infiltrating the heart in patients with severe heart failure supported by a left ventricular assist device. *J Heart Lung Transplant*. 2006;25:174-80.
55. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, Falk V, Gonzalez-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GM, Ruilope LM, Ruschitzka F, Rutten FH and van der Meer P. 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 J Heart Fail*. 2016. Jul 14;37(27):2129-2200.
56. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Jr., Colvin MM, Drazner MH, Filippatos G, Fonarow GC, Givertz MM, Hollenberg SM, Lindenfeld J, Masoudi FA, McBride PE, Peterson PN, Stevenson LW and Westlake C. 2016 ACC/AHA/HFSA Focused Update on New Pharmacological Therapy for Heart Failure: An Update of the 2013 ACCF/AHA Guideline for the Management of Heart Failure: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines and the Heart Failure Society of America. *Circulation*. 2017 Aug 8;136(6):e137-e161.
57. de Boer RA, Nayor M, deFilippi CR, Enserro D, Bhambhani V, Kizer JR, Blaha MJ, Brouwers FP, Cushman M, Lima JAC, Bahrami H, van der Harst P, Wang TJ, Gansevoort RT, Fox CS, Gaggin HK, Kop WJ, Liu K, Vasan RS, Psaty BM, Lee DS, Hillege HL, Bartz TM, Benjamin EJ, Chan C, Allison M, Gardin JM, Januzzi JL, Jr., Shah SJ, Levy D, Herrington DM, Larson MG, van Gilst WH, Gottdiener JS, Bertoni AG and Ho JE. Association of Cardiovascular Biomarkers With Incident Heart Failure With Preserved and Reduced Ejection Fraction. *JAMA Cardiol*. 2018;3:215-224.
58. Ledwidge M, Gallagher J, Conlon C, Tallon E, O'Connell E, Dawkins I, Watson C, O'Hanlon R, Bermingham M, Patle A, Badabhagni MR, Murtagh G, Voon V, Tilson L, Barry M, McDonald L, Maurer

B and McDonald K. Natriuretic peptide-based screening and collaborative care for heart failure: the STOP-HF randomized trial. *Jama*. 2013;310:66-74.
59. Shen Y, Ding FH, Sun JT, Pu LJ, Zhang RY, Zhang Q, Chen QJ, Shen WF and Lu L. Association of elevated apoA-I glycation and reduced HDL-associated paraoxonase1, 3 activity, and their interaction with angiographic severity of coronary artery disease in patients with type 2 diabetes mellitus. *Cardiovasc Diabetol*. 2015;14:52.
60. Floresta G, Pistara V, Amata E, Dichiara M, Marrazzo A, Prezzavento O and Rescifina A. Adipocyte fatty acid binding protein 4 (FABP4) inhibitors. A comprehensive systematic review. *Eur J Med Chem*. 2017;138:854-873.
61. Hobaus C, Herz CT, Pesau G, Wrba T, Koppensteiner R and Schernthaner GH. FABP4 and Cardiovascular Events in Peripheral Arterial Disease. *Angiology*. 2018;69:424-430.
62. Leiherer A, Muendlein A, Kinz E, Vonbank A, Rein P, Fraunberger P, Malin C, Saely CH and Drexel H. High plasma chemerin is associated with renal dysfunction and predictive for cardiovascular events - Insights from phenotype and genotype characterization. *Vascul Pharmacol*. 2016;77:60-8.
63. Goralski KB, McCarthy TC, Hanniman EA, Zabel BA, Butcher EC, Parlee SD, Muruganandan S and Sinal CJ. Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. *J Biol Chem*. 2007;282:28175-88.
64. Tromp J, Westenbrink BD, Ouwerkerk W, van Veldhuisen DJ, Samani NJ, Ponikowski P, Metra M, Anker SD, Cleland JG, Dickstein K, Filippatos G, van der Harst P, Lang CC, Ng LL, Zannad F, Zwinderman AH, Hillege HL, van der Meer P and Voors AA. Identifying Pathophysiological Mechanisms in Heart Failure With Reduced Versus Preserved Ejection Fraction. *J Am Coll Cardiol*. 2018;72:1081-1090.
65. Mak TW, Hauck L, Grothe D and Billia F. p53 regulates the cardiac transcriptome. *Proc Natl Acad Sci U S A*. 2017;114:2331-2336.
66. Sama IE and Huynen MA. Measuring the physical cohesiveness of proteins using physical interaction enrichment. *Bioinformatics*. 2010;26:2737-43.

Figure 1. Induced network analysis: protein interactions, biochemical interactions, and gene regulatory interactions

Legend: Please see the supplemental table 4 for the full names of the biomarkers and intermediates.

Table 1. Characteristics of the study population for both discovery and replication phases

Characteristics	Discovery					Replication				
	Cases		Controls		p-value	Cases		Controls		p-value
	N=286		N=591			N=276		N=280		
Age-years	74.4	(3.5)	74.4	(3.5)	0.94	75.1	(3.5)	75.2	(3.6)	0.81
Male sex-Nos.(%)	154	(53.8)	320	(54.1)	0.93	157	(56.9)	158	(56.4)	0.91
Cohort-Nos.(%)					0.74					0.57
HABC	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-Nos.(%)					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-mmHg	142.4	(23.4)	141.2	(23.0)	0.48	148.2	(22.9)	145.9	(22.7)	0.25
Diastolic blood pressure-mmHg	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-Nos.(%)										
Hypertension	219	(76.8)	408	(69.2)	0.02	184	(66.9)	177	(63.4)	0.39
Diabetes	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
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-Nos.(%)										
Anti-hypertensives	192	(67.1)	318	(54.0)	<0.001	198	(71.7)	174	(62.1)	0.02
ACEi	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
Beta-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

Legend: CVD, cardiovascular diseases; CAD, coronary artery diseases; PAD, peripheral arterial diseases; ACEi, angiotensin converting enzyme inhibitors; CCB, calcium channel blockers, ARBs, angiotensin receptors blockers.

Table 2. Odds ratios (OR) and 95% confidence intervals (CI) 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

Protein name	Discovery			Replication		
	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
NTPROBNP	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
GDF15	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
MMP12	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
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
IL16	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

Legend: BNP, brain natriuretic peptide; NTPROBNP, N-terminal prohormone brain natriuretic peptide; TRAILR2, TNF-related apoptosis-inducing ligand receptor 2; TNFRSF13B, tumor necrosis factor receptor superfamily member 13B; GAL9, galectin-9; FGF23, fibroblast growth factor 23; TNFRSF10A, tumor necrosis factor receptor superfamily member 10A, REN, renin; TNFRSF11A, tumor necrosis factor receptor superfamily member 11A; GDF15, growth/differentiation factor 15; FABP4, fatty acid-binding protein; SLAMF7, SLAM family member 7; CCL16, C-C motif chemokine 16; TWEAK, tumor necrosis factor (Ligand) superfamily, member 12; KIM1, kidney injury molecule 1; CD4, T-cell surface glycoprotein CD4; VSIG2, v-set and immunoglobulin domain-containing protein 2; PON3, paraoxonase; PLGF, placenta growth factor; MMP12, matrix metalloproteinase-12; ADM, adrenomedullin; RARRES2, retinoic acid receptor responder protein 2; CEACAM8, carcinoembryonic antigenrelated cell adhesion molecule 8; SLAMF1, signaling lymphocytic activation molecule; tnfr1, tumor necrosis factor receptor 1; AGRP, agouti-related protein; TNFR2, tumor necrosis factor receptor 2; IGFBP7, insulin-like growth factor-binding protein 7; UPAR, urokinase plasminogen activator surface receptor; PAR1, proteinase-activated receptor 1; PLC, perlecan; ACE2, angiotensin-converting enzyme 2; IL16, pro-interleukin-16; TFF3, trefoil factor 3; OPN, osteopontin; SPON2, spondin-2; IL4RA, interleukin-4 receptor subunit alpha; TNFRSF14, tumor necrosis factor receptor superfamily member 14.

Supplemental Material

Supplemental Table 1. Protein names and respective Olink® panel sorted in alphabetical order

Protein full name	**Entry name**	**Olink® Panel***	**Uniprot ID****
Angiotensin-converting enzyme 2	ACE2	CVD II	Q9BYF1
Adenosine Deaminase	ADA	INF	P00813
Adisintegrin and metalloproteinase with thrombospondin motifs 13	ADAMTS13	CVD II	Q76LX8
ADM	ADM	CVD II	P35318
Agouti-related protein	AGRP	CVD II	O00253
CD166 antigen	ALCAM	CVD III	Q13740
Protein AMBP	AMBP	CVD II	P02760
Angiopoietin-1	ANG1	CVD II	Q15389
Aminopeptidase N	APN	CVD III	P15144
Axin-1	AXIN1	INF	O15169
Tyrosine-protein kinase receptor UFO	AXL	CVD III	P30530
Azurocidin	AZU1	CVD III	P20160
Brain-derived neurotrophic factor	BDNF	INF	P23560
Beta-nerve growth factor	BETANGF	INF	P01138
Bleomycin hydrolase	BLMHYDROLASE	CVD III	Q13867
Bone morphogenetic protein 6	BMP6	CVD II	P22004
Natriuretic peptides B	BNP	CVD II	P16860
Eukaryotic translation initiation factor 4E-binding protein 1	BP1_4E	INF	Q13541
Carbonic anhydrase 5A, mitochondrial	CA5A	CVD II	P35218
Caspase-3	CASP3	CVD III	P42574
Caspase 8	CASP8	INF	Q14790
Eotaxin-1	CCL11	INF	P51671
C-C motif chemokine 15	CCL15	CVD III	Q16663
C-C motif chemokine 16	CCL16	CVD III	O15467
C-C motif chemokine 17	CCL17	CVD II	Q92583
C-C motif chemokine 19	CCL19	INF	Q99731
C-C motif chemokine 20	CCL20	INF	P78556
C-C motif chemikine 22	CCL22	CVD III	O00626
C-C motif chemokine 23	CCL23	INF	P55773
C-C motif chemokine 24	CCL24	CVD III	O00175
C-C motif chemokine 25	CCL25	INF	O15444
C-C motif chemokine 28	CCL28	INF	Q9NRJ3
C-C motif chemokine 3	CCL3	CVD II	P10147
C-C motif chemokine 4	CCL4	INF	P13236
Scavenger receptor cysteine-rich type 1 protein M130	CD163	CVD III	Q86VB7
Natural killer cell receptor 2B4	CD244	INF	Q9BZW8
T-cell surface glycoprotein CD4	CD4	CVD II	P01730
CD40L receptor	CD40	INF	P25942
CD40 ligand	CD40L	CVD II	P29965
T-cell surface glycoprotein CD5	CD5	INF	P06127
T cell surface glycoprotein CD6	CD6	INF	P30203
SLAM family member 5	CD84	CVD II	Q9UIB8
Complement component C1q receptor	CD93	CVD III	Q9NPY3
CUB domain-containing protein 1	CDCP1	INF	Q9H5V8
Cadherin-5	CDH5	CVD III	P33151
Carcinoembryonic antigenrelated cell adhesion molecule 8	CEACAM8	CVD II	P31997

Chitinase-3-like protein 1	CHI3L1	CVD III	P36222
Chitotriosidase-1	CHIT1	CVD III	Q13231
Contactin-1	CNTN1	CVD III	Q12860
Collagen alpha-1(I) chain	COL1A1	CVD III	P02452
Carboxypeptidase A1	CPA1	CVD III	P15085
Carboxypeptidase B	CPB1	CVD III	P15086
Macrophage colony-stimulating factor 1	CSF1	INF	P09603
Cystatin D	CST5	INF	P28325
Cystatin-B	CSTB	CVD III	P04080
Chymotrypsin C	CTRC	CVD II	Q99895
Cathepsin D	CTSD	CVD III	P07339
Cathepsin L1	CTSL1	CVD II	P07711
Cathepsin Z	CTSZ	CVD III	Q9UBR2
Fractalkine	CX3CL1	INF	P78423
C-X-C motif chemokine 1 (CVD2)	CXCL1	CVD II	P09341
C-X-C motif chemokine 10	CXCL10	INF	P02778
C-X-C motif chemokine 11	CXCL11	INF	O14625
C-X-C motif chemokine 16	CXCL16	CVD III	Q9H2A7
C-X-C motif chemokine 5	CXCL5	INF	P42830
C-X-C motif chemokine 6	CXCL6	INF	P80162
C-X-C motif chemokine 9	CXCL9	INF	Q07325
Decorin	DCN	CVD II	P07585
2,4-dienoyl-CoA reductase, mitochondrial	DECR1	CVD II	Q16698
Dickkopf-related protein 1	DKK1	CVD II	O94907
Azurocidin	DLK1	CVD III	P80370
Delta and Notch-like epidermal growth factor-related receptor	DNER	INF	Q8NFT8
Epidermal growth factor receptor	EGFR	CVD III	P00533
Protein S100-A12	ENRAGE	INF	P80511
Epithelial cell adhesion molecule	EPCAM	CVD III	P16422
Ephrin type-B receptor 4	EPHB4	CVD III	P54760
Fatty acid-binding protein, intestinal	FABP2	CVD II	P12104
Fatty acid-binding protein, adipocyte	FABP4	CVD III	P15090
Tumor necrosis factor receptor superfamily member 6	FAS	CVD III	P25445
Fibroblast growth factor 19	FGF19	INF	O95750
Fibroblast growth factor 21 (CVD2)	FGF21	CVD II	Q9NSA1
Fibroblast growth factor 23 (CVD2)	FGF23	CVD II	Q9GZV9
Fibroblast growth factor 5	FGF5	INF	P12034
Fms-related tyrosine kinase 3 ligand	FLT3L	INF	P49771
Follistatin	FS	CVD II	P19883
Galectin-3	GAL3	CVD III	P17931
Galectin-4	GAL4	CVD III	P56470
Galectin-9	GAL9	CVD II	O00182
Growth/differentiation factor 15	GDF15	CVD III	Q99988
Growth/differentiation factor 2	GDF2	CVD II	Q9UK05
Growth hormone	GH	CVD II	P01241
Gastric intrinsic factor	GIF	CVD II	P27352
Lactoylglutathione lyase	GLO1	CVD II	Q04760
Granulins	GRN	CVD III	P28799
Gastrotropin	GT	CVD II	P51161
Hydroxyacid oxidase 1	HAOX1	CVD II	Q9UJM8
Proheparin-binding EGF-like growth factor	HBEGF	CVD II	Q99075

Glial cell line-derived neurotrophic factor	HGDNF	INF	P39905
Hepatocyte growth factor	HGF	INF	P14210
Heme oxygenase 1	HO1	CVD II	P09601
Osteoclast-associated immunoglobulin-like receptor	HOSCAR	CVD II	Q8IYS5
Heat shock 27 kDa protein	HSP27	CVD II	P04792
Intercellular adhesion molecule 2	ICAM2	CVD III	P13598
Alpha-L-iduronidase	IDUA	CVD II	P35475
Insulin-like growth factor-binding protein 1	IGFBP1	CVD III	P08833
Insulin-like Growth Factor-Binding Protein 2	IGFBP2	CVD III	P18065
Insulin-like growth factor-binding protein 7	IGFBP7	CVD III	Q16270
Low affinity immunoglobulin gamma Fc region receptor II-b	IGGFCRECEPTORIIB	CVD II	P31994
Interleukin-10	IL10	INF	P22301
Interleukin-10 receptor subunit alpha	IL10RA	INF	Q13651
Interleukin-10 receptor subunit beta	IL10RB	INF	Q08334
Interleukin-12 subunit beta	IL12B	INF	P29460
Interleukin-13	IL13	INF	P35225
Interleukin-15 receptor subunit alpha	IL15RA	INF	Q13261
Pro-interleukin-16	IL16	CVD II	Q14005
Interleukin-17A	IL17A	INF	Q16552
Interleukin-17C	IL17C	INF	Q9P0M4
Interleukin-17D	IL17D	CVD II	Q8TAD2
Interleukin-17 receptor A	IL17RA	CVD III	Q96F46
Interleukin-18 (CVD2)	IL18	CVD II	Q14116
Interleukin-18-binding protein	IL18BP	CVD III	O95998
Interleukin-18 receptor 1	IL18R1	INF	Q13478
Interleukin-1 receptor antagonist protein	IL1RA	CVD II	P18510
Interleukin-1 receptor-like 2	IL1RL2	CVD II	Q9HB29
Interleukin-1 receptor type 1	IL1RT1	CVD III	P14778
Interleukin-1 receptor type 2	IL1RT2	CVD III	P27930
Interleukin-20 receptor subunit alpha	IL20RA	INF	Q9UHF4
Interleukin-27	IL27	CVD II	Q8NEV9
Interleukin-2 receptor subunit alpha	IL2RA	CVD III	P01589
Interleukin-4 receptor subunit alpha	IL4RA	CVD II	P24394
Interleukin-6 (CVD2)	IL6	CVD II	P05231
Interleukin-6 receptor subunit alpha	IL6RA	CVD III	P08887
Interleukin-7	IL7	INF	P13232
Interleukin-8	IL8	INF	P10145
Melusin	ITGB1BP2	CVD II	Q9UKP3
Integrin beta-2	ITGB2	CVD III	P05107
Junctional adhesion molecule A	JAMA	CVD III	Q9Y624
Kidney injury molecule 1	KIM1	CVD II	Q96D42
Kallikrein-6	KLK6	CVD III	Q92876
Latency-associated peptide transforming growth factor beta 1	LAPTGFBETA1	INF	P01137
Low-density lipoprotein receptor	LDLRECEPTOR	CVD III	P01130
Leptin	LEP	CVD II	P41159
Leukemia inhibitory factor receptor	LIFR	INF	P42702
Lectin-like oxidized LDL receptor 1	LOX1	CVD II	P78380
Lipoprotein lipase	LPL	CVD II	P06858
Lymphotoxin-beta receptor	LTBR	CVD III	P36941
Macrophage receptor MARCO	MARCO	CVD II	Q9UEW3

Myoglobin	MB	CVD III	P02144
Monocyte chemotactic protein 1	MCP1_CVD3	CVD III	P13500
Monocyte chemotactic protein 2	MCP2	INF	P80075
Monocyte chemotactic protein 3	MCP3	INF	P80098
Monocyte chemotactic protein 4	MCP4	INF	Q99616
Matrix extracellular phosphoglycoprotein	MEPE	CVD III	Q9NQ76
Tyrosine-protein kinase Mer	MERTK	CVD II	Q12866
Matrix metalloproteinase-1	MMP1	INF	P03956
Matrix metalloproteinase-10	MMP10	INF	P09238
Matrix metalloproteinase-12	MMP12	CVD II	P39900
Matrix metalloproteinase-2	MMP2	CVD III	P08253
Matrix metalloproteinase-3	MMP3	CVD III	P08254
Matrix metalloproteinase-7	MMP7	CVD II	P09237
Matrix metalloproteinase-9	MMP9	CVD III	P14780
Myeloperoxidase	MPO	CVD III	P05164
NF-kappa-B essential modulator	NEMO	CVD II	Q9Y6K9
Neurogenic locus notch homolog protein 3	NOTCH3	CVD III	Q9UM47
Neurotrophin-3	NT3	INF	P20783
N-terminal prohormone brain natriuretic peptide	NTPROBNP	CVDII	P16860
Osteoprotegerin	OPG_CVD3	CVD III	O00300
Osteopontin	OPN	CVD III	P10451
Oncostatin-M	OSM	INF	P13725
Plasminogen activator inhibitor 1	PAI	CVD III	P05121
Pappalysin-1	PAPPA	CVD II	Q13219
Proteinase-activated receptor 1	PAR1	CVD II	P25116
Poly [ADP-ribose] polymerase 1	PARP1	CVD II	P09874
Proprotein convertase subtilisin/kexin type 9	PCSK9	CVD III	Q8NBP7
Platelet-derived growth factor subunit A	PDGFSUBUNITA	CVD III	P04085
Platelet-derived growth factor subunit B	PDGFSUBUNITB	CVD II	P01127
Programmed cell death 1 ligand 1	PDL1	INF	Q9NZQ7
Programmed cell death 1 ligand 2	PDL2	CVD II	Q9BQ51
Platelet endothelial cell adhesion molecule	PECAM1	CVD III	P16284
Peptidoglycan recognition protein 1	PGLYRP1	CVD III	O75594
Elafin	PI3	CVD III	P19957
Polymeric immunoglobulin receptor	PIGR	CVD II	P01833
Perlecan	PLC	CVD III	P98160
Placenta growth factor	PLGF	CVD II	P49763
Paraoxonase (PON 3)	PON3	CVD III	Q15166
Prolargin	PRELP	CVD II	P51888
Brother of CDO	PROTEINBOC	CVD II	Q9BWV1
Serine protease 27	PRSS27	CVD II	Q9BQR3
Prostasin	PRSS8	CVD II	Q16651
Myeloblastin	PRTN3	CVD III	P24158
P-selectin glycoprotein ligand 1	PSGL1	CVD II	Q14242
Pulmonary surfactant-associated protein D	PSPD	CVD III	P35247
Pentraxin-related protein PTX3	PTX3	CVD II	P26022
Receptor for advanced glycosylation end products	RAGE	CVD II	Q15109
Retinoic acid receptor responder protein 2	RARRES2	CVD III	Q99969
Renin	REN	CVD II	P00797
Resistin	RETN	CVD III	Q9HD89
Stem cell factor (CVD2)	SCF	CVD II	P21583

Secretoglobin family 3A member 2	SCGB3A2	CVD III	Q96PL1
E-selectin	SELE	CVD III	P16581
P-selectin	SELP	CVD III	P16109
Serpin A12	SERPINA12	CVD II	Q8IW75
Tyrosine-protein phosphatase non-receptor type substrate 1	SHPS1	CVD III	P78324
SIR2-like protein 2	SIRT2	INF	Q8IXJ6
Signaling lymphocytic activation molecule	SLAMF1	INF	Q13291
SLAM family member 7	SLAMF7	CVD II	Q9NQ25
Superoxide dismutase [Mn], mitochondrial	SOD2	CVD II	P04179
Sortilin	SORT1	CVD II	Q99523
Spondin-1	SPON1	CVD III	Q9HCB6
Spondin-2	SPON2	CVD II	Q9BUD6
Proto-oncogene tyrosine-protein kinase Src	SRC	CVD II	P12931
Sulfotransferase 1A1	ST1A1	INF	P50225
ST2 protein	ST2	CVD III	Q01638
STAM-binding protein	STAMPB	INF	O95630
Serine/threonine-protein kinase 4	STK4	CVD II	Q13043
Tissue factor	TF	CVD II	P13726
Trefoil factor 3	TFF3	CVD III	Q07654
Tissue factor pathway inhibitor	TFPI	CVD III	P10646
Transforming growth factor alpha	TGFALPHA	INF	P01135
Protein-glutamine gamma-glutamyltransferase 2	TGM2	CVD II	P21980
Thrombospondin-2	THBS2	CVD II	P35442
Thrombopoietin	THPO	CVD II	P40225
Angiopoietin-1 receptor	TIE2	CVD II	Q02763
Metalloproteinase inhibitor 4	TIMP4	CVD III	Q99727
Trem-like transcript 2 protein	TLT2	CVD III	Q5T2D2
Thrombomodulin	TM	CVD II	P07204
Tumor necrosis factor	TNF	INF	P01375
TNF-beta	TNFB	INF	P01374
Tumor necrosis factor receptor 1	TNFR1	CVD III	P19438
Tumor necrosis factor receptor 2	TNFR2	CVD III	P20333
Tumor necrosis factor receptor superfamily member 10A	TNFRSF10A	CVD II	O00220
Tumor necrosis factor receptor superfamily member 10C	TNFRSF10C	CVD III	O14798
Tumor necrosis factor receptor superfamily member 11A	TNFRSF11A	CVD II	Q9Y6Q6
Tumor necrosis factor receptor superfamily member 13B	TNFRSF13B	CVD II	O14836
Tumor necrosis factor receptor superfamily member 14	TNFRSF14	CVD III	Q92956
Tumor necrosis factor receptor superfamily member 9	TNFRSF9	INF	Q07011
Tumor necrosis factor ligand superfamily member 13B	TNFSF13B	CVD III	Q9Y275
Tumor necrosis factor ligand superfamily member 14	TNFSF14	INF	O43557
Tissue-type plasminogen activator	TPA	CVD III	P00750
Transferrin receptor protein 1	TR	CVD III	P02786
TNF-related apoptosis-inducing ligand	TRAIL	INF	P50591
TNF-related apoptosis-inducing ligand receptor 2	TRAILR2	CVD II	O14763
TNF-related activation-induced cytokine	TRANCE	INF	O14788
Tartrate-resistant acid phosphatase type 5	TRAP	CVD III	P13686
Tumor necrosis factor (Ligand) superfamily, member 12	TWEAK	INF	O43508
Urokinase-type plasminogen activator	UPA_CVD3	CVD III	P00749
Urokinase plasminogen activator surface receptor	UPAR	CVD III	Q03405
Vascular endothelial growth factor A	VEGFA	INF	P15692
Vascular endothelial growth factor D	VEGFD	CVD II	O43915

V-set and immunoglobulin domain-containing protein 2	VSIG2	CVD II	Q96IQ7
von Willebrand factor	VWF	CVD III	P04275
Lymphotactin	XCL1	CVD II	P47992

Legend: *CVII: cardiovascular II; CVIII: cardiovascular III; INF: inflammation panels; **UniProt ID from UniProt Knowledgebase

Supplemental Table 2. Study proteins order by p-value (from lowest to highest in the discovery set) in both discovery and replication phases

Protein name	Discovery (phase 1a)								Replication (phase 1b)							
	Adjusted for matching variables				Adjusted for matching variables and risk factors				Adjusted for matching variables				Adjusted for matching variables and risk factors			
	OR	(95% CI)	P-value	Z	OR	(95% CI)	P-value	Z	OR	(95% CI)	P-value	Z	OR	(95% CI)	P-value	Z
NTPROBNP	1.74	(1.46, 2.06)	<0.0001	6.3	1.79	(1.49, 2.14)	<0.0001	6.3	2	(1.64, 2.44)	<0.0001	6.9	2.06	(1.66, 2.54)	<0.0001	6.6
BNP	1.62	(1.42, 1.86)	<0.0001	7	1.59	(1.37, 1.84)	<0.0001	6.3	1.73	(1.50, 2.01)	<0.0001	7.3	1.77	(1.51, 2.07)	<0.0001	7.1
GDF15	2.74	(2.08, 3.61)	<0.0001	7.2	2.34	(1.72, 3.19)	<0.0001	5.4	1.89	(1.44, 2.47)	<0.0001	4.6	1.55	(1.15, 2.09)	0.0036	2.9
TRAILR2	3.26	(2.32, 4.60)	<0.0001	6.8	2.7	(1.86, 3.92)	<0.0001	5.2	3.22	(2.21, 4.69)	<0.0001	6.1	2.65	(1.72, 4.07)	<0.0001	4.4
CSTB	2.1	(1.67, 2.66)	<0.0001	6.2	1.84	(1.43, 2.37)	<0.0001	4.8	1.3	(1.07, 1.60)	0.0101	2.6	1.16	(0.93, 1.44)	0.1808	1.3
UPAR	2.7	(1.94, 3.75)	<0.0001	5.9	2.27	(1.61, 3.21)	<0.0001	4.6	1.67	(1.23, 2.28)	0.0011	3.3	1.38	(1.00, 1.92)	0.0519	1.9
VEGFA	2.65	(1.91, 3.67)	<0.0001	5.9	2.27	(1.59, 3.22)	<0.0001	4.5	1.52	(1.09, 2.11)	0.0129	2.5	1.27	(0.89, 1.80)	0.1837	1.3
HGF	2.49	(1.78, 3.48)	<0.0001	5.3	2.12	(1.50, 3.01)	<0.0001	4.2	1.5	(1.11, 2.02)	0.0077	2.7	1.29	(0.94, 1.77)	0.119	1.6
CXCL9	1.56	(1.30, 1.87)	<0.0001	4.7	1.52	(1.25, 1.84)	<0.0001	4.2	1.09	(0.89, 1.34)	0.3813	0.9	1.03	(0.83, 1.28)	0.7895	0.3
IL6	1.51	(1.29, 1.78)	<0.0001	5.1	1.42	(1.20, 1.68)	<0.0001	4.1	1.21	(1.03, 1.43)	0.021	2.3	1.09	(0.92, 1.30)	0.3325	1
OPN	2	(1.52, 2.63)	<0.0001	5	1.81	(1.36, 2.41)	<0.0001	4.1	1.5	(1.16, 1.95)	0.0021	3.1	1.36	(1.03, 1.79)	0.0298	2.2
ADM	2.56	(1.82, 3.59)	<0.0001	5.4	2.12	(1.48, 3.05)	<0.0001	4.1	1.8	(1.32, 2.45)	0.0002	3.7	1.41	(1.02, 1.96)	0.0389	2.1
CD40	2.26	(1.68, 3.04)	<0.0001	5.4	1.93	(1.41, 2.66)	<0.0001	4.1	1.27	(0.94, 1.72)	0.1228	1.5	1.05	(0.76, 1.46)	0.7743	0.3
MCP3	1.7	(1.35, 2.15)	<0.0001	4.5	1.61	(1.27, 2.05)	<0.0001	3.9	1.18	(0.89, 1.55)	0.2444	1.2	1.08	(0.81, 1.44)	0.6164	0.5
TFF3	1.61	(1.32, 1.95)	<0.0001	4.8	1.52	(1.23, 1.87)	0.0001	3.9	1.45	(1.15, 1.84)	0.0019	3.1	1.25	(0.96, 1.62)	0.0924	1.7
FABP4	1.82	(1.51, 2.19)	<0.0001	6.2	1.57	(1.25, 1.97)	0.0001	3.9	1.57	(1.29, 1.92)	<0.0001	4.5	1.28	(1.01, 1.61)	0.0383	2.1
OPG_CVD3	2.31	(1.63, 3.27)	<0.0001	4.7	2.05	(1.42, 2.95)	0.0001	3.9	1.45	(1.02, 2.06)	0.0385	2.1	1.25	(0.86, 1.82)	0.2381	1.2
CXCL11	1.34	(1.17, 1.55)	<0.0001	4.1	1.33	(1.15, 1.54)	0.0001	3.8	1.11	(0.96, 1.29)	0.1425	1.5	1.13	(0.96, 1.32)	0.1375	1.5
TNFRSF10A	2.63	(1.88, 3.69)	<0.0001	5.6	2.01	(1.40, 2.88)	0.0002	3.8	2.56	(1.74, 3.77)	<0.0001	4.8	1.98	(1.31, 3.01)	0.0013	3.2
TNFR1	2.4	(1.75, 3.28)	<0.0001	5.5	1.98	(1.38, 2.84)	0.0002	3.7	1.63	(1.23, 2.16)	0.0007	3.4	1.26	(0.92, 1.73)	0.1488	1.4
SPON1	2.25	(1.55, 3.26)	<0.0001	4.3	2.05	(1.40, 2.98)	0.0002	3.7	1.76	(1.16, 2.67)	0.0083	2.6	1.42	(0.91, 2.21)	0.1225	1.5
GAL9	2.96	(1.95, 4.49)	<0.0001	5.1	2.3	(1.48, 3.58)	0.0002	3.7	3.14	(2.01, 4.91)	<0.0001	5	2.59	(1.61, 4.19)	<0.0001	3.9
FGF23	1.7	(1.40, 2.07)	<0.0001	5.3	1.48	(1.20, 1.81)	0.0002	3.7	1.84	(1.44, 2.36)	<0.0001	4.8	1.69	(1.30, 2.20)	<0.0001	3.9
TNFRSF11A	2.09	(1.61, 2.72)	<0.0001	5.6	1.72	(1.28, 2.31)	0.0003	3.6	1.95	(1.47, 2.59)	<0.0001	4.6	1.53	(1.12, 2.11)	0.0085	2.6
IL2RA	1.74	(1.33, 2.28)	<0.0001	4.1	1.66	(1.25, 2.22)	0.0005	3.5	1.42	(1.09, 1.84)	0.0092	2.6	1.26	(0.94, 1.67)	0.1163	1.6
TNFR2	2.11	(1.58, 2.82)	<0.0001	5.1	1.75	(1.27, 2.42)	0.0007	3.4	1.55	(1.20, 2.00)	0.0008	3.3	1.29	(0.98, 1.71)	0.0743	1.8
RETN	1.82	(1.42, 2.33)	<0.0001	4.7	1.58	(1.21, 2.06)	0.0008	3.3	1.37	(1.05, 1.77)	0.0182	2.4	1.22	(0.92, 1.62)	0.1584	1.4
TNFRSF13B	1.74	(1.34, 2.24)	<0.0001	4.2	1.58	(1.21, 2.06)	0.0008	3.3	2.61	(1.82, 3.74)	<0.0001	5.2	2.33	(1.58, 3.44)	<0.0001	4.3
AGRP	1.82	(1.38, 2.40)	<0.0001	4.2	1.64	(1.23, 2.20)	0.0009	3.3	1.9	(1.31, 2.76)	0.0007	3.4	1.62	(1.08, 2.44)	0.0193	2.3
TNFRSF14	2.09	(1.55, 2.82)	<0.0001	4.9	1.76	(1.26, 2.46)	0.0009	3.3	1.52	(1.15, 2.01)	0.0034	2.9	1.26	(0.93, 1.71)	0.1394	1.5
GAL4	1.89	(1.47, 2.42)	<0.0001	5	1.57	(1.19, 2.05)	0.0012	3.2	1.44	(1.10, 1.87)	0.0071	2.7	1.18	(0.89, 1.57)	0.2553	1.1
RARRES2	3.33	(2.02, 5.51)	<0.0001	4.7	2.45	(1.42, 4.23)	0.0014	3.2	2.05	(1.38, 3.05)	0.0004	3.5	1.67	(1.09, 2.56)	0.0183	2.4
ACE2	1.6	(1.27, 2.02)	<0.0001	4	1.48	(1.16, 1.89)	0.0014	3.2	1.52	(1.17, 1.98)	0.0016	3.2	1.4	(1.06, 1.84)	0.0173	2.4
PLGF	2.17	(1.54, 3.06)	<0.0001	4.4	1.86	(1.27, 2.72)	0.0014	3.2	1.95	(1.38, 2.75)	0.0002	3.8	1.61	(1.11, 2.34)	0.0131	2.5
PGLYRP1	1.68	(1.31, 2.15)	<0.0001	4.1	1.53	(1.17, 1.98)	0.0016	3.2	1.36	(1.06, 1.74)	0.0151	2.4	1.2	(0.92, 1.56)	0.1893	1.3
TGFALPHA	1.9	(1.40, 2.59)	<0.0001	4.1	1.66	(1.21, 2.28)	0.0016	3.2	1.38	(1.07, 1.78)	0.0122	2.5	1.23	(0.94, 1.61)	0.1345	1.5
MMP12	1.64	(1.32, 2.03)	<0.0001	4.6	1.42	(1.13, 1.79)	0.0023	3	1.54	(1.23, 1.92)	0.0002	3.8	1.33	(1.04, 1.70)	0.0236	2.3
TIMP4	1.72	(1.32, 2.23)	<0.0001	4	1.54	(1.17, 2.04)	0.0024	3	1.2	(0.93, 1.53)	0.1568	1.4	1.08	(0.83, 1.41)	0.5465	0.6
SLAMF7	1.55	(1.26, 1.91)	<0.0001	4.1	1.41	(1.13, 1.76)	0.0024	3	2.16	(1.52, 3.06)	<0.0001	4.3	2	(1.38, 2.91)	0.0003	3.7
TNFRSF9	1.54	(1.25, 1.90)	<0.0001	4	1.41	(1.13, 1.76)	0.0025	3	1.33	(1.03, 1.72)	0.0318	2.1	1.15	(0.86, 1.53)	0.3411	1
IL1RA	1.54	(1.27, 1.86)	<0.0001	4.4	1.37	(1.11, 1.69)	0.003	3	1.25	(1.07, 1.46)	0.0048	2.8	1.17	(0.99, 1.39)	0.059	1.9
CCL16	1.47	(1.21, 1.79)	<0.0001	3.9	1.37	(1.11, 1.68)	0.003	3	1.72	(1.34, 2.22)	<0.0001	4.2	1.55	(1.18, 2.02)	0.0015	3.2
CD4	2.06	(1.49, 2.85)	<0.0001	4.3	1.67	(1.18, 2.35)	0.0035	2.9	2.17	(1.49, 3.16)	<0.0001	4	1.86	(1.25, 2.77)	0.0021	3.1

LTBR	1.93	(1.40, 2.68)	<0.0001	4	1.7	(1.18, 2.45)	0.004	2.9	1.42	(1.06, 1.90)	0.0203	2.3	1.22	(0.88, 1.68)	0.2287	1.2
IL15RA	2.12	(1.47, 3.05)	<0.0001	4	1.74	(1.18, 2.56)	0.0053	2.8	1.75	(1.07, 2.85)	0.0258	2.2	1.46	(0.86, 2.49)	0.1579	1.4
KIM1	1.41	(1.19, 1.68)	<0.0001	4	1.29	(1.07, 1.55)	0.0065	2.7	1.51	(1.23, 1.84)	<0.0001	4	1.31	(1.05, 1.62)	0.0159	2.4
PLC	2.26	(1.59, 3.21)	<0.0001	4.5	1.77	(1.17, 2.68)	0.0071	2.7	1.76	(1.24, 2.49)	0.0015	3.2	1.43	(0.97, 2.11)	0.0686	1.8
CEACAM8	1.51	(1.22, 1.86)	0.0001	3.8	1.47	(1.18, 1.83)	0.0006	3.4	1.49	(1.19, 1.86)	0.0005	3.5	1.36	(1.08, 1.73)	0.0098	2.6
ICAM2	1.91	(1.37, 2.66)	0.0001	3.8	1.73	(1.22, 2.44)	0.0019	3.1	1.16	(0.86, 1.57)	0.3262	1	1.06	(0.77, 1.45)	0.7229	0.4
EPHB4	1.96	(1.40, 2.76)	0.0001	3.9	1.62	(1.11, 2.37)	0.0121	2.5	1.38	(1.00, 1.91)	0.0499	2	1.19	(0.84, 1.69)	0.3334	1
HSP27	2.13	(1.42, 3.22)	0.0003	3.6	2.03	(1.32, 3.14)	0.0013	3.2	1.32	(1.02, 1.71)	0.0357	2.1	1.27	(0.97, 1.68)	0.0826	1.7
LAPTGFBETA1	1.68	(1.27, 2.22)	0.0003	3.6	1.58	(1.18, 2.12)	0.0021	3.1	1.15	(0.88, 1.49)	0.3102	1	1.11	(0.84, 1.47)	0.4747	0.7
PAR1	1.86	(1.33, 2.60)	0.0003	3.6	1.64	(1.16, 2.31)	0.0048	2.8	1.74	(1.24, 2.45)	0.0014	3.2	1.53	(1.07, 2.19)	0.0186	2.4
TWEAK	0.51	(0.36, 0.73)	0.0003	3.6	0.58	(0.39, 0.85)	0.0052	2.8	0.53	(0.39, 0.71)	<0.0001	4.1	0.58	(0.42, 0.80)	0.0008	3.4
CXCL16	1.95	(1.36, 2.79)	0.0003	3.6	1.66	(1.13, 2.46)	0.0101	2.6	1.29	(0.93, 1.81)	0.1317	1.5	1.12	(0.78, 1.60)	0.5478	0.6
JAMA	1.43	(1.18, 1.73)	0.0003	3.6	1.29	(1.06, 1.58)	0.0122	2.5	1.11	(0.94, 1.32)	0.2257	1.2	1.07	(0.90, 1.28)	0.4462	0.8
IL18BP	1.71	(1.28, 2.29)	0.0003	3.6	1.5	(1.09, 2.07)	0.013	2.5	1.39	(1.06, 1.83)	0.0192	2.3	1.18	(0.87, 1.59)	0.2842	1.1
CCL15	1.51	(1.21, 1.88)	0.0003	3.6	1.29	(1.00, 1.65)	0.0463	2	1.39	(1.08, 1.79)	0.0117	2.5	1.18	(0.89, 1.57)	0.238	1.2
CHI3L1	1.33	(1.14, 1.56)	0.0004	3.5	1.25	(1.06, 1.47)	0.0086	2.6	1.2	(1.01, 1.42)	0.0352	2.1	1.08	(0.90, 1.29)	0.4317	0.8
CSF1	2.27	(1.43, 3.61)	0.0005	3.5	1.91	(1.18, 3.09)	0.0088	2.6	1.37	(0.78, 2.42)	0.2774	1.1	1.05	(0.57, 1.93)	0.8707	0.2
PDL1	1.64	(1.24, 2.16)	0.0005	3.5	1.47	(1.09, 1.98)	0.0107	2.6	1.3	(0.93, 1.81)	0.1215	1.5	0.96	(0.67, 1.39)	0.8473	0.2
PDL2	1.96	(1.34, 2.87)	0.0005	3.5	1.68	(1.13, 2.50)	0.011	2.5	1.77	(1.18, 2.65)	0.006	2.7	1.62	(1.05, 2.50)	0.0287	2.2
FAS	1.78	(1.29, 2.46)	0.0005	3.5	1.49	(1.06, 2.09)	0.0223	2.3	1.52	(1.10, 2.11)	0.012	2.5	1.29	(0.90, 1.84)	0.1601	1.4
GRN	2.09	(1.38, 3.16)	0.0005	3.5	1.66	(1.07, 2.57)	0.0239	2.3	1.41	(0.96, 2.08)	0.0811	1.7	1.16	(0.76, 1.75)	0.4918	0.7
SLAMF1	1.44	(1.17, 1.76)	0.0005	3.5	1.27	(1.02, 1.58)	0.0293	2.2	1.62	(1.23, 2.13)	0.0005	3.5	1.42	(1.06, 1.89)	0.0175	2.4
CTSZ	1.74	(1.27, 2.39)	0.0005	3.5	1.44	(1.03, 2.02)	0.0321	2.1	1.32	(0.97, 1.78)	0.0748	1.8	1.09	(0.79, 1.52)	0.5884	0.5
IGFBP7	1.66	(1.24, 2.23)	0.0006	3.4	1.42	(1.04, 1.94)	0.0259	2.2	1.6	(1.21, 2.11)	0.001	3.3	1.42	(1.05, 1.91)	0.021	2.3
IL16	1.54	(1.20, 1.97)	0.0007	3.4	1.38	(1.06, 1.79)	0.016	2.4	1.46	(1.15, 1.86)	0.0019	3.1	1.34	(1.04, 1.72)	0.0232	2.3
CCL25	1.51	(1.18, 1.94)	0.0009	3.3	1.35	(1.04, 1.75)	0.0234	2.3	0.96	(0.72, 1.29)	0.8091	0.2	0.85	(0.62, 1.17)	0.3243	1
IL12B	1.4	(1.14, 1.72)	0.0011	3.3	1.3	(1.05, 1.62)	0.0173	2.4	1.25	(1.00, 1.56)	0.0476	2	1.12	(0.88, 1.43)	0.3453	0.9
PON3	0.71	(0.58, 0.87)	0.0011	3.3	0.83	(0.66, 1.04)	0.0977	1.7	0.64	(0.51, 0.80)	<0.0001	3.9	0.72	(0.56, 0.91)	0.0064	2.7
IGFBP1	1.26	(1.09, 1.45)	0.0013	3.2	1.41	(1.19, 1.66)	<0.0001	4.1	1.08	(0.92, 1.26)	0.3564	0.9	1.14	(0.95, 1.37)	0.153	1.4
CCL23	1.6	(1.20, 2.14)	0.0014	3.2	1.74	(1.28, 2.37)	0.0005	3.5	1.08	(0.79, 1.47)	0.6256	0.5	0.98	(0.70, 1.37)	0.9144	0.1
DECR1	1.19	(1.07, 1.33)	0.0014	3.2	1.17	(1.04, 1.31)	0.0085	2.6	1.03	(0.93, 1.15)	0.5695	0.6	1.02	(0.91, 1.15)	0.7041	0.4
SPON2	2.86	(1.50, 5.48)	0.0015	3.2	2.66	(1.36, 5.21)	0.0044	2.8	2.4	(1.36, 4.25)	0.0026	3	2.1	(1.16, 3.79)	0.0137	2.5
TM	1.9	(1.27, 2.82)	0.0016	3.2	1.63	(1.07, 2.48)	0.0233	2.3	1.5	(1.03, 2.19)	0.0354	2.1	1.24	(0.85, 1.82)	0.2637	1.1
REN	1.31	(1.11, 1.55)	0.0018	3.1	1.18	(0.98, 1.41)	0.0772	1.8	1.54	(1.29, 1.85)	<0.0001	4.7	1.38	(1.13, 1.68)	0.0014	3.2
MMP3	1.4	(1.13, 1.74)	0.0021	3.1	1.37	(1.09, 1.73)	0.0062	2.7	1.17	(0.94, 1.45)	0.1521	1.4	1.05	(0.83, 1.33)	0.673	0.4
CASP8	1.26	(1.09, 1.47)	0.0021	3.1	1.22	(1.04, 1.43)	0.0126	2.5	1.19	(1.03, 1.37)	0.0148	2.4	1.18	(1.02, 1.37)	0.0286	2.2
TLT2	1.49	(1.16, 1.93)	0.0021	3.1	1.39	(1.06, 1.82)	0.0169	2.4	1.06	(0.80, 1.40)	0.6846	0.4	0.98	(0.73, 1.32)	0.9002	0.1
MMP2	1.64	(1.19, 2.24)	0.0023	3	1.55	(1.11, 2.16)	0.0096	2.6	1.38	(1.01, 1.88)	0.0452	2	1.38	(0.99, 1.91)	0.0553	1.9
IL10RB	1.77	(1.22, 2.56)	0.0024	3	1.56	(1.06, 2.30)	0.0247	2.2	1.27	(0.83, 1.92)	0.2708	1.1	0.92	(0.58, 1.46)	0.7309	0.3
GAL3	1.52	(1.16, 2.00)	0.0025	3	1.44	(1.08, 1.92)	0.012	2.5	1.17	(0.89, 1.54)	0.2714	1.1	1.02	(0.76, 1.37)	0.887	0.1
CD163	1.46	(1.14, 1.87)	0.0025	3	1.35	(1.05, 1.75)	0.0198	2.3	1.07	(0.84, 1.35)	0.581	0.6	0.99	(0.77, 1.27)	0.9236	0.1
VSIG2	1.43	(1.14, 1.81)	0.0025	3	1.24	(0.96, 1.59)	0.0967	1.7	1.76	(1.33, 2.32)	<0.0001	4	1.46	(1.06, 2.01)	0.02	2.3
TR	1.37	(1.12, 1.69)	0.0028	3	1.29	(1.04, 1.61)	0.0202	2.3	1.24	(0.99, 1.55)	0.0663	1.8	1.12	(0.88, 1.43)	0.3645	0.9
CCL20	1.21	(1.07, 1.38)	0.003	3	1.17	(1.02, 1.33)	0.0223	2.3	1.06	(0.94, 1.21)	0.3405	1	1.03	(0.90, 1.18)	0.6877	0.4
NEMO	1.19	(1.06, 1.34)	0.0032	2.9	1.16	(1.02, 1.31)	0.0224	2.3	1.07	(0.93, 1.23)	0.3387	1	1.04	(0.90, 1.21)	0.5799	0.6
IL4RA*	1.52	(1.15, 2.02)	0.0033	2.9	1.42	(1.06, 1.90)	0.0171	2.4	1.62	(1.18, 2.22)	0.0029	3	1.45	(1.03, 2.03)	0.0309	2.2
MERTK	1.66	(1.18, 2.33)	0.0038	2.9	1.6	(1.12, 2.28)	0.0101	2.6	1.38	(0.96, 1.99)	0.0863	1.7	1.29	(0.87, 1.91)	0.2016	1.3
IL17C	1.41	(1.12, 1.77)	0.0038	2.9	1.29	(1.01, 1.65)	0.0398	2.1	1.07	(0.80, 1.44)	0.6416	0.5	1.02	(0.75, 1.39)	0.8845	0.1

CXCL10	1.28	(1.08, 1.52)	0.0042	2.9	1.26	(1.05, 1.51)	0.0116	2.5	1.05	(0.88, 1.25)	0.5963	0.5	1.08	(0.89, 1.29)	0.437	0.8
ENRAGE	1.26	(1.08, 1.47)	0.0042	2.9	1.23	(1.04, 1.46)	0.0137	2.5	1.54	(1.25, 1.90)	<0.0001	4	1.44	(1.15, 1.81)	0.0016	3.2
BETANGF	1.66	(1.16, 2.36)	0.005	2.8	1.38	(0.95, 2.00)	0.0917	1.7	1.15	(0.77, 1.72)	0.4889	0.7	1.06	(0.70, 1.62)	0.7691	0.3
LOX1	1.39	(1.10, 1.76)	0.0054	2.8	1.36	(1.07, 1.74)	0.0129	2.5	1.49	(1.19, 1.86)	0.0005	3.5	1.46	(1.15, 1.86)	0.0018	3.1
MMP10	1.34	(1.09, 1.66)	0.0055	2.8	1.25	(1.00, 1.55)	0.0463	2	0.88	(0.68, 1.14)	0.3391	1	0.76	(0.57, 1.00)	0.0518	1.9
PECAM1	1.51	(1.13, 2.01)	0.0058	2.8	1.41	(1.04, 1.91)	0.0267	2.2	1.05	(0.81, 1.35)	0.7106	0.4	1.06	(0.81, 1.38)	0.6884	0.4
IL10	1.35	(1.09, 1.67)	0.0062	2.7	1.31	(1.05, 1.63)	0.0161	2.4	1.03	(0.84, 1.27)	0.7601	0.3	0.96	(0.78, 1.20)	0.742	0.3
LIFR	1.68	(1.16, 2.45)	0.0067	2.7	1.44	(0.97, 2.14)	0.0679	1.8	1.15	(0.75, 1.76)	0.5178	0.6	0.94	(0.60, 1.49)	0.7975	0.3
HOSCAR	2.11	(1.23, 3.64)	0.0069	2.7	1.93	(1.09, 3.39)	0.023	2.3	1.93	(1.12, 3.32)	0.0175	2.4	1.68	(0.95, 2.97)	0.0764	1.8
IGFBP2	1.35	(1.08, 1.68)	0.0072	2.7	1.55	(1.21, 1.99)	0.0005	3.5	1.25	(0.99, 1.58)	0.0628	1.9	1.35	(1.03, 1.77)	0.0298	2.2
CDCP1	1.34	(1.08, 1.66)	0.0074	2.7	1.26	(1.01, 1.59)	0.0431	2	0.98	(0.76, 1.26)	0.8736	0.2	0.85	(0.65, 1.11)	0.2395	1.2
MCP2	1.36	(1.09, 1.70)	0.0075	2.7	1.35	(1.07, 1.71)	0.0127	2.5	1.13	(0.91, 1.41)	0.2737	1.1	1.11	(0.88, 1.41)	0.3724	0.9
CX3CL1	1.48	(1.11, 1.97)	0.0075	2.7	1.3	(0.96, 1.77)	0.0926	1.7	1.18	(0.87, 1.60)	0.2787	1.1	1.01	(0.72, 1.41)	0.9603	0
PRTN3	1.35	(1.08, 1.68)	0.0081	2.6	1.3	(1.04, 1.64)	0.0238	2.3	1.26	(0.99, 1.59)	0.0557	1.9	1.18	(0.92, 1.51)	0.1911	1.3
AXIN1	1.16	(1.04, 1.29)	0.0085	2.6	1.13	(1.01, 1.26)	0.0403	2.1	0.96	(0.84, 1.10)	0.5615	0.6	0.94	(0.81, 1.09)	0.4114	0.8
MPO	1.43	(1.09, 1.86)	0.009	2.6	1.36	(1.03, 1.79)	0.0323	2.1	1.17	(0.90, 1.51)	0.2337	1.2	1.09	(0.83, 1.43)	0.524	0.6
GT	1.27	(1.06, 1.53)	0.0101	2.6	1.19	(0.97, 1.44)	0.088	1.7	1.3	(1.02, 1.67)	0.037	2.1	1.26	(0.96, 1.65)	0.092	1.7
SORT1	1.93	(1.17, 3.18)	0.0103	2.6	1.99	(1.17, 3.38)	0.0107	2.6	1.68	(1.08, 2.62)	0.0218	2.3	1.8	(1.12, 2.89)	0.0149	2.4
ST1A1	1.16	(1.04, 1.31)	0.0103	2.6	1.13	(1.00, 1.27)	0.0593	1.9	1.02	(0.91, 1.15)	0.7111	0.4	1.01	(0.89, 1.15)	0.8863	0.1
CCL11	1.45	(1.09, 1.93)	0.0104	2.6	1.41	(1.05, 1.91)	0.0242	2.3	1.46	(1.06, 1.99)	0.019	2.3	1.36	(0.97, 1.91)	0.0734	1.8
CST5	1.35	(1.07, 1.70)	0.0113	2.5	1.19	(0.92, 1.54)	0.1858	1.3	1.01	(0.76, 1.35)	0.9334	0.1	0.85	(0.62, 1.18)	0.3325	1
SELP	1.33	(1.07, 1.67)	0.0121	2.5	1.26	(1.00, 1.60)	0.0529	1.9	1.12	(0.91, 1.37)	0.3031	1	1.11	(0.89, 1.39)	0.3584	0.9
BP1_4E	1.17	(1.04, 1.33)	0.0121	2.5	1.14	(1.00, 1.30)	0.0541	1.9	1.17	(1.02, 1.34)	0.0254	2.2	1.12	(0.97, 1.29)	0.135	1.5
SIRT2	1.13	(1.03, 1.24)	0.0131	2.5	1.11	(1.00, 1.22)	0.049	2	1.05	(0.94, 1.17)	0.3818	0.9	1.03	(0.92, 1.15)	0.6376	0.5
ST2	1.37	(1.07, 1.75)	0.0131	2.5	1.25	(0.97, 1.62)	0.0861	1.7	1.42	(1.09, 1.83)	0.0083	2.6	1.33	(1.01, 1.76)	0.0395	2.1
CASP3	1.15	(1.03, 1.28)	0.0133	2.5	1.12	(1.00, 1.26)	0.0486	2	1.04	(0.93, 1.17)	0.4996	0.7	1.02	(0.90, 1.16)	0.7465	0.3
PRSS8	1.62	(1.11, 2.38)	0.0133	2.5	1.39	(0.93, 2.07)	0.1063	1.6	1.3	(1.04, 1.63)	0.0193	2.3	1.21	(0.96, 1.53)	0.1148	1.6
VWF	1.18	(1.04, 1.35)	0.0135	2.5	1.15	(1.00, 1.32)	0.0539	1.9	1.18	(1.03, 1.36)	0.0153	2.4	1.17	(1.01, 1.35)	0.0347	2.1
FGF21	1.15	(1.03, 1.28)	0.0139	2.5	1.08	(0.96, 1.21)	0.2096	1.3	1.15	(1.03, 1.30)	0.0161	2.4	1.07	(0.95, 1.22)	0.272	1.1
TGM2	1.26	(1.05, 1.51)	0.0144	2.4	1.22	(1.01, 1.48)	0.0383	2.1	1.43	(1.18, 1.73)	0.0003	3.6	1.55	(1.25, 1.91)	<0.0001	4
IL1RT1	1.58	(1.09, 2.28)	0.0145	2.4	1.39	(0.94, 2.06)	0.0991	1.6	1.18	(0.86, 1.62)	0.3017	1	1.07	(0.76, 1.49)	0.704	0.4
CD244	1.48	(1.08, 2.04)	0.0154	2.4	1.36	(0.97, 1.90)	0.0737	1.8	0.79	(0.55, 1.12)	0.1841	1.3	0.7	(0.48, 1.02)	0.0603	1.9
MCP1_CVD3	1.27	(1.05, 1.55)	0.0162	2.4	1.23	(1.02, 1.49)	0.0308	2.2	1.14	(0.91, 1.43)	0.265	1.1	1.09	(0.86, 1.38)	0.4702	0.7
CD93	1.6	(1.09, 2.35)	0.0163	2.4	1.38	(0.91, 2.09)	0.1272	1.5	1.62	(1.12, 2.35)	0.0107	2.6	1.44	(0.96, 2.15)	0.0793	1.8
PCSK9	1.43	(1.07, 1.93)	0.0171	2.4	1.32	(0.97, 1.79)	0.0791	1.8	1	(0.74, 1.36)	0.982	0	0.91	(0.66, 1.25)	0.5486	0.6
PI3	1.27	(1.04, 1.54)	0.0174	2.4	1.08	(0.87, 1.35)	0.4985	0.7	1.21	(1.01, 1.45)	0.0384	2.1	1.09	(0.90, 1.32)	0.3972	0.8
IL6RA	1.5	(1.07, 2.10)	0.0192	2.3	1.58	(1.10, 2.25)	0.0126	2.5	1.21	(0.89, 1.63)	0.228	1.2	1.17	(0.85, 1.61)	0.3472	0.9
VEGFD	1.52	(1.07, 2.17)	0.0205	2.3	1.75	(1.19, 2.58)	0.0045	2.8	1.81	(1.29, 2.54)	0.0007	3.4	1.98	(1.36, 2.89)	0.0004	3.6
TNFSF14	1.28	(1.04, 1.57)	0.0212	2.3	1.22	(0.98, 1.52)	0.0795	1.8	1.14	(0.94, 1.38)	0.1775	1.3	1.17	(0.96, 1.44)	0.1236	1.5
ITGB1BP2	1.11	(1.02, 1.21)	0.0213	2.3	1.09	(0.99, 1.19)	0.0842	1.7	1.03	(0.93, 1.14)	0.5949	0.5	1.01	(0.90, 1.12)	0.9105	0.1
FABP2	1.27	(1.03, 1.56)	0.0223	2.3	1.2	(0.97, 1.50)	0.0998	1.6	1.32	(1.06, 1.63)	0.0119	2.5	1.19	(0.95, 1.49)	0.1356	1.5
BLMH	1.44	(1.05, 1.96)	0.0224	2.3	1.5	(1.08, 2.09)	0.0154	2.4	0.79	(0.58, 1.07)	0.1287	1.5	0.83	(0.60, 1.15)	0.2558	1.1
SRC	1.19	(1.02, 1.38)	0.0231	2.3	1.15	(0.99, 1.35)	0.0733	1.8	1.07	(0.92, 1.26)	0.3867	0.9	1.03	(0.87, 1.21)	0.7691	0.3
IL8	1.2	(1.03, 1.40)	0.0232	2.3	1.18	(1.00, 1.39)	0.0508	2	1.13	(0.94, 1.36)	0.2025	1.3	1.1	(0.90, 1.33)	0.3563	0.9
SELE	1.37	(1.04, 1.79)	0.0239	2.3	1.13	(0.85, 1.51)	0.409	0.8	1.31	(0.99, 1.75)	0.0624	1.9	1.18	(0.87, 1.60)	0.2917	1.1
LEP	1.17	(1.02, 1.35)	0.0246	2.2	1.01	(0.84, 1.21)	0.9082	0.1	1.22	(1.04, 1.43)	0.0148	2.4	1.03	(0.83, 1.28)	0.7984	0.3
PSPD	1.22	(1.02, 1.46)	0.0262	2.2	1.27	(1.05, 1.54)	0.0122	2.5	1.29	(1.03, 1.61)	0.0236	2.3	1.32	(1.05, 1.67)	0.0194	2.3
IL18R1	1.42	(1.04, 1.94)	0.0266	2.2	1.2	(0.86, 1.66)	0.2856	1.1	1.2	(0.84, 1.72)	0.3199	1	0.96	(0.65, 1.41)	0.8428	0.2

SOD2	2.01	(1.08, 3.73)	0.0274	2.2	2.3	(1.20, 4.42)	0.0122	2.5	1.46	(0.87, 2.45)	0.1539	1.4	1.53	(0.88, 2.66)	0.1353	1.5
NT3	1.37	(1.04, 1.82)	0.0274	2.2	1.29	(0.96, 1.74)	0.0929	1.7	0.95	(0.74, 1.23)	0.7178	0.4	1	(0.76, 1.30)	0.986	0
BMP6	1.23	(1.02, 1.47)	0.0278	2.2	1.17	(0.97, 1.42)	0.1076	1.6	1.09	(0.91, 1.31)	0.3624	0.9	1.08	(0.88, 1.31)	0.4708	0.7
APN	1.53	(1.05, 2.23)	0.0279	2.2	1.37	(0.92, 2.04)	0.1161	1.6	0.96	(0.68, 1.34)	0.7958	0.3	0.92	(0.64, 1.32)	0.6636	0.4
STK4	1.13	(1.01, 1.26)	0.0285	2.2	1.1	(0.98, 1.24)	0.0908	1.7	1.06	(0.94, 1.20)	0.3107	1	1.04	(0.92, 1.18)	0.5008	0.7
AMBP	1.98	(1.07, 3.64)	0.0285	2.2	1.63	(0.85, 3.12)	0.1424	1.5	2.48	(1.35, 4.55)	0.0034	2.9	1.86	(0.97, 3.53)	0.06	1.9
IL27	1.46	(1.04, 2.05)	0.0303	2.2	1.57	(1.10, 2.26)	0.014	2.5	1.83	(1.22, 2.74)	0.0036	2.9	1.77	(1.15, 2.72)	0.0095	2.6
CTSL1	1.49	(1.04, 2.13)	0.0314	2.2	1.51	(1.03, 2.20)	0.0331	2.1	1.31	(0.95, 1.79)	0.0972	1.7	1.26	(0.90, 1.76)	0.1822	1.3
TNFSF13B	1.45	(1.03, 2.05)	0.0322	2.1	1.35	(0.94, 1.94)	0.1018	1.6	1.04	(0.76, 1.42)	0.819	0.2	1.04	(0.75, 1.44)	0.8279	0.2
MMP1	1.13	(1.01, 1.28)	0.0346	2.1	1.1	(0.97, 1.24)	0.1339	1.5	1.1	(0.96, 1.25)	0.158	1.4	1.06	(0.92, 1.22)	0.398	0.8
SHPS1	1.38	(1.02, 1.86)	0.0347	2.1	1.14	(0.83, 1.58)	0.4173	0.8	1.19	(0.91, 1.54)	0.1971	1.3	1.07	(0.81, 1.42)	0.6111	0.5
MMP7	1.18	(1.01, 1.39)	0.0352	2.1	1.12	(0.95, 1.32)	0.1941	1.3	1.1	(0.96, 1.25)	0.1669	1.4	1.1	(0.96, 1.26)	0.1809	1.3
IL18	1.21	(1.01, 1.45)	0.0366	2.1	1.16	(0.97, 1.41)	0.1113	1.6	1.27	(1.07, 1.51)	0.0074	2.7	1.24	(1.03, 1.49)	0.0239	2.3
STAMPB	1.14	(1.01, 1.29)	0.0367	2.1	1.11	(0.97, 1.26)	0.1225	1.5	1.03	(0.89, 1.20)	0.6566	0.4	1	(0.86, 1.18)	0.9529	0.1
TF	1.44	(1.02, 2.03)	0.0385	2.1	1.24	(0.86, 1.78)	0.2487	1.2	1.55	(1.05, 2.31)	0.0285	2.2	1.35	(0.89, 2.05)	0.159	1.4
ITGB2	1.38	(1.02, 1.87)	0.0387	2.1	1.31	(0.95, 1.81)	0.0988	1.7	1.02	(0.79, 1.31)	0.8783	0.2	1.01	(0.77, 1.33)	0.9216	0.1
OSM	1.19	(1.01, 1.41)	0.0395	2.1	1.18	(0.99, 1.41)	0.0644	1.8	1.19	(1.01, 1.40)	0.0334	2.1	1.14	(0.96, 1.36)	0.1331	1.5
XCL1	1.26	(1.01, 1.57)	0.0403	2.1	1.18	(0.94, 1.49)	0.1584	1.4	1.33	(1.04, 1.70)	0.0235	2.3	1.26	(0.97, 1.64)	0.0808	1.7
CCL19	1.16	(1.01, 1.34)	0.0403	2.1	1.08	(0.93, 1.26)	0.3193	1	1.23	(1.04, 1.47)	0.0164	2.4	1.21	(1.01, 1.45)	0.0414	2
DKK1	1.27	(1.01, 1.61)	0.0443	2	1.27	(0.99, 1.63)	0.0579	1.9	1.19	(0.97, 1.46)	0.0952	1.7	1.21	(0.97, 1.51)	0.091	1.7
ALCAM	1.42	(1.00, 2.02)	0.0486	2	1.33	(0.92, 1.92)	0.126	1.5	1.1	(0.79, 1.52)	0.5788	0.6	1.03	(0.73, 1.46)	0.8773	0.2
PTX3	1.25	(0.99, 1.58)	0.0597	1.9	1.24	(0.97, 1.59)	0.0816	1.7	1.31	(0.97, 1.78)	0.0778	1.8	1.43	(1.03, 1.97)	0.0302	2.2
IL17D	1.38	(0.99, 1.94)	0.06	1.9	1.37	(0.96, 1.95)	0.0816	1.7	1.22	(0.79, 1.89)	0.3631	0.9	1.28	(0.80, 2.03)	0.3057	1
HGDNF	1.3	(0.98, 1.72)	0.0687	1.8	1.05	(0.78, 1.43)	0.7318	0.3	1.17	(0.80, 1.72)	0.4178	0.8	1.02	(0.68, 1.54)	0.9082	0.1
CXCL6	1.17	(0.99, 1.38)	0.069	1.8	1.18	(0.99, 1.40)	0.0677	1.8	0.99	(0.84, 1.18)	0.9326	0.1	1.01	(0.84, 1.21)	0.9013	0.1
FGF19	1.15	(0.99, 1.35)	0.0722	1.8	1.14	(0.97, 1.35)	0.1164	1.6	0.93	(0.78, 1.10)	0.387	0.9	0.89	(0.74, 1.08)	0.2396	1.2
IL17A	1.25	(0.98, 1.61)	0.0779	1.8	1.18	(0.91, 1.53)	0.2225	1.2	0.93	(0.67, 1.30)	0.6714	0.4	0.94	(0.67, 1.33)	0.7287	0.3
IGGFCRECEPTORIIB	1.23	(0.98, 1.54)	0.0784	1.8	1.25	(0.98, 1.58)	0.0672	1.8	1.18	(0.86, 1.60)	0.3064	1	1.13	(0.81, 1.57)	0.466	0.7
PARP1	1.13	(0.99, 1.29)	0.0805	1.7	1.1	(0.95, 1.27)	0.2035	1.3	1.16	(1.00, 1.36)	0.0535	1.9	1.19	(1.00, 1.40)	0.0435	2
IDUA	1.26	(0.97, 1.64)	0.0834	1.7	1.25	(0.95, 1.64)	0.1091	1.6	1.03	(0.82, 1.30)	0.7905	0.3	0.94	(0.74, 1.21)	0.6409	0.5
PDGFSUBUNITB	1.12	(0.98, 1.27)	0.0855	1.7	1.13	(0.99, 1.29)	0.0757	1.8	1.09	(0.94, 1.27)	0.2374	1.2	1.09	(0.93, 1.28)	0.2947	1
CD40L	1.1	(0.99, 1.23)	0.0899	1.7	1.09	(0.97, 1.23)	0.1281	1.5	1.07	(0.96, 1.20)	0.2244	1.2	1.09	(0.97, 1.23)	0.1406	1.5
TNFRSF10C	1.26	(0.96, 1.64)	0.0925	1.7	1.09	(0.83, 1.45)	0.5284	0.6	1.22	(0.94, 1.59)	0.1351	1.5	1.12	(0.85, 1.48)	0.4103	0.8
CTSD	1.28	(0.96, 1.70)	0.0931	1.7	1.06	(0.78, 1.43)	0.7145	0.4	1.06	(0.81, 1.38)	0.6892	0.4	0.93	(0.70, 1.24)	0.6154	0.5
NOTCH3	1.3	(0.96, 1.75)	0.0936	1.7	1.17	(0.85, 1.61)	0.3379	1	1.1	(0.82, 1.49)	0.5157	0.7	1.1	(0.80, 1.51)	0.5684	0.6
MB	1.21	(0.97, 1.50)	0.0947	1.7	1.02	(0.80, 1.30)	0.894	0.1	0.98	(0.78, 1.22)	0.8252	0.2	0.85	(0.66, 1.09)	0.1947	1.3
CD5	1.25	(0.96, 1.63)	0.0956	1.7	1.11	(0.84, 1.48)	0.4591	0.7	1.28	(0.91, 1.79)	0.1504	1.4	1.08	(0.75, 1.55)	0.6754	0.4
TPA	1.21	(0.97, 1.50)	0.0975	1.7	1.11	(0.88, 1.41)	0.3743	0.9	0.99	(0.82, 1.20)	0.9333	0.1	0.96	(0.78, 1.17)	0.6772	0.4
PAPPA	1.22	(0.96, 1.55)	0.0995	1.6	1.28	(1.00, 1.65)	0.0535	1.9	1.15	(0.88, 1.50)	0.2964	1	1.32	(0.99, 1.75)	0.0579	1.9
COL1A1	1.29	(0.95, 1.75)	0.0996	1.6	1.11	(0.81, 1.54)	0.5109	0.7	1.16	(0.84, 1.61)	0.3769	0.9	1.1	(0.78, 1.56)	0.5765	0.6
IL7	1.17	(0.97, 1.41)	0.1021	1.6	1.19	(0.98, 1.45)	0.086	1.7	0.96	(0.78, 1.17)	0.6572	0.4	0.96	(0.78, 1.19)	0.7271	0.3
CCL4	1.16	(0.96, 1.40)	0.1143	1.6	1.14	(0.94, 1.39)	0.1714	1.4	0.98	(0.81, 1.19)	0.8406	0.2	0.96	(0.78, 1.18)	0.7178	0.4
SCF	0.73	(0.50, 1.08)	0.115	1.6	0.73	(0.48, 1.09)	0.1228	1.5	1.3	(0.93, 1.82)	0.1216	1.5	1.25	(0.88, 1.77)	0.2118	1.2
CA5A	1.13	(0.97, 1.31)	0.1184	1.6	0.99	(0.84, 1.16)	0.8755	0.2	1.08	(0.90, 1.31)	0.4065	0.8	0.95	(0.77, 1.16)	0.6048	0.5
PRELP	1.58	(0.89, 2.82)	0.1185	1.6	1.36	(0.74, 2.49)	0.321	1	3.17	(1.55, 6.49)	0.0016	3.2	2.79	(1.31, 5.94)	0.008	2.7
FS	1.29	(0.93, 1.78)	0.1237	1.5	1.24	(0.88, 1.73)	0.2173	1.2	1.4	(1.02, 1.92)	0.039	2.1	1.37	(0.98, 1.91)	0.0637	1.9
CCL17	1.11	(0.97, 1.28)	0.1284	1.5	1.12	(0.97, 1.29)	0.1299	1.5	1.2	(1.04, 1.38)	0.0144	2.4	1.22	(1.04, 1.42)	0.0131	2.5
HBEGF	1.27	(0.92, 1.74)	0.1412	1.5	1.25	(0.90, 1.75)	0.1831	1.3	1.29	(0.99, 1.67)	0.0569	1.9	1.34	(1.01, 1.77)	0.04	2.1

ADA	1.21	(0.93, 1.57)	0.1589	1.4	1.11	(0.85, 1.46)	0.4453	0.8	0.84	(0.62, 1.13)	0.2553	1.1	0.83	(0.61, 1.14)	0.256	1.1
MMP9	1.12	(0.96, 1.30)	0.1601	1.4	1.11	(0.95, 1.31)	0.1942	1.3	1.21	(1.04, 1.41)	0.0135	2.5	1.18	(1.00, 1.39)	0.048	2
PAI	1.12	(0.96, 1.31)	0.1612	1.4	1.07	(0.91, 1.27)	0.4117	0.8	1.02	(0.87, 1.21)	0.7794	0.3	0.99	(0.83, 1.18)	0.9022	0.1
GIF	0.92	(0.81, 1.04)	0.165	1.4	0.94	(0.83, 1.07)	0.3446	0.9	1.1	(0.96, 1.27)	0.1642	1.4	1.12	(0.97, 1.30)	0.1313	1.5
FGF5	1.33	(0.89, 1.97)	0.1654	1.4	1.15	(0.76, 1.75)	0.5134	0.7	0.95	(0.61, 1.50)	0.8408	0.2	0.88	(0.55, 1.43)	0.6127	0.5
FLT3L	1.23	(0.92, 1.66)	0.1682	1.4	1.18	(0.86, 1.61)	0.2983	1	1.05	(0.76, 1.45)	0.7568	0.3	0.96	(0.68, 1.35)	0.82	0.2
DCN	1.28	(0.90, 1.82)	0.1728	1.4	1.15	(0.79, 1.67)	0.4694	0.7	1.43	(1.02, 2.02)	0.0391	2.1	1.33	(0.93, 1.91)	0.1236	1.5
CHIT1	1.08	(0.97, 1.20)	0.1769	1.4	1.08	(0.96, 1.20)	0.1908	1.3	1.01	(0.91, 1.12)	0.8861	0.1	1.01	(0.90, 1.13)	0.8693	0.2
CPB1	1.13	(0.94, 1.36)	0.18	1.3	1.1	(0.90, 1.34)	0.3452	0.9	1.15	(0.95, 1.40)	0.1547	1.4	1.1	(0.89, 1.36)	0.4001	0.8
CCL3	1.1	(0.95, 1.27)	0.1882	1.3	1.05	(0.90, 1.22)	0.5275	0.6	1.13	(0.91, 1.39)	0.2599	1.1	1.05	(0.84, 1.30)	0.6787	0.4
CD84	1.23	(0.90, 1.67)	0.1894	1.3	1.28	(0.93, 1.77)	0.1285	1.5	1.12	(0.81, 1.55)	0.5086	0.7	1.17	(0.83, 1.66)	0.3731	0.9
UPA_CVD3	1.22	(0.90, 1.65)	0.1925	1.3	1.2	(0.88, 1.63)	0.251	1.1	1.05	(0.75, 1.47)	0.7792	0.3	1.06	(0.74, 1.53)	0.7324	0.3
AXL	1.23	(0.90, 1.70)	0.1933	1.3	1.15	(0.82, 1.62)	0.4106	0.8	1.14	(0.85, 1.54)	0.388	0.9	1.08	(0.79, 1.48)	0.6297	0.5
TFPI	1.26	(0.87, 1.82)	0.2131	1.2	1.25	(0.85, 1.84)	0.2569	1.1	0.98	(0.71, 1.35)	0.8866	0.1	0.96	(0.68, 1.35)	0.8106	0.2
SCGB3A2	1.09	(0.95, 1.26)	0.216	1.2	1.09	(0.94, 1.27)	0.2515	1.1	1.04	(0.89, 1.22)	0.6438	0.5	0.99	(0.83, 1.17)	0.8647	0.2
ANG1	1.08	(0.95, 1.24)	0.2223	1.2	1.09	(0.95, 1.25)	0.2388	1.2	1.06	(0.92, 1.21)	0.4174	0.8	1.08	(0.93, 1.26)	0.302	1
EGFR	0.77	(0.50, 1.18)	0.2258	1.2	0.83	(0.53, 1.31)	0.4258	0.8	0.77	(0.53, 1.10)	0.1504	1.4	0.8	(0.55, 1.18)	0.2607	1.1
DNER	0.8	(0.56, 1.15)	0.2294	1.2	0.85	(0.58, 1.25)	0.4125	0.8	0.41	(0.25, 0.67)	0.0003	3.6	0.43	(0.26, 0.72)	0.0012	3.2
HO1	1.23	(0.88, 1.72)	0.2335	1.2	1.41	(0.99, 2.01)	0.0587	1.9	1.02	(0.73, 1.41)	0.9233	0.1	1.01	(0.71, 1.43)	0.9632	0
TRANCE	0.88	(0.70, 1.10)	0.2543	1.1	0.89	(0.70, 1.12)	0.3111	1	0.83	(0.64, 1.08)	0.1626	1.4	0.78	(0.59, 1.04)	0.0863	1.7
PIGR	1.44	(0.76, 2.73)	0.2581	1.1	1.6	(0.82, 3.13)	0.1693	1.4	1.89	(1.05, 3.39)	0.0333	2.1	1.9	(1.01, 3.57)	0.048	2
PDGFSUBUNITA	1.09	(0.93, 1.26)	0.2869	1.1	1.11	(0.95, 1.31)	0.1923	1.3	1.03	(0.89, 1.18)	0.7228	0.4	1.06	(0.91, 1.23)	0.4793	0.7
THPO	1.18	(0.87, 1.60)	0.2963	1	1.18	(0.86, 1.63)	0.2984	1	1.09	(0.80, 1.50)	0.5843	0.5	1.11	(0.80, 1.56)	0.5249	0.6
CTRC	0.91	(0.77, 1.09)	0.3048	1	0.94	(0.78, 1.13)	0.496	0.7	0.99	(0.83, 1.19)	0.9303	0.1	1.03	(0.85, 1.26)	0.7581	0.3
CNTN1	0.85	(0.62, 1.16)	0.3131	1	0.86	(0.62, 1.19)	0.3602	0.9	0.75	(0.54, 1.02)	0.0654	1.8	0.76	(0.54, 1.05)	0.0996	1.6
CXCL5	1.05	(0.96, 1.15)	0.3253	1	1.03	(0.94, 1.14)	0.4934	0.7	1	(0.90, 1.11)	0.9813	0	1	(0.89, 1.12)	0.9958	0
IL17RA	1.1	(0.90, 1.35)	0.3346	1	1.1	(0.89, 1.36)	0.3577	0.9	0.95	(0.74, 1.23)	0.7123	0.4	0.93	(0.71, 1.22)	0.6185	0.5
PROTEINBOC	1.19	(0.83, 1.71)	0.3396	1	1.17	(0.81, 1.71)	0.4024	0.8	1.54	(1.02, 2.32)	0.0385	2.1	1.59	(1.02, 2.48)	0.0402	2.1
THBS2	1.26	(0.78, 2.05)	0.3475	0.9	1.11	(0.67, 1.83)	0.6897	0.4	2.47	(1.51, 4.03)	0.0003	3.6	2.33	(1.39, 3.90)	0.0013	3.2
MCP4	1.1	(0.89, 1.37)	0.3822	0.9	1.07	(0.85, 1.34)	0.5679	0.6	0.97	(0.78, 1.20)	0.7668	0.3	0.99	(0.79, 1.24)	0.9367	0.1
HAOX1	1.05	(0.93, 1.18)	0.4242	0.8	1.01	(0.89, 1.14)	0.918	0.1	0.91	(0.80, 1.04)	0.1691	1.4	0.87	(0.76, 1.00)	0.0571	1.9
ADAMTS13	0.79	(0.43, 1.44)	0.4457	0.8	1.08	(0.57, 2.04)	0.8111	0.2	1.32	(0.76, 2.31)	0.3298	1	1.61	(0.86, 3.03)	0.1348	1.5
GLO1	1.07	(0.89, 1.29)	0.4772	0.7	1.05	(0.86, 1.27)	0.64	0.5	1.11	(0.90, 1.37)	0.348	0.9	1.07	(0.85, 1.34)	0.5722	0.6
TRAIL	1.14	(0.79, 1.65)	0.4776	0.7	1.12	(0.77, 1.64)	0.5556	0.6	0.45	(0.29, 0.69)	0.0003	3.6	0.42	(0.26, 0.67)	0.0003	3.6
GH	0.97	(0.89, 1.06)	0.4806	0.7	0.99	(0.90, 1.09)	0.8655	0.2	1.05	(0.95, 1.16)	0.3226	1	1.1	(0.99, 1.23)	0.0665	1.8
RAGE	1.12	(0.82, 1.53)	0.4918	0.7	1.2	(0.85, 1.68)	0.3013	1	1.25	(0.90, 1.74)	0.1809	1.3	1.31	(0.91, 1.88)	0.1528	1.4
PSGL1	1.09	(0.84, 1.41)	0.5312	0.6	1.12	(0.84, 1.48)	0.4478	0.8	1.39	(0.92, 2.09)	0.117	1.6	1.48	(0.95, 2.30)	0.0795	1.8
MARCO	0.9	(0.64, 1.27)	0.5512	0.6	0.96	(0.66, 1.38)	0.8067	0.2	1.09	(0.86, 1.38)	0.467	0.7	1.06	(0.83, 1.35)	0.6562	0.4
IL13	1.11	(0.79, 1.55)	0.5531	0.6	1.22	(0.86, 1.72)	0.2701	1.1	0.95	(0.62, 1.45)	0.8094	0.2	0.92	(0.59, 1.42)	0.6948	0.4
CCL28	1.09	(0.81, 1.46)	0.5589	0.6	1.11	(0.82, 1.51)	0.5044	0.7	1	(0.78, 1.30)	0.9758	0	1.09	(0.83, 1.44)	0.5345	0.6
CXCL1	1.04	(0.91, 1.19)	0.566	0.6	1.03	(0.89, 1.19)	0.7054	0.4	1.05	(0.89, 1.24)	0.5851	0.5	1.05	(0.88, 1.25)	0.6141	0.5
GDF2	0.95	(0.78, 1.16)	0.613	0.5	1.03	(0.83, 1.27)	0.8145	0.2	1.01	(0.81, 1.26)	0.9631	0	1.1	(0.87, 1.40)	0.4238	0.8
AZU1	1.06	(0.85, 1.32)	0.618	0.5	1.06	(0.85, 1.34)	0.6042	0.5	1.07	(0.91, 1.25)	0.4075	0.8	1.07	(0.90, 1.26)	0.4452	0.8
MEPE	1.07	(0.83, 1.38)	0.6191	0.5	0.95	(0.72, 1.26)	0.7171	0.4	0.94	(0.72, 1.23)	0.663	0.4	0.8	(0.60, 1.08)	0.1525	1.4
IL20RA	0.9	(0.56, 1.44)	0.6494	0.5	0.94	(0.57, 1.54)	0.7927	0.3	1.14	(0.46, 2.81)	0.784	0.3	1.39	(0.54, 3.57)	0.496	0.7
LPL	0.93	(0.67, 1.29)	0.6713	0.4	0.96	(0.68, 1.35)	0.8206	0.2	0.99	(0.75, 1.30)	0.9345	0.1	1	(0.75, 1.34)	0.9738	0
LDLRECEPTOR	0.96	(0.78, 1.18)	0.6782	0.4	0.97	(0.78, 1.20)	0.7566	0.3	0.89	(0.70, 1.13)	0.3295	1	0.85	(0.66, 1.09)	0.1969	1.3
KLK6	1.06	(0.79, 1.42)	0.7023	0.4	0.99	(0.71, 1.38)	0.9555	0.1	0.9	(0.70, 1.14)	0.3731	0.9	0.82	(0.62, 1.09)	0.1705	1.4

CDH5	1.06	(0.79, 1.41)	0.7034	0.4	0.97	(0.71, 1.32)	0.8371	0.2	1.06	(0.80, 1.42)	0.6711	0.4	1.02	(0.75, 1.38)	0.9126	0.1
TIE2	1.07	(0.73, 1.58)	0.721	0.4	1.07	(0.72, 1.61)	0.7347	0.3	1.41	(0.90, 2.20)	0.137	1.5	1.35	(0.85, 2.14)	0.2	1.3
SERPINA12	1.02	(0.93, 1.12)	0.7294	0.3	0.99	(0.90, 1.09)	0.8486	0.2	1.15	(0.98, 1.36)	0.0826	1.7	1.14	(0.96, 1.35)	0.127	1.5
IL1RL2	0.95	(0.72, 1.26)	0.732	0.3	0.94	(0.71, 1.26)	0.7027	0.4	1.24	(0.90, 1.72)	0.1842	1.3	1.24	(0.88, 1.74)	0.2277	1.2
IL10RA	1.06	(0.74, 1.53)	0.7411	0.3	1.12	(0.76, 1.63)	0.5672	0.6	1.14	(0.59, 2.21)	0.7014	0.4	1.3	(0.65, 2.60)	0.4622	0.7
EPCAM	0.98	(0.86, 1.12)	0.7485	0.3	0.96	(0.84, 1.11)	0.6088	0.5	0.97	(0.83, 1.14)	0.7455	0.3	0.94	(0.79, 1.11)	0.4395	0.8
TNFB	0.96	(0.73, 1.27)	0.7851	0.3	0.98	(0.73, 1.32)	0.9046	0.1	0.83	(0.62, 1.12)	0.222	1.2	0.84	(0.61, 1.14)	0.263	1.1
DLK1	1.02	(0.84, 1.26)	0.812	0.2	0.88	(0.71, 1.11)	0.2825	1.1	1.1	(0.87, 1.38)	0.4338	0.8	0.96	(0.75, 1.25)	0.7821	0.3
TRAP	0.97	(0.71, 1.32)	0.8421	0.2	0.98	(0.71, 1.35)	0.8888	0.1	0.86	(0.65, 1.14)	0.3037	1	0.8	(0.60, 1.08)	0.1446	1.5
TNF	1.02	(0.75, 1.39)	0.8775	0.2	1.05	(0.76, 1.44)	0.7876	0.3	0.99	(0.69, 1.41)	0.9465	0.1	1.03	(0.71, 1.51)	0.8614	0.2
CCL24	0.99	(0.85, 1.15)	0.8876	0.1	1.04	(0.89, 1.21)	0.6233	0.5	0.97	(0.82, 1.14)	0.6845	0.4	0.96	(0.81, 1.15)	0.668	0.4
BDNF	1	(0.96, 1.05)	0.9166	0.1	1.01	(0.96, 1.05)	0.8295	0.2	1.03	(0.98, 1.09)	0.2125	1.2	1.05	(0.99, 1.11)	0.095	1.7
CPA1	1.01	(0.85, 1.20)	0.918	0.1	1	(0.83, 1.20)	0.9837	0	1.06	(0.88, 1.28)	0.5252	0.6	1.02	(0.84, 1.25)	0.8099	0.2
CD6	0.99	(0.79, 1.24)	0.9181	0.1	1.04	(0.82, 1.31)	0.7607	0.3	0.91	(0.70, 1.19)	0.5062	0.7	0.91	(0.69, 1.22)	0.5401	0.6
IL1RT2	1.01	(0.77, 1.34)	0.9375	0.1	0.99	(0.74, 1.32)	0.9496	0.1	0.97	(0.72, 1.30)	0.8275	0.2	0.92	(0.67, 1.26)	0.6147	0.5
CCL22	1	(0.89, 1.14)	0.9398	0.1	1.01	(0.89, 1.15)	0.8543	0.2	1.03	(0.88, 1.19)	0.7421	0.3	1.07	(0.91, 1.25)	0.4181	0.8
PRSS27	1.01	(0.77, 1.32)	0.9562	0.1	0.88	(0.66, 1.17)	0.3814	0.9	1.4	(1.01, 1.94)	0.0443	2	1.3	(0.92, 1.85)	0.1386	1.5

*FDR 1% threshold in the discovery phase

Supplemental Table 3. Odds ratios (OR) and 95% confidence intervals (CI) for the selected proteins (significant in both discovery and replication sets with a false discovery rate of 1%) after adjustment for the matching variables (age, sex, and follow-up time), clinical risk factors (smoking, diabetes, coronary artery disease, serum creatinine, body mass index, heart rate, anti-hypertensive medication use) and correction for multiple comparisons in both discovery and replication sets

Protein name	Discovery			Replication		
	OR	(95% CI)	P-value	OR	(95% CI)	P-value
BNP	1.59	(1.38, 1.84)	<0.0001	1.78	(1.52, 2.08)	<0.0001
NTPROBNP	1.79	(1.50, 2.14)	<0.0001	2.05	(1.66, 2.53)	<0.0001
TRAILR2	2.70	(1.86, 3.92)	<0.0001	2.65	(1.72, 4.06)	<0.0001
TNFRSF13B	1.58	(1.21, 2.07)	0.0008	2.30	(1.56, 3.39)	<0.0001
GAL9	2.30	(1.48, 3.57)	0.0002	2.46	(1.53, 3.94)	0.0002
FGF23	1.48	(1.21, 1.82)	0.0002	1.71	(1.31, 2.22)	<0.0001
TNFRSF10A	2.02	(1.41, 2.90)	0.0001	2.00	(1.32, 3.03)	0.0010
REN	1.17	(0.98, 1.41)	0.0798	1.36	(1.12, 1.66)	0.0019
TNFRSF11A	1.73	(1.29, 2.32)	0.0002	1.54	(1.12, 2.11)	0.0074
GDF15	2.34	(1.72, 3.19)	<0.0001	1.58	(1.18, 2.12)	0.0024
FABP4	1.56	(1.24, 1.96)	0.0001	1.29	(1.03, 1.63)	0.0290
SLAMF7	1.42	(1.14, 1.77)	0.0019	2.02	(1.39, 2.93)	0.0002
CCL16	1.37	(1.11, 1.68)	0.0028	1.57	(1.20, 2.05)	0.0009
TWEAK	0.60	(0.41, 0.87)	0.0075	0.58	(0.42, 0.80)	0.0008
KIM1	1.30	(1.08, 1.55)	0.0050	1.33	(1.07, 1.65)	0.0093
CD4	1.67	(1.18, 2.35)	0.0034	1.82	(1.23, 2.70)	0.0026
VSIG2	1.25	(0.97, 1.60)	0.0857	1.46	(1.06, 2.00)	0.0196
PON3	0.83	(0.66, 1.03)	0.0962	0.72	(0.57, 0.91)	0.0070
PLGF	1.86	(1.28, 2.72)	0.0013	1.61	(1.11, 2.34)	0.0119
MMP12	1.43	(1.14, 1.80)	0.0019	1.35	(1.06, 1.72)	0.0168
ADM	2.10	(1.46, 3.02)	<0.0001	1.40	(1.01, 1.94)	0.0408
RARRES2	2.42	(1.41, 4.18)	0.0015	1.69	(1.10, 2.58)	0.0158
CEACAM8	1.47	(1.18, 1.83)	0.0006	1.37	(1.09, 1.74)	0.0081
SLAMF1	1.28	(1.03, 1.59)	0.0254	1.43	(1.07, 1.90)	0.0142
TNFR1	1.98	(1.38, 2.84)	0.0002	1.27	(0.93, 1.74)	0.1353
AGRP	1.65	(1.23, 2.21)	0.0008	1.66	(1.11, 2.48)	0.0139
TNFR2	1.75	(1.27, 2.42)	0.0007	1.29	(0.97, 1.70)	0.0779
IGFBP7	1.44	(1.05, 1.96)	0.0219	1.43	(1.06, 1.92)	0.0183
UPAR	2.27	(1.61, 3.21)	<0.0001	1.39	(1.01, 1.93)	0.0464
PAR1	1.65	(1.17, 2.33)	0.0040	1.53	(1.08, 2.19)	0.0176
PLC	1.78	(1.17, 2.70)	0.0065	1.42	(0.97, 2.08)	0.0743
ACE2	1.49	(1.17, 1.89)	0.0012	1.41	(1.08, 1.86)	0.0131
IL16	1.39	(1.07, 1.80)	0.0131	1.34	(1.04, 1.72)	0.0219
TFF3	1.52	(1.23, 1.87)	0.0001	1.26	(0.97, 1.63)	0.0834
OPN	1.81	(1.36, 2.40)	<0.0001	1.36	(1.03, 1.79)	0.0304
SPON2	2.64	(1.35, 5.18)	0.0047	2.04	(1.14, 3.66)	0.0166
IL4RA	1.42	(1.07, 1.90)	0.0164	1.45	(1.04, 2.02)	0.0299
TNFRSF14	1.77	(1.26, 2.47)	0.0009	1.26	(0.93, 1.71)	0.1309

Legend: same as in figure 1.

Supplemental Table 4. Spearman correlation between the clinical risk score variables and the selected biomarkers

	AGE	SMK	DM	CAD	HTN	BMI	HR	CR
BNP	0.12	0.02	-0.02	0.18	0.06	-0.03	-0.12	0.08
NTPROBNP	0.13	-0.02	-0.07	0.18	0.07	-0.08	-0.13	0.06
TRAILR2	0.12	0.10	0.08	0.09	0.07	0.08	-0.01	0.27
TNFRSF13B	0.00	0.10	0.07	-0.01	0.03	0.09	-0.05	0.13
GAL9	-0.10	0.18	0.05	-0.04	-0.03	0.14	-0.07	-0.02
FGF23	0.00	-0.03	0.05	0.06	0.11	0.12	-0.03	0.16
TNFRSF10A	0.01	0.06	0.08	0.06	0.06	0.14	-0.01	0.10
REN	0.04	0.06	0.19	0.08	0.18	0.12	-0.02	0.17
TNFRSF11A	0.08	0.04	0.10	0.06	0.09	0.16	-0.06	0.22
GDF15	0.15	0.04	0.13	0.09	0.15	0.03	-0.01	0.31
FABP4	-0.01	-0.04	0.07	-0.02	0.16	0.36	0.05	0.04
SLAMF7	-0.04	0.02	0.11	0.04	0.04	0.08	0.00	0.10
CCL16	-0.02	0.03	0.01	0.00	0.10	0.08	-0.01	0.20
TWEAK	-0.01	0.02	-0.11	-0.04	-0.12	-0.04	-0.10	-0.05
KIM1	0.02	0.03	0.16	0.02	0.09	0.08	0.00	0.12
CD4	-0.11	0.20	0.10	-0.01	-0.06	0.10	-0.05	0.00
VSIG2	0.03	0.11	0.15	0.00	0.03	0.00	-0.07	0.14
PON3	-0.05	0.07	-0.08	-0.13	-0.18	-0.26	-0.08	-0.11
PLGF	0.13	0.05	0.03	0.08	0.04	0.05	-0.05	0.34
MMP12	0.09	0.12	0.07	0.15	0.12	-0.01	-0.03	0.20
ADM	0.05	0.07	0.03	0.05	0.07	0.20	-0.04	0.16
RARRES2	-0.01	0.06	0.00	-0.03	0.09	0.12	0.00	0.14
CEACAM8	0.17	-0.07	-0.02	0.06	0.12	0.06	0.03	0.19
SLAMF1	-0.08	0.10	0.09	0.03	0.01	0.13	-0.04	0.15
TNFR1	0.10	0.06	0.06	0.00	0.10	0.10	-0.04	0.28
AGRP	0.10	-0.05	0.05	0.10	0.08	-0.02	-0.02	0.22
TNFR2	0.05	0.09	0.05	0.00	0.07	0.06	-0.05	0.20
IGFBP7	0.05	0.03	0.06	0.01	0.08	0.03	-0.07	0.18
UPAR	0.07	0.04	0.01	0.03	0.08	0.04	-0.02	0.09
PAR1	-0.05	0.17	0.09	-0.08	-0.08	0.07	-0.01	0.01
PLC	0.12	-0.03	0.00	0.03	0.11	0.13	-0.06	0.33
ACE2	-0.09	0.12	0.10	0.03	-0.04	0.08	-0.03	0.02
IL16	0.00	0.08	0.03	-0.04	0.02	0.14	-0.05	0.03
TFF3	0.08	0.02	0.06	0.02	0.16	-0.06	0.01	0.24
OPN	0.01	0.14	0.00	0.03	0.00	0.01	-0.07	0.08
SPON2	-0.16	0.22	0.04	-0.06	-0.14	0.04	-0.12	-0.09
IL4RA	-0.04	0.15	0.06	-0.01	-0.10	0.01	-0.10	0.01
TNFRSF14	0.11	0.05	0.06	0.00	0.09	0.03	-0.01	0.25

Legend: SMK, smoking, DM, diabetes mellitus; CAD, coronary artery disease; HTN, hypertension; BMI, body mass index; HR, heart rate; CR, creatinine.

Supplemental Table 5. Final adjusted stepwise forward model starting with the 39 proteins independently selected in both discovery and replication sets (discovery and replication sets "pooled" for this analysis)

Biomarker	**OR (95%CI)**	**P-value**
BNP	1.43 (1.25-1.64)	<0.0001
TWEAK	0.38 (0.28-0.50)	<0.0001
NTPROBNP	1.39 (1.17-1.66)	<0.0001
REN	1.28 (1.10-1.49)	0.001
TRAILR2	1.56 (1.13-2.15)	0.007
PON3	0.78 (0.65-0.94)	0.008
CCL16	1.27 (1.06-1.53)	0.008
SLAMF1	1.24 (1.01-1.52)	0.036

Model adjusted for matching variables (age, sex, cohort, phase, follow-up time) and clinical variables (smoking, diabetes, history of coronary artery disease, serum creatinine, body mass index, systolic blood pressure, use of antihypertensive medication, and heart rate (the clinical model) with a p-value for inclusion set at 0.05.

AUC clinical model =0.68; AUC clinical model + final protein selection =0.78 (ΔAUC p-value <0.001).

Legend: BNP, brain natriuretic peptide; TWEAK, tumor necrosis factor (Ligand) superfamily, member 12; NTPROBNP, N-terminal prohormone brain natriuretic peptide; REN, renin; TRAILR2, TNF-related apoptosis-inducing ligand receptor 2; PON3, paraoxonase; CCL16, C-C motif chemokine 16; SLAMF1, signalling lymphocytic activation molecule.

Supplemental Table 6. Nodes of induced network linking the top proteins to incident heart failure.

Level	Name (sorted by)	Uniprot ID	Protein / Gene	Cluster
seed	ACE2	Q9BYF1	Angiotensin-converting enzyme 2	Blood pressure regulation
seed	ADM	P35318	Adrenomedullin	Blood pressure regulation
seed	AGRP	O00253	Agouti-related protein	Metabolism
seed	BNP	P16860	Natriuretic peptides B / N-terminal prohormone brain natriuretic peptide	Blood pressure regulation
seed	CCL16	O15467	C-C motif chemokine 16	Inflammation and apoptosis
seed	CD4	P01730	T-cell surface glycoprotein CD4	Inflammation and apoptosis
seed	CEACAM8	P31997	Carcinoembryonic antigen related cell adhesion molecule 8	Extracellular matrix remodelling, angiogenesis and growth
seed	FABP4	P15090	Fatty acid-binding protein, adipocyte	Metabolism
seed	FGF23	Q9GZV9	Fibroblast growth factor 23	Blood pressure regulation
seed	GAL9	O00182	Galectin-9	Extracellular matrix remodelling, angiogenesis and growth
seed	GDF15	Q99988	Growth/differentiation factor 15	Extracellular matrix remodelling, angiogenesis and growth
seed	IGFBP7	Q16270	Insulin-like growth factor-binding protein 7	Inflammation and apoptosis
seed	IL16	Q14005	Pro-interleukin-16	Inflammation and apoptosis
seed	IL4RA	P24394	Interleukin-4 receptor subunit alpha	Inflammation and apoptosis
seed	KIM1	Q96D42	Kidney injury molecule 1/Hepatitis A virus cellular receptor 1	Inflammation and apoptosis
seed	MMP12	P39900	Matrix metalloproteinase-12	Extracellular matrix remodelling, angiogenesis and growth
seed	OPN	P10451	Osteopontin	Extracellular matrix remodelling, angiogenesis and growth
seed	PAR1	P25116	Proteinase-activated receptor 1 / Coagulation Factor II Thrombin Receptor	Blood pressure regulation
seed	PLC	P98160	Perlecan	Extracellular matrix remodelling, angiogenesis and growth
seed	PLGF	P49763	Placenta growth factor	Extracellular matrix remodelling, angiogenesis and growth
seed	PON3	Q15166	Paraoxonase 3	Metabolism
seed	RARRES2	Q99969	Retinoic acid receptor responder 2	Metabolism
seed	REN	P00797	Renin	Blood pressure regulation
seed	SLAMF1	Q13291	Signaling lymphocytic activation molecule	Inflammation and apoptosis
seed	SLAMF7	Q9NQ25	SLAM family member 7	Inflammation and apoptosis
seed	SPON2	Q9BUD6	Spondin 2	Extracellular matrix remodelling, angiogenesis and growth
seed	TFF3	Q07654	Trefoil factor 3	Inflammation and apoptosis
seed	TNFR1	P19438	Tumor necrosis factor receptor 1	Inflammation and apoptosis
seed	TNFR2	P20333	Tumor necrosis factor receptor 2	Inflammation and apoptosis

Level	Name (sorted by)	Uniprot ID	Protein / Gene	Cluster
seed	TNFRSF10A	O00220	Tumor necrosis factor receptor superfamily member 10A	Inflammation and apoptosis
seed	TNFRSF11A	Q9Y6Q6	Tumor necrosis factor receptor superfamily member 11A	Inflammation and apoptosis
seed	TNFRSF13B	O14836	Tumor necrosis factor receptor superfamily member 13B	Inflammation and apoptosis
seed	TNFRSF14	Q92956	Tumor necrosis factor receptor superfamily member 14	Inflammation and apoptosis
seed	TRAILR2	O14763	TNF-related apoptosis-inducing ligand receptor 2	Inflammation and apoptosis
seed	TWEAK	O43508	Tumor necrosis factor (Ligand) superfamily, member 12	Inflammation and apoptosis
seed	UPAR	Q03405	Urokinase plasminogen activator surface receptor	Extracellular matrix remodelling, angiogenesis and growth
seed	VSIG2	Q96IQ7	V-set and immunoglobulin domain-containing protein 2	Inflammation and apoptosis
intermediate	AGT	P01019	Angiotensinogen	Blood pressure regulation
intermediate	Angiotensin I		Angiotensin I	Blood pressure regulation
intermediate	Angiotensin II		Angiotensin II	Blood pressure regulation
intermediate	CERCAM	Q5T4B2	Inactive glycosyltransferase 25 family member 3 / Cerebral endothelial cell adhesion molecule	Extracellular matrix remodelling, angiogenesis and growth
intermediate	CIP2A	Q8TCG1	Cancerous inhibitor of PP2A	Inflammation and apoptosis
intermediate	DNAJA1	P31689	DnaJ homolog subfamily A member 1	Inflammation and apoptosis
intermediate	ERAP1	Q9NZ08	Endoplasmic reticulum aminopeptidase 1	Inflammation and apoptosis
intermediate	FAP	Q12884	Prolyl endopeptidase FAP	Extracellular matrix remodelling, angiogenesis and growth
intermediate	FAS	P25445	Fas Cell Surface Death Receptor/ Tumor necrosis factor receptor superfamily member 6	Inflammation and apoptosis
intermediate	FBLN1	P23142	Fibulin 1	Extracellular matrix remodelling, angiogenesis and growth
intermediate	FBN1	P35555	Fibrillin-1	Extracellular matrix remodelling, angiogenesis and growth
intermediate	FUT11	Q495W5	Fucosyltransferase 11	Extracellular matrix remodelling, angiogenesis and growth
intermediate	GALNT18	Q6P9A2	Polypeptide N-Acetylgalactosaminyltransferase 18	Extracellular matrix remodelling, angiogenesis and growth
intermediate	GHRL	Q9UBU3	Ghrelin	Metabolism
intermediate	GRN	P28799	Granulin/Epithelin	Inflammation and apoptosis /Extracellular matrix remodelling, angiogenesis and growth
intermediate	HIF1A/ARNT	/	Hypoxia-inducible factor 1-alpha/Aryl hydrocarbon receptor nuclear translocator complex	
intermediate	HNF1B	P35680	Hepatocyte nuclear factor 1-beta	
intermediate	KRT18	P05783	Keratin, type I cytoskeletal 18	Inflammation and apoptosis
intermediate	LAMA1	P25391	Laminin Subunit Alpha 1	Extracellular matrix remodelling, angiogenesis and growth
intermediate	LARGE1	O95461	LARGE xylosyl- and glucuronyltransferase 1	Extracellular matrix remodelling, angiogenesis and growth
intermediate	LTA	P01374	Lymphotoxin Alpha	Inflammation and apoptosis
intermediate	NAGLU	P54802	N-Acetyl-Alpha-Glucosaminidase	Extracellular matrix remodelling, angiogenesis and growth
intermediate	NFkB	/	Nuclear factor NF-kappa-B complex	Inflammation and apoptosis

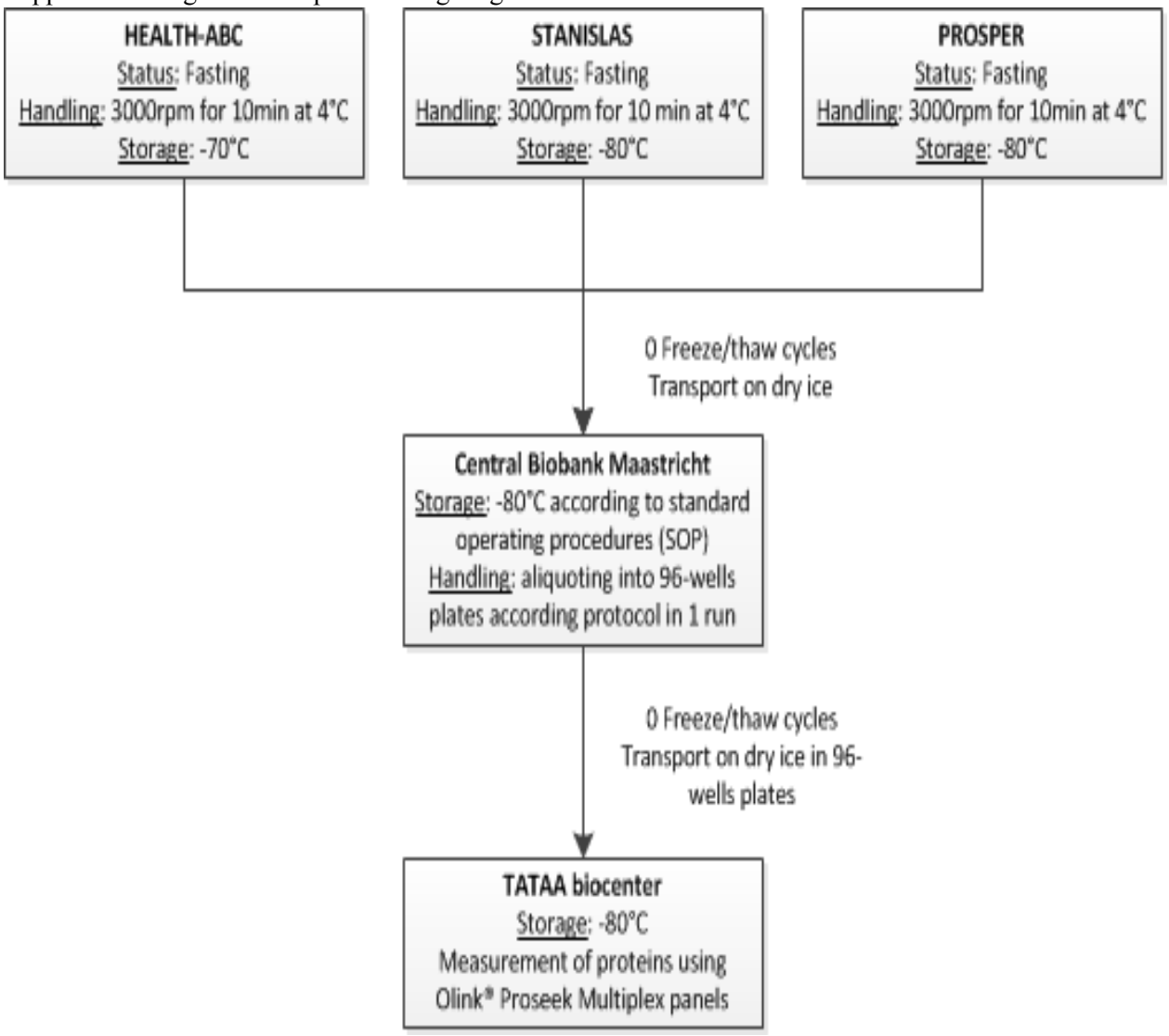
Level	Name (sorted by)	Uniprot ID	Protein / Gene	Cluster
intermediate	NID1	P14543	Nidogen 1/Entactin	Extracellular matrix remodelling, angiogenesis and growth
intermediate	NID2	Q14112	Nidogen 2/Osteonidogen	Extracellular matrix remodelling, angiogenesis and growth
intermediate	S100A10	P60903	S100 Calcium Binding Protein A10	Inflammation and apoptosis
intermediate	SERTAD3	Q9UJW9	SERTA domain-containing protein 3	
intermediate	SLC1A1	P43005	Excitatory amino acid transporter 3	Inflammation and apoptosis
intermediate	SSR4	P51571	Translocon-associated protein subunit delta	Inflammation and apoptosis
intermediate	STAT6	P42226	Signal Transducer And Activator Of Transcription 6 dimer	
intermediate	TNFRSF25	Q93038	TNF Receptor Superfamily Member 25	Inflammation and apoptosis
intermediate	TNFSF10	P50591	TNF Superfamily Member 10	Inflammation and apoptosis
intermediate	TNFSF13	O75888	Tumor Necrosis Factor (Ligand) Superfamily, Member 13	Inflammation and apoptosis
intermediate	TP53	P04637	Tumor Protein P53	
intermediate	TP73	O15350	Tumor Protein P73	
intermediate	TRADD	Q15628	Tumor necrosis factor receptor type 1-associated DEATH domain protein	Inflammation and apoptosis
intermediate	TRAF2	Q12933	TNF Receptor Associated Factor 2	Inflammation and apoptosis
intermediate	TRAF3	Q13114	TNF Receptor Associated Factor 3	Inflammation and apoptosis
intermediate	TRAF5	O00463	TNF receptor-associated factor 5	Inflammation and apoptosis
intermediate	VEGFA	P15692	Vascular Endothelial Growth Factor A	Extracellular matrix remodelling, angiogenesis and growth

Supplemental Table 7. Results of overrepresentation analysis on GO for selected top proteins against all measured proteins as background.

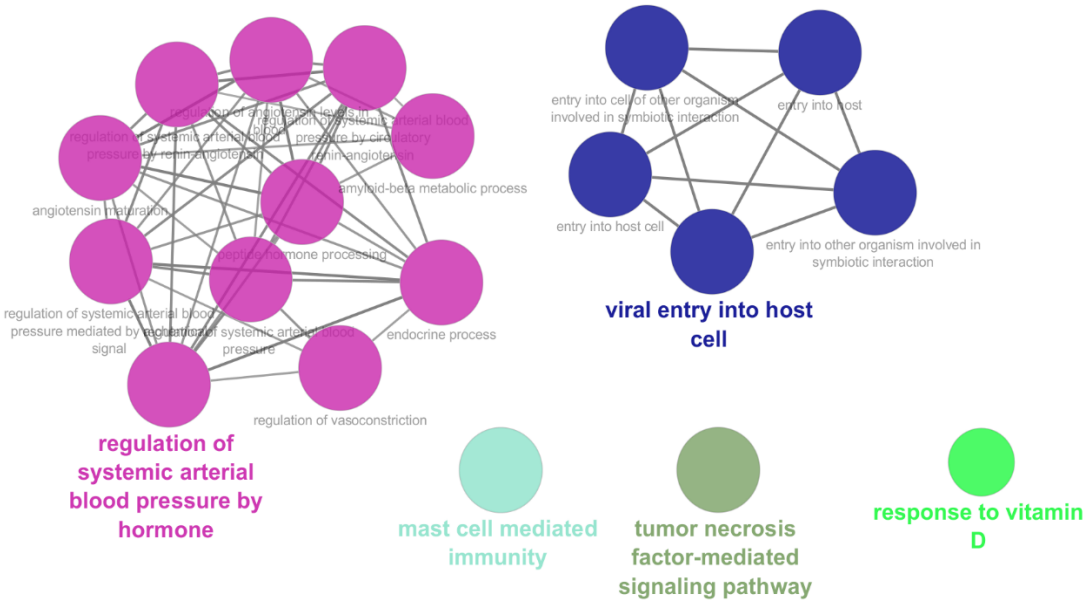
GO process and function	Number of proteins in GO item	Number of proteins on OLINK panel	Proteins in GO process on OLINK panel (with Entrez Gene ID)	Number of selected proteins	Selected proteins (with Entrez Gene ID)	p-value
death receptor activity (GO:0005035)	11	5	7132 TNFRSF1A : TNF receptor superfamily member 1A 7133 TNFRSF1B : TNF receptor superfamily member 1B 8792 TNFRSF11A : TNF receptor superfamily member 11a 8797 TNFRSF10A : TNF receptor superfamily member 10a 8764 TNFRSF14 : TNF receptor superfamily member 14	5 (100.0%)	7132 TNFRSF1A : TNF receptor superfamily member 1A 7133 TNFRSF1B : TNF receptor superfamily member 1B 8792 TNFRSF11A : TNF receptor superfamily member 11a 8797 TNFRSF10A : TNF receptor superfamily member 10a 8764 TNFRSF14 : TNF receptor superfamily member 14	5.47E-05
tumor necrosis factor-activated receptor activity (GO:0005031)	9	4	7132 TNFRSF1A : TNF receptor superfamily member 1A 7133 TNFRSF1B : TNF receptor superfamily member 1B 8792 TNFRSF11A : TNF receptor superfamily member 11a 8764 TNFRSF14 : TNF receptor superfamily member 14	4 (100.0%)	7132 TNFRSF1A : TNF receptor superfamily member 1A 7133 TNFRSF1B : TNF receptor superfamily member 1B 8792 TNFRSF11A : TNF receptor superfamily member 11a 8764 TNFRSF14 : TNF receptor superfamily member 14	0.000409
renal system process (GO:0003014)	116	5	133 ADM : adrenomedullin 2149 F2R/PAR1 : coagulation factor II thrombin receptor 4879 NPPB : natriuretic peptide B 5155 PDGFB : platelet derived growth factor subunit B 5972 REN : renin	4 (80.0%)	133 ADM : adrenomedullin 2149 F2R/PAR1 : coagulation factor II thrombin receptor 4879 NPPB : natriuretic peptide B 5972 REN : renin	0.001827

GO process and function	Number of proteins in GO item	Number of proteins on OLINK panel	Proteins in GO process on OLINK panel (with Entrez Gene ID)	Number of selected proteins	Selected proteins (with Entrez Gene ID)	p-value
regulation of systemic arterial blood pressure (GO:0003073)	91	8	133 ADM : adrenomedullin 1522 CTSZ : cathepsin Z 2149 F2R/PAR1 : coagulation factor II thrombin receptor 4879 NPPB : natriuretic peptide B 5155 PDGFB : platelet derived growth factor subunit B 5972 REN : renin 6648 SOD2 : superoxide dismutase 2 59272 ACE2 : angiotensin I converting enzyme 2	5 (62.5%)	133 ADM : adrenomedullin 2149 F2R/PAR1 : coagulation factor II thrombin receptor 4879 NPPB : natriuretic peptide B 5972 REN : renin 59272 ACE2 : angiotensin I converting enzyme 2	0.002169

Supplemental Figure 1. Sample handling diagram



Supplemental Figure 2. The two major clusters inflammatory/apoptosis and blood pressure regulation were also detected as the main groups using the ClueGO network analysis



Supplemental addenda 1. Material and Methods for the Olink® technology

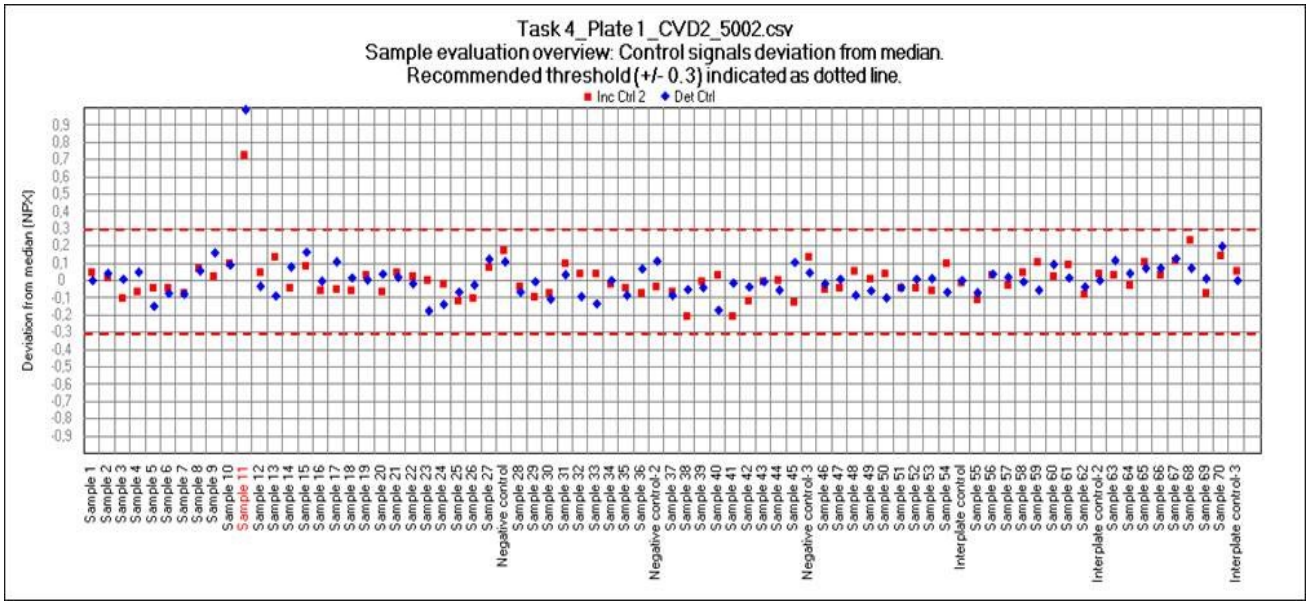
Proteins were measured using the Olink® CVDII, CVDIII, and Inflammation panels* (Olink Proteomics AB, Uppsala, Sweden) according to the manufacturer's instructions. The Proximity Extension Assay (PEA) technology used for the Olink protocol has been well described (Assarsson et al, 2014; accessible at: https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0095192), and enables 92 analytes to be analyzed simultaneously, using 1µL of each sample. In brief, pairs of oligonucleotide-labeled antibody probes bind to their targeted protein, and if the two probes are brought in close proximity the oligonucleotides will hybridize in a pair-wise manner. The addition of a DNA polymerase leads to a proximity-dependent DNA polymerization event, generating a unique PCR target sequence. The resulting DNA sequence is subsequently detected and quantified using a microfluidic real-time PCR instrument (Biomark HD, Fluidigm). The resulting Ct-data is then quality controlled and normalized using a set of internal and external controls. The final assay read-out is presented in Normalized Protein eXpression (NPX) values, which is an arbitrary unit on a log2-scale where a high value corresponds to a higher protein expression. The four internal controls are designed to mimic and monitor the different steps of the PEA. They consist of two non-human proteins with matching antibody-probes that functions as incubation/immuno controls, an IgG antibody with two matching probes attached to it that functions as an extension control and a complete double-stranded amplicon that function as detection control. The internal controls are introduced to all samples as well as to the external controls. The external controls consist of a triplicate of negative controls used to calculate the limit of detection (LOD), as well as a triplicate of interplate controls (IPCs) containing 92 sets of antibodies with both matching probes for each assay attached to them that are used for normalization. For each sample and assay NPX is calculated by the following equations: 1. $Ct_{(analyte)} - Ct_{(extension\ control)} = dCt_{(analyte)}$ (to decrease technical variation) 2. $dCt_{(analyte)} - Ct_{(median\ IPC)} = ddCt_{(analyte)}$ (to improve inter plate variation) 3. $Correction\ factor_{(analyte)} - ddCt_{(analyte)} = NPX_{(analyte)}$ (for more intuitive data). The correction factor is a set variable unique for each assay and reagent lot.

Quality control of the data is performed in two steps: First, the standard deviation for each of the incubation/immuno controls and the detection control is calculated for each run. A run will only pass quality control if the standard deviation for each of the controls is below 0.2. Secondly, each sample is quality controlled using incubation control 2 and the detection control. The run median of each of the controls is calculated and all samples within the run is compared to that. Samples that fall more than +/- 0.3 NPX from the plate median with regards to these two controls will fail the quality control and receive a QC warning in the data output file.

All assay validation data (detection limits, intra- and inter-assay precision data, etc.) are available on manufacturer's website (www.olink.com).

* Previously branded as Olink® Multiplex panels.

An example of a QC-plot is provided below:



Technichal description Olink Proximity Extension Assay

Overview

Olink Proteomics has developed a unique protein detection system called Proximity Extension Assay (PEA), which enables highly specific and sensitive multiplex immunoassays for the detection of 92 different proteins in 90 samples simultaneously, generating 9260 data points in a single run. These proteins can be measured in a large variety of human sample types using as little as one microliter volume of sample. To date, >25 different biological matrices have been tested, all of which have proven compatible with PEA. Olink Proteomics currently offers assays for more than 977 proteins. During PEA, protein targets are ID-tagged by unique DNA amplicons that are subsequently detected and quantified by real-time PCR using Fluidigm's BioMark HD system. In doing so, the platform harnesses the highly parallel nature of Fluidigm's Dynamic Array IFC (Integrated Fluidic Circuit) technology, bringing the assays to the high throughput, reproducibility and superior sensitivity realm of the BioMark HD.

Measurements performed with the Proseek® Multiplex assays are semi-quantitative, where changes in protein levels in one group or population are quantified relative to another. The assays generate quantitation cycle (Cq) values that are then normalized and converted to Olink's arbitrary Normalized Protein eXpression (NPX) unit, which can be used for further statistical analyses. NPX gives relative quantification. NPX is on a log2 scale. NPX should be compared between samples for each assay separately.

The analytical performance is thoroughly validated during product development to ensure the highest standards of sensitivity, precision, specificity and dynamic range. Since traceable calibrators are not yet available for all proteins in Olink's portfolio, performance is evaluated by using full length recombinant proteins as well as well characterized normal and diseased plasma/serum samples.

Repeatability of the measurements is reflected by an average intra-assay variation of 5-10%CV, and inter-assay variation 10-20%CV.

Multiple head-to-head comparisons have been performed against golden-standard single-plex ELISA assays in clinical settings (e.g. NT-pro-BNP, OPN, ST2). So far, these tests have demonstrated strong positive correlation, and have indicated similar, and sometimes superior, levels of sensitivity for Olink's PEA-based assays.

The real strength of Olink's technology is that it offers a high-throughput capacity coupled to a growing list of analytes, therefore placing the platform in a strong position for biomarker selection. Furthermore, the development of new multiplex assays is very rapid thank to a conjugation procedure that works well on all antibody subtypes tested so far, universal incubation conditions and minimal probe concentrations used in our assays. Olink Proteomics currently offers 12 pre-designed 92-plex panels.

Detailed technical description

Measurements

1. Accuracy and reproducibility of the measurements:

Olink's multiplex assays are validated following the standard procedure for immunoassays. Proseek® Multiplex measurements are semi-quantitative, where changes in protein levels are determined and compared for each protein separately. Due to the nature of relative quantification, no calibration standards are needed leaving room for more biological samples on the plate and, thus, higher throughput.

The output from the Proseek Multiplex protocol is in quantitation cycles (Cq) produced by the BioMark's Real-Time PCR Software. To minimize variation within and between runs, the data is normalized using both an internal control (Extension Control) and an Inter-Plate Control (IPC), and data is transformed using a pre-determined correction factor. The pre-processed data will be in the arbitrary unit NPX (Normalized Protein eXpression), which can be used for further statistical analyses.

2. Volume:

As little as 1 □l of sample is required to analyse 92 proteins in a single measurement. The amount of data generated increases proportionally with the volume of analysed sample, so that 2 □l produces data for 184 proteins and so forth.

3. Number of proteins:

92 proteins are quantified for each sample in a single run.

4. Sample types:

Proseek® Multiplex assays are designed for detection of proteins in complex human biofluids. Proseek® Multiplex assays are validated using both EDTA plasma and serum samples. Furthermore, a wide range of additional sample types are compatible with the technology, such as citrate and heparin plasma, CSF, urine, saliva, tissue and cell lysates, cell culture media, dried blood spots and

many more. See Table 1 at the end of this document for information about some of the sample types that have been analyzed so far. Olink is open to discussion and exploration of untested sample types.

5. Correlation and agreement:

Correlation studies have been carried out for a subset of proteins to compare Olink's Proseek® Multiplex assays to commercial singleplex ELISA/conventional analyses in clinical settings. Olink assays have so far displayed high correlations with these established tests, both in Olink's own Service Laboratory and external, customer-led evaluations, demonstrating the accuracy and parallelism of the PEA technology. PEA is described by a peer-reviewed publication in PLOS one: Homogenous 96-Plex PEA Immunoassay Exhibiting High Sensitivity, Specificity, and Excellent Scalability. Assarsson and Lundberg *et al.,* 2014.

6. Validation:

During the validation procedure, Olink analyzes matched plasma (three different anti-coagulants) and serum samples, and reports their respective protein levels relative to the measurements in EDTA plasma. This varies for each biomarker and is presented in the Validation Data package for each panel. Intra- and inter-assays precision values are also determined for each protein and presented in the Validation Data Packages which can be found on http://www.olink.com/proseek-multiplex/.

In a recent pilot wellness study, EDTA plasma was taken from 88 well-characterized individuals at three different time points (every 3 months) and each sample was analyzed using multiple Proseek® Multiplex panels. A large proportion of biomarkers correlated significantly well across the different time points for each individual, while some proteins revealed distinct expression patterns that varied with time. It is also worth mentioning that the inflammation panel was able to identify the signs of infection and single out an individual who had been suffering from illness during one of the three time points (data not shown).

These data further strengthen the case for the robustness of the technology and its potential for future longitudinal studies.

7. Information:

The platform was recently used to investigate the effects of freezer storage, chronological age at sampling, season and month of the year on the abundance levels of 108 proteins in 380 plasma samples collected from 106 Swedish women: "Effects of Long-Term Storage Time and Original Sampling Month on Biobank Plasma Protein Concentrations" Enroth *et al*., 2016.

The study found that storage time can explain 4.8-34.9% of the observed variance, and that the chronological age at sample collection after adjustment for storage-time explains 1.1-33.5% of the observed variance.

Another recent study performed at the Karolinska Institute, using samples from the BBMRI BioBank in Stockholm, found that proteins are stable for consistent measurements by PEA, even after 8 short, successive freeze-thaw cycles (manuscript in preparation).

The first Olink panel was launched on the 1st of March 2013 and since then the technology has been used to analyse over 235 000 samples. This has so far led to 57 publications, in the form of technology reviews and customer applications, demonstrating that Olink's semi-quantitative approach for measuring biomarker levels is approved by referees. More publications are in the pipeline as Olink continues to analyse protein biomarkers for life science researchers around the world. For a complete list of publications see; http://www.olink.com/data-you-can-trust/publications/

Olink will work closely with customers to produce smaller and focused custom panels, to support the future development of diagnostic tools based on clinically relevant protein signatures.

Finally, with the help of bio-informatics tools, Olink proteomics is currently developing ways of extending the biological information provided about the proteins in each panel. In doing so, Olink intends to go beyond the simple provision of technology and reagents by simultaneously catering knowledge for the field of protein biomarker discovery. Olink Proteomics provides first-class support to our customers. With our unparalleled experience and skills working with Proseek® Multiplex panels, the support team quickly delivers the best assistance, free of charge. Olink Proteomics also offers professional statistical analysis services. Our in-house team of biostatisticians can help you with customized statistical analyses tailored to your requirements.

All assay validation data (detection limits, intra- and inter-assay precision data, etc) are available on manufacturer's website (www.olink.com).

Instrumentation

1. Automation:

The Fluidigm BioMark HD combined with a controller or Juno is able to load all reagents into the microfluidic circuits and can be left to operate protocols unattended during the run. The Dynamic Arrays have an integrated network of channels, chambers and valves that automatically combine the reactions, conserving time, reagents and sample volumes. Initial pipetting is needed to pipette samples and reagents into the micro-well section of the dynamic arrays. Automatic sample and assay mixing of Fluidigm integrated fluidic circuit (IFCs) reduces pipetting by 95% compared to conventional PCR microplate-based systems. The IFC is subsequently placed in the controller (either HX or Juno). The samples do not come in contact with the interface plate which is within the controllers. However, it is possible to remove the interface plate and clean with 70% Ethanol as an additional measure between runs. Furthermore, it is possible to automate this using liquid handling instruments as the dynamic arrays have a Society for Biomolecular Sciences (SBS)-compatible format enabling manual and/or 384-well plate format handling and standard dispensing equipment.

By using Fluidigm 96.96 IFCs it is possible to analyse 90 samples + 6 controls in a single run on the BioMark HD. At a rate of 3 to 4 IFCs per day, the platform is largely capable of supporting the proposed scale of proteomic studies. Dedicated staff can achieve up to 360 samples per day (4 IFCs), which translates into 4 x 9216 = 36864 data points in one day per BioMark HD system.

2. Accuracy and Reproducibility:

The repeatability and reproducibility of Olink multiplex assays is determined by using spiked and unspiked serum and plasma samples. These reference samples are generated to cover a wide concentration range for each protein target in order to mimic large biological variation, and are analysed by several (external) operators/service providers. Olink assays display an average intra-assay variation of 5-10% and a 10-20% inter-assay variation. The variation between runs is naturally slightly higher, with the highest variation observed for assays that measure samples close to the Limit of Detection (LOD).

Sample types:

Table 1. Assay detectability in different sample types: Number of proteins detected in healthy blood donors

Sample Type	**Panels**				
	CVD II	**CVD III**	**ONC II**	**INF I**	**NEU I**
Whole blood	NT	NT	NT	NT	NT
Plasma EDTA	AS 99% in >75% of samples	AS 98% in >75% of samples	AS 100% in >75% of samples	AS 80% in >75% of samples	AS 100% in >75% of samples
Plasma Heparin	AS	AS	AS	AS	AS
Plasma Citrate	AS	AS	AS	AS	AS
Serum	AS 97% in >75% of samples	AS 99% in >75% of samples	AS 100% in >75% of samples	AS 82% in >75% of samples	AS 98% in >75% of samples
Urine	NT	NT	NT	AS 37% in >75% of samples	NT
PBMC	NT	NT	NT	AS 73% in >75% of samples	NT

Additional sample-types include, but are not limited to:

CSF	AS 70% in >75% samples	AS 61% in >75% samples	AS 76% in >75% samples	AS 62% in >75% samples	AS 85% in >75% samples
Exosomes	AS 48% in >75% samples (plasma origin)	AS 38% in >75% samples (plasma origin)	AS 37% in >75% samples (cell origin)	AS 27% in >75% samples (cell origin)	NT
Saliva	NT	NT	NT	AS 63% in >75% of samples	NT
Synovial fluid	AS 46% in >75% of samples	NT	NT	AS 41% in >75% of samples	NT
Cell lysates	AS 33% in >75% of samples	AS 34% in >75% of samples	AS 41% in >75% of samples	AS 38% in >75% of samples	NT
Tissue lysates	NT	NT	NT	AS 79 % in >75% samples	AS 86% in >75% samples
Cell culture supernatants	NT	NT	AS 34% in >75% of samples	AS 47% in >75% samples	NT
Dried blood spots	NT	NT	NT	AS 73% in >75% of samples	AS 54% in >75% of samples

AS - Run in Analysis Service

NT - Not Tested

References

- Assarsson E, Lundberg M, Holmquist G, Björkesten J, Thorsen SB, Ekman D, Eriksson A, Rennel Dickens E, Ohlsson S, Edfeldt G, Andersson A-C, Lindstedt P, Stenvang J, Gullberg M, and Fredriksson S. Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. *PLOS one* (2014).
- Enroth S, Johansson Å, Bosdotter Enroth S and Ulf Gyllensten U. Strong effects of genetic and lifestyle factors on biomarker variation and use of personalized cutoffs. *Nature Communications*. (2014).
- Chen H, Zucknick M, Werner S, Knebel P, Brenner H. Head-to-Head Comparison and Evaluation of 92 Plasma Protein Biomarkers for Early Detection of Colorectal Cancer in a True Screening Setting. *Clin Cancer Res*. (2015).
- Andreasson U, Blennow K and Zetterberg H. Update on ultrasensitive technologies to facilitate research on blood biomarkers for central nervous system disorders. *Alzheimer's & Dementia* (2016).

NPX, QC and CV

Calculation of NPX

Normalization of the Ct values derived from the qPCR detection step improves precision and makes data more intuitive (higher value = higher protein concentration). NPX (Normalized Protein eXpression) represents the relative signal (on log2 scale) and is used for downstream statistical analyses.

1. Extension Control:
   $Ct_{analyte} - Ct_{Ext\ Ctrl} = dCt_{analyte}$
2. Interplate Control:
   $dCt_{analyte} - dCt_{Interplate\ Ctrl} = ddCt$
3. Normalization against a correction factor:
   Correction factor - ddCt = NPX

- Protein expression changes are measured for each protein separately by relative quantification
- Even if two different proteins have the same NPX values, their actual concentration may differ
- NPX should be compared between samples within a run for each assay separately
- NPX cannot be directly compared between runs

Quality control

Olink has built-in quality controls in all multiplex panels. Each 92-plex panel contains 96 assays. Four of those are internal controls that allow for an in-process quality control designed to monitor different steps of the protocol: immuno reaction, extension and amplification/ detection.

- The two incubation controls consist of two different non-human antigens measured with PEA assays: Incubation Control 1 and 2. These controls monitor potential variation in all three steps of the reaction.
- The Extension Control is an antibody coupled to both DNA-tags (hence always in proximity). This control monitors the extension and amplification/detection step.
- The Detection Control is a complete double stranded DNA amplicon which does not require any proximity binding or extension step. This control monitors the amplification/detection step.

The internal controls are used for both sample and run QC. The quality control of data is performed separately for each sample plate.

Sample QC

Each of the internal controls are spiked into the samples in the same concentration. The signal for these are therefore expected to be the same over the plate. Sample QC is performed using the Detection Control and Incubation Control 2. Within each run, the levels of these controls are monitored for each sample and compared against the median of all samples. If either of the controls deviate more than the acceptance criteria allow (see below), the sample is flagged.

The Extension Control is used in the normalization step and in generation of NPX, and hence not included in the quality control of data.

The IPC cannot flag on the Detection control. This function was removed already from the start, as the large number of extensions that is required for the IPC made the IPC to always be a bit behind the rest of the samples. I.e., the IPC would always flag on the Detection control. It is however always constant, and flagging on the Detection control was therefore excluded.

Acceptance criteria for passing a sample:

- Incubation Control 2 and Detection Control deviates < +/- 0.3 NPX from plate median.

Run QC

The internal controls are used also in the run QC. This QC assesses the variation over the plate for each of the Incubation Controls 1 and 2 and the Detection Control. If the variation for one of the controls is too large (see below) the entire run is considered unreliable.

Acceptance criteria for passing a run:

- Standard deviation of Incubation Control 1 & 2 and Detection Control: < 0.2 NPX.
- Number of flagged samples: ≤ 1/6 of total number of samples (i.e. ≤ 16 in a full plate).

Correction factor

The correction factor is calculated during the validation of the panels. It is a pre-determined value, a fixed background, used to shift signals to a more intuitive value. Several runs during the validation process is used for this. Data from all assays are used but only IPC and negative control are needed from the samples. A minimum of 12 runs is performed. All runs should have at least three IPCs and three negative controls. Median is used in the final steps of the calculations to make the analysis less sensitive for extreme values.

Calculation of correction factor

1. Normalize using extension control:

   $Ct_{\text{sample assay (x)}} - Ct_{\text{sample assay (ext ctrl)}} = dCt_{\text{sample assay (x)}}$

2. Normalize using median IPC (per experiment):

   $dCt_{\text{sample assay (x)}} - \text{Median } dCt_{\text{IPC assay (x)}} = ddCt_{\text{sample assay (x)}}$

3. Calculate average value for background (per experiment):

   $\text{Average } (ddCt_{\text{background assay(x)}}) = \text{Bg IPC}_{(x)}$

4. Calculate median value for the background of all assays:

   $\text{Median } (\text{Bg IPC}_{(x)})$

Since the background is shifting within an interval, this interval is usually estimated by assuming normal distribution and selecting which interval to use. Olink uses a confidence interval of 99% (+/- 3xSD from the background). As the customer is recommended to run only three background samples in every run, this will not be enough to exactly calculate the standard deviation for the background. Therefore, we have a fixed value for this, which is selected from the same data as the Correction Factor. In contrast to the correction factor, fixed SD has a function and impact on the data.

Normalization

Olink NPX Manager has three available methods for data normalization and minimization of systematic biases between sample plates. These methods are described below. An important concept when deciding on normalization procedure is randomization, which in this context applies to the sample placement across the plates. For randomized studies with more than one plate, intensity normalization is the default normalization. For other studies, IPC normalization is default.

IPC normalization

Three inter-plate controls (IPC) are included on each plate and run as normal samples. The inter-plate control is a pool of 92 antibodies, each with one of the pairs of unique DNA tags on it positioned in fixed proximity (i.e. 92 Extension Controls).

The median of the three IPCs is used as normalizer for each assay, and this compensates for potential variation between runs/plates. This method is completely independent on the samples included on the plate and is therefore recommended for projects where complete randomization of samples cannot be guaranteed.

Intensity normalization

The intensity normalization adjusts the data so that the median NPX for a protein on each plate is equal to the overall median. Each plate is adjusted so that the median of all assays is the same on all plates.

This method requires that the true median of each plate is the same. One way of ensuring this is to randomize the samples beforehand. If there is total randomization, this method outperforms other normalization methods. If there are specific types of samples that are only available on certain plates, this normalization method should not be used.

Control normalization

The control normalization works in the same way as the IPC normalization, but with one addition. While the IPC adjusts each protein to the level of a reference, the control normalization also adjusts the total intensity of entire plates. This is useful if you have plates or projects that differ on average in a way that cannot be explained biologically. Control normalization can be used to reduce systematic variation in non-randomized studies. This normalization method requires the addition of at least two control samples on each plate.

NOTE This is an optional normalization method that should only be used when standard normalizations are not sufficient.

Coefficient of variation

%CV is calculated using linear NPX values from replicate control samples, for assays detected above LOD.

Intra-CV represents the CV within a plate and inter-CV represents the CV between plates. The reported %CV is the mean %CV over all assays.

Coefficient of Variation = (Standard Deviation / Mean) * 100

- Reference value for Inter %CV: < 25%
- Reference value for Intra %CV: < 15%