ESC HEART FAILURE
ESC Heart Failure 2020; **7**: 4151–4158
Published online 13 October 2020 in Wiley Online Library (wileyonlinelibrary.com) **DOI:** 10.1002/ehf2.13036

ORIGINAL RESEARCH ARTICLE

Proteomic exploration of common pathophysiological pathways in diabetes and cardiovascular disease

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Abstract

Aims The epidemiological association between diabetes and cardiovascular disease is well established, but the pathophysiological link is complex and multifactorial. We investigated seven proteins, previously linked to incident diabetes mellitus, and their association with cardiovascular disease and mortality.

Methods and results Plasma samples from 1713 individuals from the Swedish population-based Malmö Preventive Project (mean age 67.4 ± 6.0 years; 29.1% women) were analysed with a proximity extension assay panel. Seven proteins [scavenger receptor cysteine rich type 1 protein M130 (CD163), fatty acid-binding protein 4 (FABP4), plasminogen activator inhibitor 1 (PAI), insulin-like growth factor-binding protein 2 (IGFB2), cathepsin D (CTSD), galectin-4 (GAL4), and paraoxonase-3 (PON3)] previously shown to be associated with incident diabetes were analysed for associations with all-cause mortality (ACM), cardiovascular mortality (CVM), incident coronary events (CEs), and incident heart failure (HF). After exclusion of prevalent cases of respective outcome, proteins that met Bonferroni-corrected significance were analysed in multivariable Cox regression models. Significant associations were identified between five proteins [GAL4 (hazard ratio; 95% confidence interval: 1.17–1.41), CTSD (1.15–1.37), CD163 (1.09–1.30), IGFBP2 (1.05–1.30), and FABP4 (1.04–1.29)] and ACM and four proteins [GAL4 (1.38–1.56), CTSD (1.14–1.43), CD163 (1.09–1.36), and IGFBP2 (1.03–1.35)] with CVM. Three proteins [GAL4 (1.14–1.57), CTSD (1.12–1.50), and FABP4 (1.05–1.55)] were significantly associated with incident CE and two [GAL4 (1.03–1.54) and CTSD (1.01–1.46)] were associated with incident HF after adjusting for traditional risk factors including N-terminal pro-brain natriuretic peptide.

Conclusions In a general Swedish population, four proteins previously shown to be associated with diabetes were associated with ACM and CVM. Three proteins were associated with incident CE. Finally, GAL4 and CTSD displayed novel associations with incident HF and were the only proteins associated with all outcomes.

Keywords Cardiometabolic disease; Cardiovascular disease; Cathepsin D; Diabetes; Galectin-4; Proteomics

Received: 4 May 2020; Revised: 7 August 2020; Accepted: 15 September 2020
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Introduction

Cardiovascular disease (CVD) is the leading cause of death in patients with type 2 diabetes and occurs more frequently and earlier in life than in subjects without diabetes.[1] Subjects with diabetes carry up to a quadrupled risk of cardiovascular mortality and morbidity compared with those without diabetes.[2] Furthermore, their arteries are associated with an accelerated and more extensive atherosclerotic process, resulting in narrower lumen, higher plaque burden, and plaques more prone to ulceration and rupture.[3] The difference in heart failure (HF) burden is even more discouraging, with HF surpassing both myocardial infarction and stroke as the most common complication of type 2 diabetes.[4] Furthermore, the

prognosis in subjects with concomitant diabetes and HF is significantly worse than in subjects with HF alone.[5]

Although the macrovascular, microvascular, and non-vascular complications of diabetes are well established from an epidemiological perspective, the underlying pathophysiological mechanisms are complex and not fully understood.[6] Most efforts to reduce cardiovascular events and mortality by glucose-lowering agents have been largely unsuccessful and sometimes even harmful,[7–9] which emphasizes the need for novel mechanism-based therapeutic strategies.

Multiplex proteomic platforms represent an appealing approach for exploration of pathophysiological pathways and novel associations between multiple proteins and disease, with possible diagnostic, prognostic, and therapeutic implications. We recently used such a panel[10] based on proximity extension assay technology to explore associations between 92 proteins and incident diabetes in a population-based cohort. The study identified seven proteins associated with incident diabetes, four of which were novel associations.[11]

The aim of this observational study was to explore how these seven proteins associate with CVD and mortality and to explore possible common pathophysiological pathways, in an attempt to bridge the knowledge gap in cardiometabolic disease.

Methods

Study sample

During 1974–1992, specific birth cohorts between 1921 and 1949 of inhabitants in Malmö, Sweden, were invited to participate in the Malmö Preventive Project (MPP), a large screening study with a total of 33 346 individuals attending (attendance rate 71%). Re-examination of 18 238 MPP survivors, who were still residing in the Malmö area [the MPP Re-Examination Study (MPP-RES)], was conducted during 2002–2006 (attendance rate 72%). In a subsample of 1792 participants, echocardiography was performed.[11] These subjects were randomly selected from groups defined by glucometabolic status: normal fasting glucose, impaired fasting glucose, new-onset diabetes, and prevalent diabetes, with oversampling in the groups with glucometabolic disturbances to ensure numerical balance, as described previously.[12]

Clinical examination

Height and weight were measured and body mass index (kg/m^2) subsequently calculated. Heart rate and blood pressure were measured twice in the supine position after 10 min of rest, and blood samples were drawn after an overnight fast and plasma stored at −80°C. Data on smoking and current medication were self-reported. Diabetes status, prevalence of atrial fibrillation, prevalence of HF, and prevalence of CVD (myocardial infarction and/or stroke) were retrieved through regional and national registers.

All participants signed a written informed consent form before entering MPP-RES. The study was approved by the Regional Ethical Review board at Lund University, Sweden (LU 244-02) and complied with the Helsinki Declaration.

Laboratory assays

Blood samples were drawn after an overnight fast. The samples were centrifuged, and plasma was stored at −20°C until the time of analysis. N-terminal pro-brain natriuretic peptide (NT-proBNP) was measured with an electrochemiluminescence immunoassay (Elecsys, Roche Diagnostics, Basel, Switzerland) at the Department of Clinical Chemistry, Akershus University Hospital, Lørenskog, Norway.

Proteomic profiling

A total of 1737 individuals provided blood samples that were successfully analysed with the Olink proximity extension assay. Plasma levels of proteins were analysed by the proximity extension assay technique using the Proseek Multiplex CVD III 96 × 96 reagents kit (Olink Bioscience, Uppsala, Sweden). The CVD III panel consists of 92 proteins with either established or proposed associations with CVD, inflammation, and metabolism. All data are presented as arbitrary units. One protein was below detectable limits in >15% samples (NT-proBNP); therefore, we used measurements of NT-proBNP as carried out by the local laboratory (for details, see Laboratory assays). Across all 92 assays, the mean intra-assay and inter-assay variations were 8.1% and 11.4%, respectively. Validation data and coefficients of variance for all proteins can be found in the Supporting Information (validation data CVD III), and further technical information about the assays are available on the Olink website (http://www.olink.com). In this study, we analysed seven proteins, previously linked to incident diabetes: scavenger receptor cysteine rich type 1 protein M130 (CD163), fatty acid-binding protein 4 (FABP4), plasminogen activator inhibitor 1 (PAI), insulin-like growth factor-binding protein 2 (IGFB2), cathepsin D (CTSD), galectin-4 (GAL4), and paraoxonase-3 (PON3).[11]

Endpoints

Participants were followed in local and national registers for incident coronary events (CEs) and incident HF. CE was defined as coronary revascularization and/or fatal or non-fatal myocardial infarction. Data on all-cause mortality (ACM) and cardiovascular mortality (CVM) were retrieved through

the Swedish Board on Health and Welfare and Statistics, Sweden. Follow-up ended on 31 December 2018. Diagnoses of incident CE and incident HF were retrieved from record linkage using the Swedish personal identification number with the Swedish Hospital Discharge Register, the Swedish Cause of Death Register, and the Swedish Coronary Angiography and Angioplasty Registry. International Classification of Diseases, Ninth and Tenth Revision (ICD-9 and ICD-10) codes are found in the **Supporting Information.**

Statistical analysis

All skewed variables were ln-transformed [the seven proteins, cystatin C, and high-density lipoprotein cholesterol (HDL)]. The analysed proteins were subsequently standardized by *z* score transformation. The proportional hazards assumption was tested using partial residuals. Cox regression was carried out adjusted for age and sex *(Model 1),* and a Bonferroni-corrected *P* value of 0.007 (0.05/7) was considered statistically significant. Proteins that were significantly associated with the outcome of interest were then analysed further using models adjusted for other relevant covariates, in which a *P* value of 0.05 was considered significant. All analyses were adjusted for age, sex, body mass index, smoking, prevalent diabetes, systolic blood pressure, anti-hypertensive treatment, prevalent atrial fibrillation, and cystatin C. For the analysis of ACM and CVM, prevalent CVD, prevalent HF, total cholesterol, and HDL were included (*Model 2a*). For the analysis of incident CE, total cholesterol and HDL were included, and prevalent cases of CVD and HF were excluded prior to analysis (*Model 2a*). For the analysis of HF, prevalent CVD, heart rate, and NT-proBNP were included and prevalent cases of HF excluded (*Model 2b*). All analyses were carried out using SPSS 25.0.

Results

Baseline characteristics of all subjects (*n* = 1713), those deceased (*n* = 590), with incident CE (*n* = 189), and with incident HF (*n* = 130) are presented in *Table 1*. The overall study population had a mean age of 67.4 years, male predominance (70.9%), and more than a third had prevalent diabetes at baseline (35.3%). The overall prevalence of CVD was 10.8%, and almost half of the population was on anti-hypertensive treatment (46.8%). There were no interactions between the investigated proteins and diabetes in the endpoint analyses.

Analyses of all-cause mortality

In age-adjusted and sex-adjusted analyses, five of the seven proteins (GAL4, CTSD, IGFBP2, CD163, and FABP4) were significantly associated with ACM. All associations remained significant after further adjusting according to *Model 2a* [median follow-up time 12.7 years, interquartile range (IQR) 25–75: 11.2–13.6 years; 590 deaths; *Table 2*].

Analyses of cardiovascular mortality

Five proteins (GAL4, CTSD, IGFBP2, CD163, and FABP4) yielded significant associations with CVM in age-adjusted

Table 1 Baseline characteristics of the study population

	All subjects *n* = 1713	Deceased *n* = 590	Incident HF *n* = 130	Incident CE *n* = 189
Demographics				
Age (years)	67.4 (±6.0)	70.9 (±4.9)	70.4 (±4.8)	69.0 (±5.6)
Women, *n* (%)	498 (29.1)	179 (30.3)	28 (21.5)	28 (14.8)
Smoking, *n* (%)	303 (17.7)	125 (21.2)	23 (17.7)	32 (16.9)
Clinical profile				
BMI (kg/m^2)	28.3 (±4.3)	28.5 (±4.6)	29.5 (±5.0)	28.0 (±3.9)
Systolic BP (mmHg)	146.6 (±20.2)	146.5 (±21.1)	148.2 (±21.5)	151.6 (±20.8)
Heart rate (BPM)	71.8 (±12.5)	72.0 (12.5)	70.5 (±14.4)	71.1 (±12.5)
Medical history				
Diabetes, *n* (%)	604 (35.3)	298 (50.5)	72 (55.4)	88 (46.6)
Prevalent HF, *n* (%)	30 (1.8)	25 (4.3)	N/A	N/A
Prevalent AF, *n* (%)	97 (5.7)	65 (11.0)	16 (12.3)	13 (6.9)
Prevalent CVD, *n* (%)	185 (10.8)	100 (16.9)	41 (31.5)	N/A
AHT, *n* (%)	802 (46.8)	348 (59.0)	86 (66.2)	104 (55.0)
Laboratory				
Cystatin C (mg/L)	1.06 (0.95–1.20)	1.14 (0.99–1.33)	1.16 (1.0–1.3)	1.12 (0.99.1.29)
NT-proBNP (pg/mL)	12 (6–25)	21 (10–45)	32 (15–69)	14 (7–33)
Cholesterol (mmol/L)	5.40 (4.6–6.2)	5.2 (4.4–6.0)	5.0 (4.1–5.7)	5.5 (4.7–6.2)
HDL-C (mmol/L)	1.25 (1.03–1.52)	1.24 (1.03–1.50)	1.20 (1.0–1.4)	1.22 (0.97–1.43)

AF, atrial fibrillation; AHT, anti-hypertensive treatment; BMI, body mass index; BP, blood pressure; BPM, beats per minute; CE, coronary event; CVD, cardiovascular disease; HDL, high-density lipoprotein; HF, heart failure; N/A not applicable, excluded prior to analysis; NT-proBNP; N-terminal pro-B-type natriuretic peptide.
Values are displayed as means (±standard deviation) or, for skewed variables, medians and interquartile (25–75) range.

Table 2 Cox regression analysis for risk of all-cause mortality

	Model 1				Model 2a			
$n = 590$	HR	95% CI		*P* value	HR	95% CI		*P* value
GAL4	1.42	1.30	1.56	2.9×10^{-14}	1.29	1.17	1.41	1.9×10^{-7}
CTSD	1.33	1.23	1.45	6.6×10^{-12}	1.26	1.15	1.37	2.1×10^{-7}
FABP4	1.32	1.21	1.45	1.7×10^{-9}	1.16	1.04	1.29	0.010
CD163	1.22	1.12	1.33	3.0×10^{-6}	1.19	1.09	1.30	0.00009
IGFBP2	1.19	1.08	1.30	0.0003	1.17	1.05	1.30	0.004
PON3	0.90	0.84	0.97	0.008				
PAI	1.09	0.99	1.19	0.057				

CD163, scavenger receptor cysteine rich type 1 protein M130; CTSD, cathepsin D; FABP4, fatty acid-binding protein 4; GAL4, galectin-4; IGFBP2, insulin-like growth factor-binding protein 2; PAI, plasminogen activator inhibitor 1; PON3, paraoxonase-3.
Values are hazard ratios (HRs) and 95% confidence intervals (95% CI) for all-cause mortality. *Model 1* is adjusted for age and sex. *Model 2a* is adjusted for age, sex, body mass index, systolic blood pressure, anti-hypertensive treatment, smoking, diabetes, prevalent atrial fibrillation, prevalent heart failure, prevalent cardiovascular disease, cystatin C, total cholesterol, and high-density lipoprotein.

and sex-adjusted Cox regression analyses (median follow-up time 12.7 years, IQR 11.2–13.6 years; 353 deaths; *Table 3*). After further adjustment according to *Model 2*, all but FABP4 remained significantly associated with CVM (*Table 3*).

Analyses of incident coronary events

In age-adjusted and sex-adjusted Cox regression analyses, three proteins (GAL4, CTSD, and FABP) yielded significant associations with incident CE (median follow-up time 10.7 years, IQR 10.0–11.7 years; 164 events) that all remained after further adjustment per *Model 2a* (*Table 4*).

Table 3 Cox regression analysis for risk of cardiovascular mortality

	Model 1				Model 2a			
$n = 353$	HR	95% CI		*P* value	HR	95% CI		*P* value
GAL4	1.56	1.38	1.75	2.8×10^{-13}	1.38	1.22	1.56	4.8×10^{-7}
CTSD	1.35	1.22	1.50	2.1×10^{-8}	1.28	1.14	1.43	6.7×10^{-5}
FABP4	1.35	1.20	1.52	4.5×10^{-7}	1.14	0.99	1.32	0.065
CD163	1.25	1.12	1.40	5.4×10^{-5}	1.21	1.09	1.36	0.001
IGFBP2	1.26	1.12	1.42	1.7×10^{-4}	1.18	1.03	1.35	0.020
PAI	1.08	0.97	1.21	0.16				
PON3	0.92	0.84	1.02	0.12				

CD163, scavenger receptor cysteine rich type 1 protein M130; CTSD, cathepsin D; FABP4, fatty acid-binding protein 4; GAL4, galectin-4; IGFBP2, insulin-like growth factor-binding protein 2; PAI, plasminogen activator inhibitor 1; PON3, paraoxonase-3.
Values are hazard ratios (HRs) and 95% confidence intervals (95% CI) for cardiovascular mortality. *Model 1* is adjusted for age and sex. *Model 2a* is adjusted for age, sex, body mass index, systolic blood pressure, anti-hypertensive treatment, smoking, diabetes, prevalent atrial fibrillation, prevalent heart failure, prevalent cardiovascular disease, cystatin C, total cholesterol, and high-density lipoprotein.

Table 4 Cox regression analysis for risk of incident coronary events

	Model 1				Model 2a			
$n = 189$	HR	95% CI		*P* value	HR	95% CI		*P* value
GAL4	1.44	1.23	1.68	5.0×10^{-6}	1.34	1.14	1.57	0.0004
CTSD	1.38	1.20	1.58	9.0×10^{-6}	1.30	1.12	1.50	0.001
FABP4	1.36	1.16	1.60	1.2×10^{-4}	1.27	1.05	1.55	0.015
IGFBP2	1.20	1.03	1.41	0.023				
CD163	1.20	1.02	1.40	0.027				
PAI	1.14	0.98	1.33	0.86				
PON3	0.95	0.83	1.09	0.486				

CD163, scavenger receptor cysteine rich type 1 protein M130; CTSD, cathepsin D; FABP4, fatty acid-binding protein 4; GAL4, galectin-4; IGFBP2, insulin-like growth factor-binding protein 2; PAI, plasminogen activator inhibitor 1; PON3, paraoxonase-3.
Values are hazard ratios (HRs) and 95% confidence intervals (95% CI) for incident coronary events. *Model 1* is adjusted for age and sex. *Model 2a* is adjusted for age, sex, body mass index, systolic blood pressure, anti-hypertensive treatment, smoking, diabetes, cystatin C, total cholesterol, and high-density lipoprotein.

Analyses of incident heart failure

Four proteins (GAL4, CTSD, FABP4, and PON3) yielded significant associations with incident HF in age-adjusted and sex-adjusted Cox regression analyses (median follow-up time 10.8 years, IQR 10.2–11.7; 105 events), but only GAL4 and CTSD remained significantly associated with incident HF after further adjustment for *Model 2b* (*Table 5*).

Discussion

In this community-based sample of 1713 individuals, we analysed seven diabetes-associated proteins in relation to ACM, CVM, incident CE, and incident HF. Four proteins (GAL4, CTSD, CD163, and IGFBP2) were associated with both

Table 5 Cox regression analysis for risk of incident heart failure

	Model 1				Model 2b			
$n = 130$	HR	95% CI		*P* value	HR	95% CI		*P* value
GAL4	1.49	1.23	1.81	4.9×10^{-5}	1.26	1.03	1.54	0.024
CTSD	1.30	1.09	1.55	0.003	1.21	1.01	1.46	0.044
FABP4	1.38	1.13	1.67	0.001	0.95	0.75	1.19	0.64
PON3	0.80	0.69	0.93	0.003	0.95	0.81	1.12	0.57
IGFBP2	1.01	0.84	1.22	0.91				
CD163	1.11	0.92	1.33	0.28				
PAI	1.00	0.84	1.20	0.99				

CD163, scavenger receptor cysteine rich type 1 protein M130; CTSD, cathepsin D; FABP4, fatty acid-binding protein 4; GAL4, galectin-4; IGFBP2, insulin-like growth factor-binding protein 2; PAI, plasminogen activator inhibitor 1; PON3, paraoxonase-3.
Values are hazard ratios (HRs) and 95% confidence intervals (95% CI) for incident heart failure. *Model 1* is adjusted for age and sex. *Model 2b* is adjusted for age, sex, body mass index, systolic blood pressure, heart rate, anti-hypertensive treatment, smoking, diabetes, prevalent atrial fibrillation, prevalent cardiovascular disease, cystatin C, and N-terminal pro-B-type natriuretic peptide.

ACM and CVM. For incident CE, associations for three proteins (GAL4, CTSD, and FABP4) remained significant in the fully adjusted model, with GAL4 and CTSD as novel findings. Finally, both GAL4 and CTSD were associated with incident HF after adjustment for traditional risk factors including NT-proBNP. In the end, GAL4 and CTSD were the only proteins associated with all outcomes and represent novel findings to the best of our knowledge. Below follows a brief description of the proteins with known associations with CVD and mortality and a more extensive discussion regarding our novel findings of GAL4 and CTSD. However, PON3 and PAI did not predict any outcome and will not be further discussed.

Proteins with previously established associations with cardiovascular outcomes and mortality

Scavenger receptor cysteine rich type 1 protein M130

CD163 is a marker of macrophage activation and has previously been linked to diabetes,[11,13] atherosclerosis,[14] and increased mortality in several acute and chronic inflammatory diseases.[15] However, our findings of CD163 being associated with CVM and ACM in a general population have, to the best of our knowledge, not been described earlier.

Insulin-like growth factor-binding protein 2

In a recently published study from the Framingham Heart Study that also used the proteomic approach, IGFBP2 was associated with both ACM and CVM,[16] which our findings support. However, in contrast to our findings, IGFB2 was associated with incident HF, but not with atherosclerotic CVD in the Framingham Heart Study. This could very well be an effect of the difference in baseline characteristics between the two cohorts, particularly the predominance of men in our study (71% vs. 47%) and prevalence of diabetes (41% vs. 11%).

Fatty acid-binding protein 4

FABP4 is expressed and secreted from adipocytes and acts as an adipokine for insulin resistance and atherosclerosis.[17] In a recent review,[17] FABP4's role in cardiovascular and metabolic disease, as both a biomarker and as potential treatment target, was discussed. Prior studies have shown increased circulating FABP4 levels to be associated with type 2 diabetes, hypertension, dyslipidaemia, atherosclerosis, HF, and long-term cardiovascular events and mortality, which is in line with our findings.

Proteins with a novel association with cardiovascular outcomes and mortality

Galectin-4

To the best of our knowledge, our study is the first to demonstrate a relationship between GAL4 and both ACM and CVM, incident CE, and incident HF. GAL4 is a part of the galectin family of 15 small lectin proteins, and unlike its siblings, GAL4 is expressed almost exclusively in the gastrointestinal tract of healthy subjects. This has made it an interesting candidate as a cancer marker because it is induced by several malignancies.[18] GAL4 most likely performs several functions, including cell adhesion and induction of intracellular signalling.[18,19] Another function of GAL4 is the stabilization of lipid rafts for the apical transport of proteins from the Golgi apparatus to the apical membrane of the enterocyte.[20] This is interesting because one of the transported proteins is the protease dipeptidyl peptidase-4 (DPP-4). In GAL4-depleted mice, DPP-4 is misguided when transported and accumulates intracellularly, as opposed to being expressed at the apical membrane of the enterocyte in the presence of GAL4.[20] DPP-4 is well known for cleavage and inactivation of our two most common incretins: glucose-dependent insulinotropic polypeptide and proglucagon-derived peptide glucagon-like peptide-1 (GLP-1). The inactivation of glucose-dependent insulinotropic polypeptide and GLP-1 by DPP-4 leads to several cardiometabolically adverse effects, including endothelial dysfunction, insulin resistance, and hyperlipidaemia.[21] Thus, the introduction of incretin-based anti-diabetic medication in the form of DPP-4 inhibitors or GLP-1 agonists represented a major advance in diabetes treatment, without risks of hypoglycaemia or weight gain.[22] However, the use of DPP-4 inhibitors has elicited some concern due to increased hospitalization for HF and a recent meta-analysis consisting of both randomized clinical trials and observational studies suggesting an increased risk in certain patients.[23] In contrast to this, the GLP-1 agonist liraglutide was the first anti-diabetic medication to be approved as therapy to reduce CVD in patients with diabetes.[24] Accordingly, one possible explanation for our findings is that increased levels of GAL4 lead to an increased expression of DPP-4 and thus reduced activity of the beneficial incretin GLP-1. This would also support our original finding of GAL4's association with incident diabetes.[11]

Finally, researchers from Sweden, also using the Olink Proseek CVD III panel,[25] reported that GAL4 was associated with aortic stenosis requiring surgery in two separate cohorts and that GAL4 in the discovery cohort was associated with aortic stenosis in the absence of coronary artery disease. The last finding could, however, not be replicated in the validation cohort. Their findings could possibly be explained by the same mechanism described above, with increased levels of GAL4 leading to increased activity of DPP-4, as DPP-4 has been shown to induce aortic valve calcification.[26]

Cathepsin D

Together with GAL4, CTSD was the only protein to be associated with all outcomes, where the finding of CTSD's association with ACM, CVM, and incident HF in a general population appears to represent novel findings. CTSD has,

however, previously been linked to incident CE in a recent Swedish study.[27]

CTSD has more often been studied as an independent prognostic factor and potential target for anti-cancer treatment.[28] CTSD is a lysosomal endopeptidase whose main functions include intracellular protein turnover and extracellular matrix breakdown, but due to its ability to cleave many different target proteins, there are several possible biological functions.[28]

CTSD is also present extracellularly in human atherosclerotic lesions. It is possible that CTSD released by macrophages participates in the modification of low-density lipoprotein, resulting in both extracellular and intracellular accumulation of lipids in the arterial intima.[29] CTSD has also been suggested to participate in the apoptosis of foam cells, a determinant of plaque instability.[30] These described mechanisms can add to the understanding of our findings. Furthermore, in patients with diabetes, CTSD has been shown to truncate ApoA1 (the main protein of HDL) to ApoA1Δ (1–38), which binds to low-density lipoprotein and increases its susceptibility to oxidation, possibly contributing to the increased risk of CVD in diabetes.[31]

In the setting of an acute coronary syndrome (ACS), previous studies of CTSD are somewhat conflicting. Vivanco *et al.* used a proteomic approach to demonstrate that CTSD was significantly higher in the plasma of ACS patients as compared with healthy subjects.[32] However, in a Turkish study of patients presenting with ACS, levels of CTSD were significantly and independently lower in patients who had a higher rate of in-hospital mortality, more severe coronary artery disease, and lower left ventricular ejection fraction.[33] Finally, in another Turkish study based on 88 patients presenting with ST-elevation myocardial infarction, CTSD levels were increased at admission as compared with controls. However, at 6-month follow-up, the relationship was inverse, with lower levels of CTSD being associated with new-onset HF and recurrent adverse CE.[34] The authors speculated that the lower levels of CTSD were a marker for impaired endogenous phagocytosis and remodelling.

These results are based on small studies and hard to interpret. Further, our findings of CTSD's associations with mortality and cardiovascular outcomes in a general population might not be comparable with the findings in populations that were in acute distress.

Study strengths and limitations

The use of a well-characterized, prospective cohort with many participants and a long follow-up time is a significant strength of the current study; however, all diagnoses were based on retrieval from national registries and not clinical re-examination, which could potentially skew the results. Because we investigated a variety of outcomes with several known risk factors/markers contributing to their different pathogeneses, these risk factors/markers should be considered when conclusions are drawn regarding associations. The observational nature of this study prevents any conclusions to be drawn regarding causality. Furthermore, it was not possible to repeat or confirm measurements of the proteins through an additional method. The original selection of the population with oversampling of groups for glucometabolic disturbances mentioned in the Methods section may raise concerns of this cohort's representation of the background population. Moreover, our data were collected at a single regional centre, comprising subjects of predominantly European descent, limiting generalizability of the results.

Conclusions

In this observational, prospective study, we identified the diabetes-associated proteins GAL4 and CTSD being associated with all investigated outcomes: ACM, CVM, incident CE, and incident HF in a general population. All findings, with the exception of CTSD's association with incident CE, represent, to the best of our knowledge, novel findings.

Conflict of interest

None declared.

Funding

M.M. was supported by grants from the Wallenberg Centre for Molecular Medicine, Lund University (ALFSKANE-675271), Medical Faculty of Lund University (ALFSKANE-432021 and ALFSKANE-436111), Skåne University Hospital, the Crafoord Foundation, the Ernhold Lundstrom's Research Foundation, Region Skåne, the Hulda and Conrad Mossfelt Foundation, the Southwest Skåne's Diabetes Foundation, the Kock's Foundation, the Research Funds of Region Skåne, and the Swedish Heart-Lung Foundation (2015-0322).

The MPP-RES study 2002–2006 was supported by The Swedish Heart-Lung Foundation, the Hulda and E Conrad Mossfelt's Foundation, and the Ernhold Lundström's Foundation to P.M.N.

Supporting information

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

Data S1. Endpoint definitions.

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