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ORIGINAL ARTICLE

Epidemiology/Genetics

Obesity-related inflammatory protein signature in cardiovascular clinical outcomes: results from the Gutenberg Health Study

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Abstract

Objective: The objective of this study was to investigate whether an obesity-related inflammatory protein signature (OIPS) is associated with adverse cardiovascular events.

Methods: The Olink Target 96 Inflammation panel was performed in 6662 participants from the population-based Gutenberg Health Study (GHS). The OIPS was selected by a logistic regression model, and its association with cardiovascular outcomes was evaluated by Cox regression analysis. The GHS-derived OIPS was externally validated in the MyoVasc study.

Results: The identified OIPS entailed 21 proteins involved in chemokine activity, tumor necrosis factor (TNF) receptor binding, and growth factor receptor binding. The signature revealed a novel positive association of axis inhibition protein 1 with obesity. The OIPS was associated with increased risk of all-cause and cardiac deaths, major adverse cardiovascular events, and incident coronary artery disease,

independent of clinical covariates and established risk instruments. A BMI-stratified analysis confirmed the association of OIPS with increased death in those with obesity and overweight and with increased risk for coronary artery disease in those with obesity. The association of OIPS with increased risk of all-cause and cardiac deaths was validated in the MyoVasc cohort.

Conclusions: The OIPS showed a significant association with adverse clinical outcomes, particularly in those with overweight and obesity, and represents a promising tool for identifying patients at higher risk for worse cardiovascular outcomes.

INTRODUCTION

Obesity remains a disease of major global concern, currently affecting around 150 million people in Europe and 650 million people worldwide. As of March 2021, the European Commission has acknowledged obesity as a chronic relapsing disease and a gateway to a number of noncommunicable diseases [1]. Obesity is a strong risk factor for type 2 diabetes mellitus, cardiovascular disease (CVD), nonalcoholic fatty liver disease, immune disorders, and various types of cancer [2, 3].

Numerous preclinical and clinical studies have confirmed that low-grade inflammation of adipose tissue mechanistically leads to obesity-associated metabolic disorders and related diseases [4]. Chronic inflammation is a common denominator for obesity and the development of metabolic complications and forms the basis for the development of atherosclerotic cardiovascular disease. CVD has been found to be responsible for more than two-thirds of deaths in the subgroup of individuals with high body mass index (BMI), independent of smoking and other health conditions [5]. Common markers of inflammation such as C-reactive protein (CRP) and fibrinogen have been repeatedly associated with cardiovascular events, independent of traditional cardiovascular risk factors (CVRF) [6, 7]. Despite the clear association, a causal relationship between CRP and future cardiovascular events has not been demonstrated [8]. In contrast, studies that have focused on upstream regulators of CRP such as interleukin (IL)-6 have reported an important link between genetic variations associated with plasma IL-6 levels and coronary events, supporting a causal link between IL-6 and cardiovascular events [9].

It is not clear whether obesity-related inflammatory pathways are associated with adverse cardiovascular events beyond the cardiovascular risk profile, medications, and traditionally used risk instruments. This study aimed to delineate an obesity-related inflammatory protein signature (OIPS) and evaluate the association with all-cause mortality, cardiac-specific cause of death, three-component major adverse cardiovascular events (MACE), and incident coronary artery disease (CAD).

Study Importance

What is already known?

- Obesity is a complex chronic disease associated with increased cardiovascular morbidity and mortality.
- Chronic inflammation is a common denominator for obesity and the development of metabolic complications and forms the basis for the development of atherosclerotic cardiovascular disease.

What does this study add?

- This is the largest study to identify a population-based obesity-related inflammatory protein signature of 21 unique proteins independent of a wide range of clinical covariates.
- A novel association of axis inhibition protein 1 with obesity was found with potential to identify novel mechanisms for the development of obesity-related complications.
- The obesity-related inflammatory protein signature was associated with all-cause mortality, cardiac death, and incident coronary artery disease, independent of established risk instruments.

How might these results change the direction of research or the focus of clinical practice?

- The present data improve the ability to identify individuals with overweight or obesity who are at higher risk for adverse cardiovascular outcomes and death.
- These findings improve the understanding of the complex interplay among obesity, inflammation, and cardiovascular disease and represent a promising avenue for future research and treatment strategies addressing obesity-related systemic inflammation.

METHODS

Population sample

The Gutenberg Health Study (GHS) (http://www.gutenberghealthstudy.org/ghs/overview.html?L=1) is a population-based, prospective, and observational cohort study of adults in the Mainz-Bingen region in midwestern Germany. The study sample consisted of 15,010 participants aged 35 to 74 years who were enrolled at their baseline examination between 2007 and 2012. Each participant spent approximately 5 h at the study center undergoing a comprehensive standardized clinical and laboratory examination. Biomaterial collected at each study visit was

bio-banked for future investigation. Inflammatory protein profiling was performed in the first 7571 GHS study participants. After excluding participants with recent cancer (cancer diagnosis in the last ≤5 years, $n = 289$), signs of infection (defined by leukocyte count >10/nL, $n = 618$), and missing data on BMI ($n = 2$), $N = 6662$ participants remained for statistical analysis.

The GHS was designed according to the revised Helsinki protocol and approved by the ethics committee of the Rhineland-Palatinate Chamber of Physicians and local data protection officials (reference no. 837.020.07 [5555]). More detailed information on the study design is available elsewhere [10].

Determination of proteins

Bio-banked EDTA-anticoagulated plasma samples, once thawed, were analyzed using proximity extension assay technology (Olink Proteomics, Uppsala, Sweden), a targeted protein quantification method that provides normalized expression values [11]. Briefly, the assay uses a pair of specific oligonucleotide-labeled antibodies targeting each protein. When two complementary oligonucleotides are in close proximity, a polymerase chain reaction (PCR) reporter sequence is formed by proximity-dependent DNA polymerization. This is then amplified, and the amount of each protein-specific sequence is subsequently quantified by real-time PCR. For the analysis in the present study, the target 96 inflammation panel quantifying the expression of 92 unique proteins was used (Table S1).

Validation cohort

A large prospective cohort of individuals with all stages of heart failure (HF), the MyoVasc study (NCT04064450), was used for external validation of the association between OIPS and clinical outcome (e.g., all-cause mortality, cardiac-specific cause of death, worsening of HF, HF hospitalization). Information on cohort design, including detailed information on study objectives and study examinations, has been reported in detail previously [12]. In the MyoVasc cohort, the investigation of targeted inflammation proteomics was performed identically to the GHS cohort in the biomarker laboratory at the Department of Clinical Epidemiology, University Medical Center, Johannes Gutenberg University Mainz. This study was reviewed and approved by the ethics committee of the Rhineland-Palatinate Chamber of Physicians and local data protection officials (reference number 837.319.12 [8420-F]). From the study sample at the baseline examination ($N = 3289$), after excluding $n = 101$ participants with missing protein profiling and $n = 505$ participants with recent cancer (cancer diagnosis in the last ≤5 years), 2683 participants remained for further analysis.

Data management and statistical and bioinformatic analysis

A central data management unit controlled and reviewed all clinical and laboratory data for completeness using predefined algorithms and plausibility criteria. Normally distributed continuous variables were described by mean and standard deviation (SD) and tested with a Student t test. Data with skewed distribution were described by median and interquartile range (IQR) and tested with a Wilcoxon rank sum test. Categorical variables were described by relative and absolute frequencies. Proteomic data transformation/scaling was performed by use of the bestNormalize R package (The R Project for Statistical Computing, Vienna, Austria) for the variables with a skewness near to 0, and winsorizing of the outliers was done by the Hampel method. Missing proteomic data were imputed by a random forest algorithm (missForest R package; The R Project for Statistical Computing).

The OIPS was selected by a multivariable logistic regression model after Bonferroni correction for multiple testing (threshold $p < 0.0004$; Table S2). Each protein level (normalized protein expression [NPX]) was considered as a dependent variable after adjustment for age, sex, CVRF, comorbidities, and medications. The covariates in the logistic regression model are listed in the online Supporting Information.

To support the interpretation of the results, STRING network analysis (http://string-db.org) was performed with a minimum required interaction score of 0.7 to identify, with high confidence, functional protein associations among obesity-related identified proteins [13]. The Markov clustering algorithm, an unsupervised stochastic clustering method for graphs that clusters strongly interconnected nodes, was applied (with an inflation parameter = 3) to the resulting network to identify additional subclusters.

After transformation by logistic regression analysis, the OIPS, calculated as predicted probability of obesity using the 21 selected inflammatory proteins, was tested for the association with all-cause mortality, cardiac death, incident CAD, and MACE (a composite of nonfatal stroke, nonfatal myocardial infarction, and cardiovascular death) by means of Kaplan–Meier curves and Cox proportional hazard models. Categorization of clinical endpoints is listed in online Supporting Information. To evaluate the performance of OIPS in relationship to an established risk instrument and a standard inflammation biomarker, the Cox regression models included the European Society for Cardiology (ESC) risk score (per 5% increase) and CRP (per 1-SD increase). Finally, a full model was calculated that included all potential confounders such as CVRF, comorbidities, and medications. A significance threshold was not defined due to the explorative nature of the study, and p values were interpreted as continuous measures of statistical evidence. To facilitate interpretation of effects and relative differences in data, 95% confidence intervals (CI) are provided. All statistical analyses were performed in R version 3.5.1 (The R Project for Statistical Computing).

RESULTS

The clinical characteristics of the study sample were stratified according to the World Health Organization (WHO)-defined classification of normal weight (18.5 kg/m^2 ≤ BMI ≤ 24.9 kg/m^2 BMI), overweight (25 kg/m^2 ≤ BMI ≤ 29.9 kg/m^2 BMI), and obesity (BMI ≥ 30 kg/m^2)

and are presented in Table 1. Participants with overweight and obesity were older, with a mean age of 58 years, compared with participants with normal weight, who had a mean age of 54 years. Waist to height ratio (WHtR) and visceral adiposity, defined by waist circumference (≥94 cm in men and ≥80 cm in women), were highest in the participants with obesity (mean [SD], WHtR = 0.66 [0.06]; visceral adiposity = 99.9%) compared with the participants with overweight (WHtR = 0.56 [0.04]; visceral adiposity = 83.4%) and normal weight (WHtR = 0.48 [0.04]; visceral adiposity = 30.5%). Participants with obesity had a higher prevalence of most traditional CVRF, namely

TABLE 1 Demographic and clinical characteristics of the study sample stratified by BMI ($N = 6662$).

	Normal weight (18.5 ≤ BMI < 25)	Overweight (25 ≤ BMI < 30)	Obesity (BMI ≥ 30)
Number	1986	2712	1964
Sex (women)	61.1% (1213)	40.0% (1084)	44.3% (871)
Age (y)	53.9 ± 11.0	57.6 ± 10.5	58.9 ± 10.2
BMI (kg/m^2)	23.1 (21.7/24.1)	27.3 (26.1/28.5)	32.9 (31.3/35.7)
WHtR	0.48 ± 0.04	0.56 ± 0.04	0.66 ± 0.06
Visceral adiposity[a]	30.5% (605)	83.4% (2263)	99.9% (1962)
CVRF			
Arterial hypertension	35.2% (699)	55.7% (1511)	73.5% (1442)
Diabetes mellitus	6.1% (120)	13.4% (363)	29.5% (579)
Smoking	19.7% (392)	17.4% (471)	14.1% (276)
Dyslipidemia	24.6% (489)	41.4% (1122)	51.4% (1007)
FH of myocardial infarction/stroke	20.9% (415)	24.2% (655)	26.4% (518)
Alcohol use (>24/12 g/d)[b]	24.4% (484)	25.1% (681)	19.5% (382)
ESC risk score (10-y risk of CVD)	1.00 (0/3.00)	2.00 (1.00/5.00)	2.00 (1.00/4.00)
Cardiovascular comorbidities			
CAD	3.5% (68)	5.8% (155)	10.3% (196)
Myocardial infarction	2.7% (54)	5.0% (135)	8.3% (161)
Stroke	1.2% (24)	2.5% (68)	3.2% (62)
Peripheral artery disease	2.5% (50)	4.4% (117)	6.6% (128)
Atrial fibrillation	1.5% (30)	3.7% (98)	4.7% (91)
Chronic HF	0.8% (16)	1.7% (46)	3.3% (65)
Venous thromboembolism	4.2% (83)	5.7% (154)	8.5% (166)
Medications (ATC code)			
Antihypertensive (C02)	0.5% (9)	1.2% (32)	2.9% (57)
Diuretics (C03)	1.9% (37)	5.5% (149)	14.4% (282)
Beta-blockers (C07)	9.9% (196)	18.1% (486)	32.6% (638)
Calcium channel blockers (C08)	3.4% (68)	8.4% (225)	15.2% (298)
Agents acting on RAAS (C09)	13.4% (265)	27.1% (729)	44.1% (863)
Lipid-modifying (C10)	9.2% (181)	17.3% (466)	25.6% (500)
Drugs used in diabetes (A10)	3.5% (70)	8.1% (219)	20.3% (397)
Insulins and analogues (A10A)	1.0% (19)	2.6% (70)	6.9% (134)
Metformin (A10BA02)	1.9% (38)	4.8% (130)	13.7% (268)
Sulfonylureas (A10BB)	1.0% (19)	1.6% (44)	3.6% (70)
Anti-inflammatory and antirheumatic (M01)	9.5% (188)	11.8% (317)	16.5% (322)
Antithrombotics (B01A)	8.9% (177)	15.9% (429)	24.3% (475)
Corticosteroids systemic (H02)	1.2% (24)	1.7% (46)	2.0% (39)

Note: Data are presented as mean ± SD, median (Q1/Q3), or relative and absolute frequencies.

Abbreviations: ATC, anatomical therapeutic chemical classification system; CAD, coronary artery disease; CVD, cardiovascular disease; CVRF, cardiovascular risk factors; ESC, European Society of Cardiology; FH, family history; HF, heart failure; Q, quartile; RAAS, renin-angiotensin-aldosterone system; WHtR, waist to height ratio.

[a]Visceral adiposity defined (WHO-based) by waist circumference (≥94 cm for men and ≥80 cm for women).

[b]Alcohol use above the tolerable upper alcohol intake levels [14].

arterial hypertension, diabetes mellitus, dyslipidemia, and family history of myocardial infarction or stroke. Smoking (14.1%) and alcohol use above the tolerable upper alcohol intake levels (19.5%) [14] were less common in those with obesity. History of CVD, i.e., myocardial infarction, stroke, peripheral arterial disease, atrial fibrillation, chronic HF, and venous thromboembolism, was more common in the groups with overweight and obesity compared with the group with normal weight. The use of medications for the treatment of CVD, lipid-modifying agents, antidiabetic drugs, antithrombotics, and anti-inflammatory nonsteroidal and corticosteroid drugs was also reported more frequently by participants with obesity and overweight compared with participants with normal weight.

The laboratory profile of the investigated participants, reported in Table S3, revealed increased concentrations for standard inflammatory parameters CRP (milligrams per liter) and fibrinogen (milligrams per deciliter) in those with obesity (CRP = 2.70; IQR: 1.50–5.00 and fibrinogen = 357; IQR: 309–413) compared with participants with overweight (CRP = 1.60; IQR: 0.89–3.00 and fibrinogen = 338; IQR: 297–388) and normal weight (CRP = 1.10; IQR: 0.50–1.90 and fibrinogen = 320; IQR: 281–363). Fasting glucose, hemoglobin A1c (percentage), C-peptide, insulin, and homeostatic model assessment of insulin resistance index were higher in the group with obesity than in the groups with overweight and normal weight. Participants with obesity presented with the highest triglyceride levels and fatty liver index scores compared with participants with overweight and normal weight. In addition, the estimated glomerular filtration rate (milliliters per minute per 1.73 m^2) was lower in both participants with obesity and overweight (mean [SD], 84.07 [15.10] and 84.79 [13.70], respectively) than in participants with normal weight (87.32 [12.86]).

OIPS and network analysis

In this study, an OIPS consisting of 21 unique proteins was identified that was independent of age, sex, cardiovascular status, and medications (Table 2). A larger number of the selected proteins showed a negative association with obesity, and seven proteins showed a positive association with obesity, i.e., hepatocyte growth factor (HGF), macrophage colony-stimulating factor 1 (CSF-1), IL-6, IL-12 subunit β

TABLE 2 The obesity-related inflammatory protein signature (OIPS).

Protein name (abbreviation)	UniProt No	OR (95% CI)	*p* value	Direction of association
Hepatocyte growth factor (HGF)	P14210	1.95 (1.71–2.22)	<0.0001	Positive
Axis inhibition protein 1 (AXIN1)	O15169	1.33 (1.14–1.54)	0.00028	Positive
Macrophage colony-stimulating factor 1 (CSF-1)	P09603	1.31 (1.15–1.48)	<0.0001	Positive
Interleukin-6 (IL-6)	P05231	1.30 (1.18–1.43)	<0.0001	Positive
Interleukin-12 subunit β (IL-12B)	P29460	1.22 (1.11–1.35)	<0.0001	Positive
TNF ligand superfamily member 11 (TRANCE)	O14788	1.20 (1.10–1.31)	<0.0001	Positive
Adenosine deaminase (ADA)	P00813	1.19 (1.10–1.30)	<0.0001	Positive
C-X-C motif chemokine ligand 9 (CXCL9)	Q07325	0.73 (0.64–0.82)	<0.0001	Negative
C-C motif chemokine ligand 28 (CCL28)	Q9NRJ3	0.75 (0.68–0.83)	<0.0001	Negative
Programmed cell death 1 ligand 1 (PDL1)	Q9NZQ7	0.77 (0.69–0.85)	<0.0001	Negative
Matrix metalloproteinase-10 (stromelysin-2) (MMP10)	P09238	0.79 (0.73–0.86)	<0.0001	Negative
Delta and notch-like epidermal growth factor-related receptor (DNER)	Q8NFT8	0.80 (0.73–0.88)	<0.0001	Negative
Fibroblast growth factor 19 (FGF19)	O95750	0.81 (0.76–0.87)	<0.0001	Negative
Stem cell factor (SCF)	P21583	0.81 (0.75–0.88)	<0.0001	Negative
Interleukin-8 (IL-8)	P10145	0.81 (0.73–0.90)	0.00015	Negative
TNF ligand superfamily member 11 (OPG)	O00300	0.81 (0.73–0.91)	0.00020	negative
Glial cell line-derived neurotrophic factor (GDNF)	P39905	0.83 (0.76–0.91)	<0.0001	Negative
Cystatin-D (CST5)	P28325	0.83 (0.77–0.90)	<0.0001	Negative
TNF ligand superfamily member 12 (TWEAK)	O43508	0.83 (0.75–0.92)	0.00033	Negative
C-C motif chemokine ligand 23 (CCL23)	P55773	0.84 (0.77–0.91)	<0.0001	Negative
Neurotrophin-3 (NT 3)	P20783	0.85 (0.79–0.92)	<0.0001	Negative

Note: Presented are proteins identified by multivariable logistic regression analysis, adjusted for age, sex, traditional cardiovascular risk factors, comorbidities, and medications (full list of covariates in online Supporting Information) after Bonferroni multiple testing correction at a threshold of $p < 0.0004$. Dependent variable was BMI-defined obesity (BMI ≥ 30 kg/m^2) with $n = 1964$ participants.
Abbreviation: OR, odds ratio.

(IL-12B), tumor necrosis factor (TNF) ligand superfamily member 11 (TRANCE), adenosine deaminase (ADA), and axis inhibition protein 1 (AXIN1). The highest odds ratios (OR) for the group with obesity were observed for HGF (OR = 1.95; 95% CI: 1.71–2.22), followed by AXIN1 (OR = 1.33; 95% CI: 1.14–1.54), CSF-1 (OR = 1.31; 95% CI: 1.15–1.48), and IL-6 (OR = 1.30; 95% CI: 1.18–1.43). There was a positive correlation between OIPS and BMI, as shown in Figure S1. As reported in Table S4, the OIPS explained 37% of the variance in BMI. Further adjustments in the linear regression model for age, sex, and CRP only slightly increased the explained variance of BMI to 39%.

Figure 1 depicts the STRING network analysis of the proteins forming the OIPS. Using the Markov clustering algorithm, the following three protein clusters were identified: a cluster with nine interconnected proteins (nodes) and fourteen known protein–protein interactions that were largely involved in chemokine activity; a second cluster with three proteins and two known protein–protein interactions involved in TNF receptor binding; and a third cluster with two proteins and one interaction involved in chemoattractant activity and growth factor receptor binding. Six proteins showed no known protein–protein interactions, of which AXIN1 was positively associated with obesity, and delta and notch-like epidermal growth factor-related receptor (DNER), C-C motif chemokine ligand 23 (CCL23), CCL28, matrix metalloproteinase-10 (stromelysin-2) (MMP10), and cystatin-D (CST5) were negatively associated with obesity.

Relationship between the OIPS and clinical outcomes

During a median follow-up of 12.9 years (IQR = 11.1–13.6 years), a total of $n = 695$ participants died, 133 of whom died of cardiac death. Figure 2 demonstrates the association of OIPS with all-cause mortality, cardiac death, MACE, and incident CAD. Cox proportional hazards regression analyses with adjustment for age and sex found that the OIPS predicted an increased risk of death from any cause (hazard ratio [HR] = 1.396; 95% CI: 1.30–1.49; $p < 0.0001$), cardiac death (HR = 1.64: 95% CI: 1.37–1.95; $p < 0.0001$), MACE (HR = 1.29; 95% CI: 1.16–1.43; $p < 0.0001$), and incident CAD (HR = 1.32; 95% CI: 1.17–1.48; $p < 0.0001$). Further adjustment of the model for CRP or ESC risk score did not change the observed associations (data not shown). In the fully adjusted Cox regression model for age, sex, traditional CVRF, comorbidities, and medications (full list of covariates listed in online Supporting Information), the OIPS remained an important independent predictor for worse cardiovascular outcomes, as shown in Table 3.

Cox proportional hazards regression analyses were further computed in BMI-stratified categories, i.e., normal weight, overweight, and obesity, for the association of OIPS, CRP, and ESC risk score and cardiovascular clinical outcomes, independent of age and sex. As shown in Table S5, the OIPS showed a robust association with death from any cause and cardiac death in all BMI categories, whereas CRP showed an

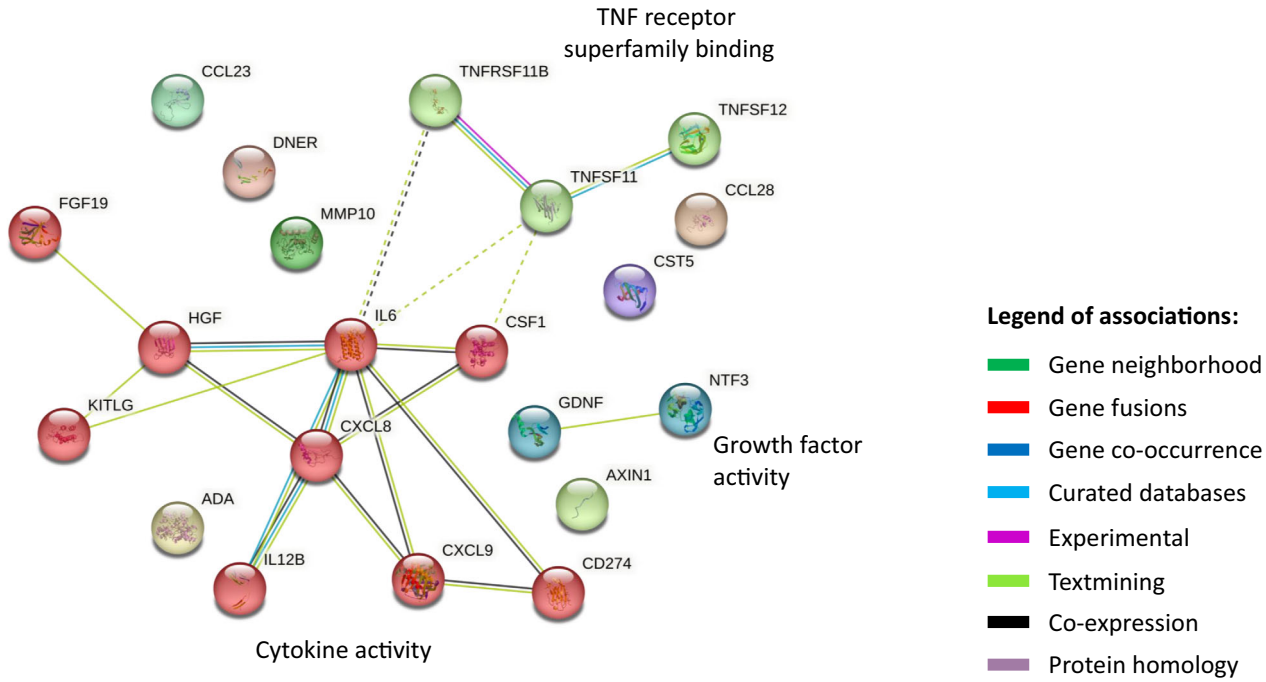

FIGURE 1 Network analysis of the GHS-derived OIPS. Node colors indicate cluster membership, as determined using the unsupervised Markov clustering algorithm. Solid lines indicate intra-cluster connections. Protein–protein connections (i.e., the edges of the graph) reflect experimentally established and from curated databases protein–protein interactions, co-expression patterns, and putative connections based on text mining. ADA, adenosine deaminase; AXIN1, axis inhibition protein 1; CCL, C-C motif chemokine ligand; CSF, colony-stimulating factor; CD274, programmed cell death 1 ligand 1; CST5, cystatin-D; CXCL, C-X-C motif chemokine ligand; DNER, delta and notch-like epidermal growth factor-related receptor; FGF, fibroblast growth factor; GDNF, glial cell line-derived neurotrophic factor; GHS, Gutenberg Health Study; HGF, hepatocyte growth factor; KITLG, KIT ligand; MMP, matrix metallopeptidase; NTF, neurotrophin; OIPS, obesity-related inflammatory protein signature; TNFRSF, TNF receptor superfamily member; TNFSF, TNF superfamily member. [Color figure can be viewed at wileyonlinelibrary.com]

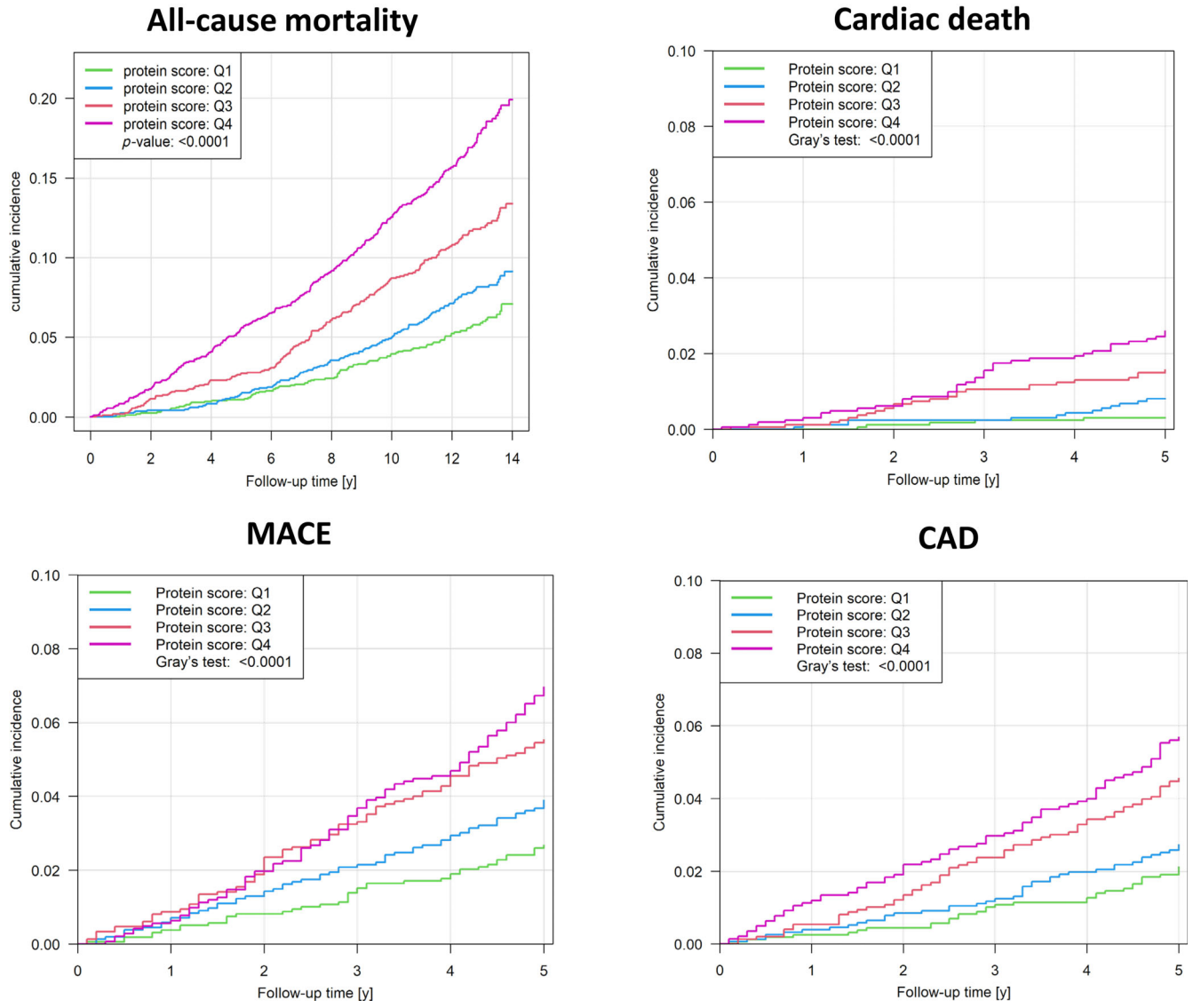

FIGURE 2 Association of OIPS with mortality, MACE, and incident CAD. Presented are Kaplan–Meier survival curves for increasing quartiles of OIPS. (A) Association with all-cause mortality. (B) Association with cardiac death. (C) Association with three-component MACE. (D) Association with incident CAD. CAD, coronary artery disease; MACE, major adverse cardiovascular events; OIPS, obesity-related inflammatory protein signature. [Color figure can be viewed at wileyonlinelibrary.com]

association with lower HR in the groups with normal weight and overweight compared with OIPS and a weaker association in those with obesity. Contrarily, ESC score was associated with higher risk of all-cause mortality and cardiac death only in the group with normal weight. For the three-component MACE, CRP showed an association for all BMI categories, whereas the OIPS showed an important association in the groups with overweight and obesity, and ESC score was associated with MACE in participants with normal weight and overweight. Interestingly, both the OIPS and CRP showed a significant association with incident CAD in participants with normal weight and overweight, whereas ESC risk score was associated with incident CAD only in the group with overweight. The association of OIPS and incident CAD was of borderline significance.

Figure 3 shows the HR for OIPS and clinical outcomes after adjustment for age, sex, ESC risk score, and CRP. The results confirmed a relevant association between OIPS and death from any cause and cardiac death in participants with overweight (all-cause death: HR = 1.32; 95% CI: 1.10–1.59; $p = 0.0027$ and cardiac death: HR = 1.44; 95% CI: 1.03–2.00; $p = 0.030$) and obesity (all-cause death: HR = 1.31; 95% CI: 1.10–1.55; $p = 0.0020$ and cardiac death: HR = 1.78; 95% CI: 1.17–2.70; $p = 0.0066$) and an increased risk of incident CAD in participants with obesity (HR = 1.51; 95% CI: 1.15–1.98; $p = 0.0033$).

Replication in the MyoVasc study

The clinical characteristics of the MyoVasc study population are presented in Table S6. The mean age was 63.8 (SD 11.2) years, and 35.9% (960) were female. There were $n = 699$ individuals with normal weight,

TABLE 3 Association of the OIPS with clinical outcome in the overall GHS sample ($N = 6662$).

OIPS	Model 1 (Age + sex), HR (95% CI)	*p* value	Model 2 (Age + sex + CVRF + comorbidities + medications), HR (95% CI)	*p* value
All-cause mortality	1.396 (1.304–1.494)	<0.0001	1.238 (1.143–1.341)	<0.0001
Cardiac death	1.635 (1.372–1.948)	<0.0001	1.448 (1.194–1.758)	0.00018
CAD	1.317 (1.172–1.479)	<0.0001	1.227 (1.067–1.411)	0.0041
MACE	1.286 (1.158–1.428)	<0.0001	1.173 (1.043–1.318)	0.0075

Note: The reported HR illustrate the association of OIPS per increase of 1 SD and the clinical outcome. Model 1 was adjusted for age and sex. Model 2 was adjusted for age; sex; CVRF, i.e., arterial hypertension, diabetes mellitus, dyslipidemia, smoking, family history of myocardial infarction or stroke, and alcohol use > 24/12 g/d; comorbidities, i.e., CAD, myocardial infarction, stroke, peripheral artery disease, atrial fibrillation, chronic heart failure, chronic kidney disease, chronic liver disease, venous thromboembolism, and chronic obstructive pulmonary disease; and medications, i.e., antidiabetic drugs (A10), antithrombotic (B01A), cardiac therapy (C01), antihypertensive (C02), diuretics (C03), beta-blockers (C07), calcium channel blockers (C08), acting on renin-angiotensin-aldosterone system (C09), lipid-modifying agents (C10), corticosteroids systemic (H02), thyroid therapy (H03), anti-inflammatory and antirheumatic (M01), and agents used in obstructive airway diseases (R03).

Abbreviations: CAD, coronary artery disease; CVRF, cardiovascular risk factors; GHS, Gutenberg Health Study; HR, hazard ratios; MACE, major adverse cardiovascular events; OIPS, obesity-related inflammatory protein signature.

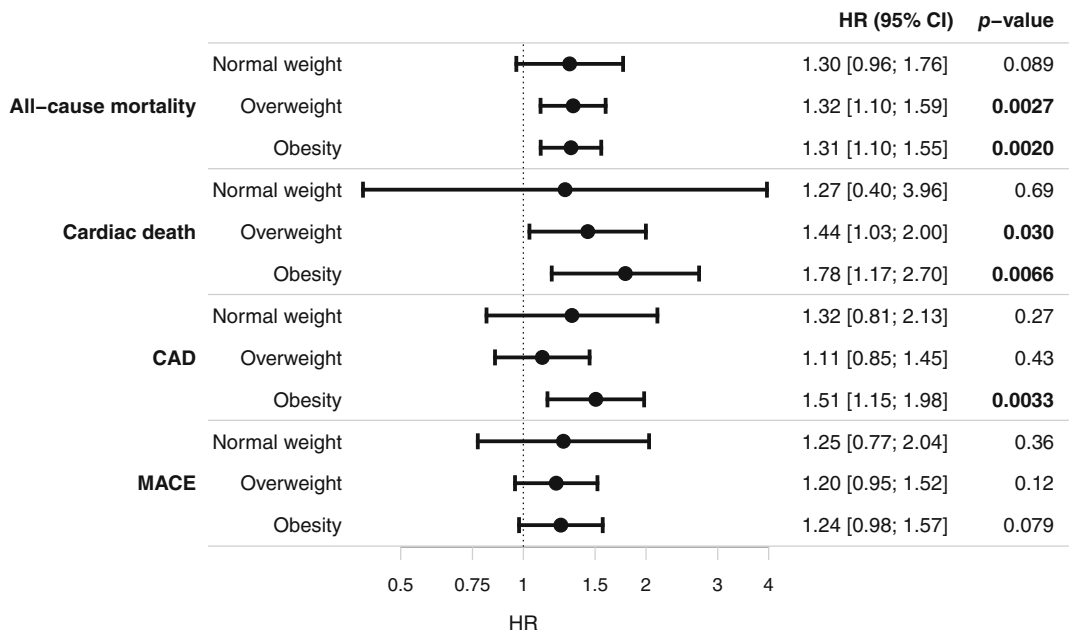

FIGURE 3 Association of OIPS and clinical outcome according to BMI categories. Presented are HR for OIPS as a predictor for all-cause mortality, cardiac death, incident CAD, and three-component MACE for participants with normal weight, overweight, and obesity after adjustment for age, sex, European Society of Cardiology risk score, and C-reactive protein. CAD, coronary artery disease; HR, hazard ratios; MACE, major adverse cardiovascular events; OIPS, obesity-related inflammatory protein signature.

$n = 1135$ with overweight, and $n = 839$ with obesity. Symptomatic HF (American Heart Association [AHA] stage C and D) was diagnosed in 36.9% of individuals with normal weight, 49.7% of individuals with overweight, and 63.6% of individuals with obesity. During a median follow-up of 5.4 years (IQR = 4.09–6.87 years), a total of $n = 317$ individuals died, $n = 116$ of whom died of cardiac death. Worsening of HF, defined as a composite of transition from asymptomatic to symptomatic HF, hospitalization due to HF, or cardiac death, occurred in $n = 437$ individuals during a median follow-up of 3.07 years (IQR = 2.07–5.01 years). The GHS-derived OIPS was highly correlated with the MyoVasc-generated inflammatory protein score (Spearman rank correlation coefficient: $r = 0.93$). In Cox proportional hazard analyses, as shown in Table 4, the GHS-derived OIPS with adjustment for age and sex was found to independently predict all-cause mortality (HR = 1.45; 95% CI: 1.30–1.60; $p < 0.0001$), cardiac death (HR = 1.41; 95% CI: 1.20–1.65; $p < 0.0001$), worsening of HF (HR = 1.32; 95% CI: 1.21–1.43; $p < 0.0001$), and HF hospitalization (HR = 1.36; 95% CI: 1.21–1.51; $p < 0.0001$) in the replication cohort. Further adjustment for CVRF, comorbidities, and medications did not significantly change the observed associations with the clinical outcome.

TABLE 4 Association of the GHS-derived OIPS with clinical outcome in the MyoVasc cohort study on HF ($N = 2683$).

GHS OIPS	Model 1 (Age + sex), HR (95% CI)	*p* value	Model 2 (Age + sex + CVRF + comorbidities + medications), HR (95% CI)	*p* value
All-cause mortality	1.445 (1.304–1.600)	<0.0001	1.268 (1.131–1.421)	<0.0001
Cardiac death	1.410 (1.202–1.653)	<0.0001	1.206 (1.010–1.441)	0.039
Worsening of HF	1.318 (1.213–1.433)	<0.0001	1.174 (1.069–1.290)	0.00079
HF hospitalization	1.355 (1.214–1.513)	<0.0001	1.140 (1.005–1.293)	0.041

Note: Model 1 was adjusted for age and sex. Model 2 was adjusted for age; sex; CVRF, i.e., arterial hypertension, diabetes mellitus, dyslipidemia, smoking, family history of myocardial infarction or stroke, and alcohol use > 24/12 g/d; comorbidities, i.e., CAD, myocardial infarction, stroke, peripheral artery disease, atrial fibrillation, chronic heart failure, chronic kidney disease, chronic liver disease, venous thromboembolism, and chronic obstructive pulmonary disease; and medications, i.e., antidiabetic drugs (A10), antithrombotic (B01A), cardiac therapy (C01), antihypertensive (C02), diuretics (C03), beta-blockers (C07), calcium channel blockers (C08), acting on renin-angiotensin-aldosterone system (C09), lipid-modifying agents (C10), corticosteroids systemic (H02), thyroid therapy (H03), anti-inflammatory and antirheumatic (M01), and agents used in obstructive airway diseases (R03).
Abbreviations: CVRF, cardiovascular risk factors; GHS, Gutenberg Health Study; HR, hazard ratios; OIPS, obesity-related inflammatory protein signature.

DISCUSSION

In this large population-based prospective cohort study, an OIPS was identified that reflected an association independent of age, sex, traditional CVRF, comorbidities, and medications. The OIPS comprised 21 unique inflammatory proteins involved mainly in chemokine activity, TNF receptor binding, or growth factor receptor binding. The obesity signature was tested for the prediction of death from any cause, cardiac death, MACE, and incident CAD and was externally validated in an independent cohort.

The major findings of this study are, first, that the OIPS had a significant association with all-cause death, cardiac death, MACE, and incident CAD, independent of the established ESC risk score for CVD and the standard marker of inflammation CRP, as well as traditional CVRF, comorbidities, and medications in the general population; second, the OIPS was particularly relevant for the relationship with worse outcomes in the subgroups with overweight and obesity; third, the association of the GHS-derived OIPS with all-cause death and cardiac death was confirmed in an external independent validation cohort of participants with cardiac and vascular dysfunction; and fourth, in this validation cohort, the obesity signature was also associated with more specific events such as worsening of HF and HF hospitalization.

Patients living with obesity as a chronic and relapsing disease have limited successful treatment modalities [15]. The difficulty of adhering to lifestyle interventions and the challenges arising from invasive approaches such as bariatric surgery, which are ultimately often followed by weight regain, place patients with obesity at ongoing long-term risk for cardiovascular events. Current ESC guidelines on CVD prevention do not consider patients with obesity as a specific group at risk and, rather, focus on patients with established atherosclerotic disease, type 2 diabetes mellitus, at risk for chronic kidney disease [16]. All of these conditions frequently complicate the life course of patients living with overweight or obesity and have direct implications with an increase in morbidity and mortality. Understanding the inflammatory molecular pattern in obesity, which emanates predominantly from adipose tissue dysfunction, could improve the identification of patients at risk for obesity-associated cardiovascular complications [17].

Several circulating inflammatory proteins have been associated with various measures of obesity such as the standard BMI, waist circumference and its ratios to height and hips, visceral adiposity index, and more complex assessments such as the body fat distribution measurement [18]. The current study confirmed the known association of IL-6, IL-8, and HGF, with HGF and IL-6 being positively associated with obesity and having the highest OR, whereas IL-8 showed an inverse association with obesity [19, 20]. IL-6 is one of the major proinflammatory cytokines expressed and released by adipose tissue cells, and it is known to stimulate increased production of CRP by the liver and induce endothelial dysfunction and has been repeatedly associated with worse cardiovascular outcomes [21, 22]. Lowering IL-6 concentrations by targeting the upstream pathway with canakinumab was associated with a lower rate of cardiovascular events, independent of lipid-lowering, highlighting the substantial pathophysiological consequences of IL-6 for CVD [23]. Increased serum HGF has also been linked with cardiovascular complications, including myocardial infarction, congestive HF, ischemic retinopathy, and peripheral arterial disease [24–27]. In addition to IL-6 and HGF, CSF-1, ADA, IL-12B, TRANCE, and AXIN1 were also positively associated with obesity. CSF-1 controls macrophage differentiation via its transmembrane receptor CSF-1R, and its circulating levels have been reported to be increased in obesity and associated with insulin resistance [28]. ADA is a polymorphic enzyme that catalyzes the irreversible deamination of adenosine to inosine and has significantly increased activity in individuals with obesity compared with those with overweight and normal weight [29]. A recent study in mice demonstrated that circulating ADA acting on RNA1 (double-stranded RNA-specific adenosine deaminase) promotes diet-induced obesity by modulating the expression and secretion of ghrelin and peptide YY [30]. Consistent with a recent population-based work, IL-12B was identified as having a positive association with obesity [31]. IL-12 family cytokines produced by macrophages, dendritic cells, and natural killer cells have been reported to be increased in obesity and associated with the development of obesity-associated comorbidities, particularly atherosclerosis, macrovascular complications, and diabetic retinopathy [32, 33]. Soluble TRANCE, an osteoclast

differentiation factor, also known as TNF ligand superfamily member 11 (TNF11) or receptor activator of nuclear factor-kB ligand (RANKL), was recently identified as having a positive association with obesity in 43 young adult monozygotic twins and in 804 participants from the population-based KORA-Fit study [18, 34]. Epidemiological and animal studies have suggested that excessive fat accumulation has harmful effects on bone mass and that this association may be explained, at least in part, by altered levels of TRANCE/RANKL and osteoprotegerin (OPG), a decoy receptor for RANKL that neutralizes its function [35]. The triad of RANKL, its receptor RANK, and OPG was further reported to have a role in atherosclerotic vascular calcification, angiogenesis, and inflammation [36]. In this work, OPG showed a negative association with BMI, as has been previously observed in individuals with obesity [37].

The present work is the first, to our knowledge, to report a positive association between AXIN1 and obesity. AXIN1 has been originally identified as a negative regulator of the Wnt/ß-catenin signaling pathway, which plays an important role in development, cell proliferation, and differentiation [38]. Because AXIN1 downregulates ß-catenin protein levels, it has been reported to function as a tumor suppressor and has been proposed as a potential therapeutic target for hepatocellular and colorectal cancers [39]. Canonical Wnt/β-catenin signaling influences diverse physiological processes because whole-body metabolism and aberration in this signaling has been linked to the development of cardiometabolic disorders, including obesity [40]. In addition, AXIN1 activates transforming growth factor-β signaling and plays an important role in a c-Jun NH_2-terminal kinase (JNK) signaling pathway, which is also relevant in obesity [41]. A role for AXIN1 in cardiomyocyte injury has been suggested in experimental conditions mimicking obstructive sleep apnea [42]. Considering the relevance of AXIN1 in canonical Wnt/β-catenin and transforming growth factor-β signaling, further characterization of the novel association in obesity holds the potential to identify novel mechanisms for the development of obesity-associated complications.

This study is the first, to our knowledge, to demonstrate an important association between the inflammatory signature related to obesity and clinical outcomes. Most of the proteins identified in this investigation have already been linked with obesity or other measures relevant to adiposity. A meta-analysis of 29 population-based prospective cohort studies found that an increase of 1 SD each in IL-6, IL-18, and TNFα was associated with an ∼10% to 25% higher risk of nonfatal myocardial infarction or death from coronary heart disease [43].

In a comprehensive approach, the present study accounted for all known clinical covariates that significantly influence the inflammatory profile. The OIPS showed a significant association with all-cause mortality, outperforming ESC risk score and CRP in participants with overweight and obesity. Whereas the standard ESC risk score did not predict an increased risk of cardiac death, incident CAD, or three-component MACE in participants with overweight and obesity, the OIPS showed strong associations with all three endpoints. Interestingly, in individuals with normal weight, the OIPS performed similarly to CRP and ESC risk score for all-cause mortality and cardiac death and outperformed ESC risk score for incident CAD. The identified signature was validated in an independent cohort of individuals with HF, in which it confirmed the association with all-cause mortality and cardiac death. In addition, this study evaluated the association with HF outcome and found that the OIPS was strongly associated with HF hospitalization and worsening of HF. This is particularly relevant considering that obesity is an important risk factor for the development of HF [44].

There are some limitations that are important to consider. By its design, this study was limited to circulating inflammatory proteins in blood plasma and did not include a direct proteomic assessment in metabolically sensitive tissues. Moreover, although statistical models were adjusted for major potential confounders, covariates such as inter-individual variation in diet and cardiovascular fitness, which have been reported to be important in the relationship between obesity and mortality [45], could not be assessed. However, the present study has several strengths that should be mentioned. Unlike previous reports that have used the same proximity extension assay technology to identify obesity-related inflammatory proteins, in the present work, we considered a wide range of potential confounders that are commonly associated with obesity. In addition, this is the largest population-based study to identify an inflammatory signature in obesity and the first study, to our knowledge, to assess the association of the signature with multiple clinical endpoints relevant to obesity. By validating the results in an independent cohort of participants presenting with HF, a disease relevant to obesity, we were able to provide additional support for the findings of this investigation.

CONCLUSION

The newly identified OIPS proves to be a promising tool for identifying individuals with overweight and obesity who are at higher risk for all-cause mortality and adverse cardiovascular outcomes. The newly identified association of AXIN1 and obesity deserves further characterization to identify novel mechanisms for the development of obesity-associated complications.O

AUTHOR CONTRIBUTIONS

Marina Panova-Noeva, Philipp S. Wild, Anita M. Hennige, and Corinna Schoelch designed the research. Andreas Schulz performed the statistical analysis. Thomas Koeck, Jürgen H. Prochaska, Matthias Michal, Konstantin Strauch, Alexander K. Schuster, Karl J. Lackner, and Thomas Münzel conducted the research. All authors participated in data interpretation. Marina Panova-Noeva and Philipp S. Wild drafted the manuscript. All authors critically revised and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

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SUPPORTING INFORMATION

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

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