ORIGINAL RESEARCH

Urinary Proteomic Profile of Arterial Stiffness Is Associated With Mortality and Cardiovascular Outcomes

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BACKGROUND: The underlying mechanisms of arterial stiffness remain not fully understood. This study aimed to identify a urinary proteomic profile to illuminate its pathogenesis and to determine the prognostic value of the profile for adverse outcomes.

METHODS AND RESULTS: We measured aortic stiffness using pulse wave velocity (PWV) and analyzed urinary proteome using capillary electrophoresis coupled with mass spectrometry in 669 randomly recruited Flemish patients (mean age, 50.2 years; 51.1% women). We developed a PWV-derived urinary proteomic score (PWV-UP) by modeling PWV with proteomics data at baseline through orthogonal projections to latent structures. PWV-UP that consisted of 2336 peptides explained the 65% variance of PWV, higher than 36% explained by clinical risk factors. PWV-UP was significantly associated with PWV (adjusted β=0.73 [95% CI, 0.67–0.79]; $P<0.0001$). Over 9.2 years (median), 36 participants died, and 75 experienced cardiovascular events. The adjusted hazard ratios (+1 SD) were 1.46 (95% CI, 1.08–1.97) for all-cause mortality, 2.04 (95% CI, 1.07–3.87) for cardiovascular mortality, and 1.39 (95% CI, 1.11–1.74) for cardiovascular events ($P≤0.031$). For PWV, the corresponding estimates were 1.25 (95% CI, 0.97–1.60), 1.35 (95% CI, 0.85–2.15), and 1.22 (95% CI, 1.02–1.47), respectively ($P≥0.033$). Pathway analysis revealed that the peptides in PWV-UP mostly involved multiple pathways, including collagen turnover, cell adhesion, inflammation, and lipid metabolism.

CONCLUSIONS: PWV-UP was highly associated with PWV and could be used as a biomarker of arterial stiffness. PWV-UP, but not PWV, was associated with all-cause mortality and cardiovascular mortality, implying that PWV-UP–associated peptides may be multifaceted and involved in diverse pathological processes beyond arterial stiffness.

Key Words: arterial stiffness ■ biomarkers ■ population science ■ proteomics ■ pulse wave velocity

Arterial stiffness reflects the altered structural and functional properties of the arterial wall attributable to vascular aging.[1] It has been demonstrated as a strong independent predictor of cardiovascular events and mortality.[2–4] As vascular stiffening affects pulse wave transmission, aortic stiffness can be quantified by carotid-femoral pulse wave velocity (cfPWV). A meta-analysis of 17 prospective studies with 15 877 participants revealed that each 1-m/s increase in aortic

pulse wave velocity (PWV) was associated with 14% increased risk of total cardiovascular events, 15% increased risk of cardiovascular mortality, and 15% increased risk of all-cause mortality.[3] Although PWV is an intuitive metric of hemodynamic alterations attributable to arterial stiffness, it does not directly reveal the underlying mechanisms of arterial stiffness. From the perspective of personalized medicine, it is necessary to propose molecular biomarkers to complement PWV.

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CLINICAL PERSPECTIVE

What Is New?

- A prospective cohort study including 669 Flemish patients investigated the urinary proteomic profile for arterial stiffness determined by pulse wave velocity and assessed its associations with cardiovascular events and mortality.
- The proteomic profile that was related to multiple pathological processes showed a high correlation with pulse wave velocity, stronger than conventional clinical risk factors.
- The proteomic profile for arterial stiffness was superior to pulse wave velocity in terms of the prediction of adverse outcomes, especially for all-cause mortality and cardiovascular mortality.

What Are the Clinical Implications?

- The potential of using urinary proteomics fingerprinting biomarkers to recognize the underlying pathophysiological processes and to facilitate the search for personalized treatment targets is described.
- Given the superior prognostic value, the proteomic profile could be used as an alternative surrogate of arterial stiffness for better prediction of adverse outcomes.

Nonstandard Abbreviations and Acronyms

CE-MS	capillary electrophoresis coupled with mass spectrometry
cfPWV	carotid-femoral pulse wave velocity
OPLS	orthogonal projections to latent structures
PWV	pulse wave velocity
PWV-UP	pulse wave velocity–derived urinary proteomic score
VIP	variable importance in the projection

Urine contains diverse proteins and peptides that predominately originate from plasma, renal tubules, or lower urinary tract.[5] Small-to-medium sized molecular proteins that leak from local tissues into blood circulation could be excreted in urine through glomerular filtration.[5] Therefore, urinary proteins/peptides have the potential to show pathological changes underlying kidney diseases and various other disorders, including cardiovascular diseases. To date, the emerging urinary proteomics have been used for biomarker discovery, risk stratification, and elucidation of pathological mechanisms.[6–9] To the best of our knowledge, there is no published study of urinary proteomics in relation to arterial stiffness. Prior proteomic studies investigated the proteomic profile of arterial stiffness in plasma and arterial tissue, but were limited to a small sample size, a cross-sectional study design, or only in young healthy adults.[10,11]

Given these circumstances, we aimed to identify a urinary proteomic profile of arterial stiffness at baseline and to determine its prognostic value for adverse outcomes in a prospective population. In addition, we further discussed the pathological processes of the proteomic profile through pathway analyses.

METHODS

The authors declare that all supporting data are available within the article and its online supplemental material.

Study Population

This study was conducted in a family-based population study, the FLEMENGHO (Flemish Study on Environment, Genes and Health Outcomes). The enrollment of the FLEMENGHO started from 1985 through 2004 with an initial participation rate of 78%.[7,12] The study was approved by the University of Leuven Ethics Committee.[7,12] All participants provided written informed consent. Participants underwent repeated follow-up. From May 2005 until May 2010, there were 688 eligible participants who had undergone PWV measurements and urinary proteomics analysis. Because of the inadequate quality of PWV measurement, 19 were excluded. Hence, 669 participants were included.

PWV Measurement

As the impaired compliance of stiffening aortic arteries accelerates the velocity of pulse wave propagation, the American Heart Association has endorsed the use of cfPWV to quantify aortic stiffness.[1] PWV was calculated as the length divided by the transit time of pulse wave travel in the segment from the right carotid artery to the femoral artery.[13] In this study, PWV was evaluated using the tonometry-based SphygmoCor Device (AtCor Medical, West Ryde, New South Wales, Australia). The robustness of this device and the reproducibility have been demonstrated in previous studies.[12,14] For quality control, recordings were excluded when the deviation of pulse wave was >10%.

Urinary Proteomics Analysis

Detailed information on sample preparation, proteome analysis using capillary electrophoresis coupled with mass spectrometry (CE-MS), data processing, and

sequencing of the peptides has been described in previous publications and the Supplemental Methods (Data S1).[15,16] Briefly, CE-MS was performed, using a P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA) coupled to a micrOTOF MS (Bruker Daltonics, Bremen, Germany). Mass spectral data were processed with MosaiquesVisu software, generating a raw list of peptides or proteins with molecular mass, migration time, and signal intensity that were calibrated using internal urinary standard peptides to assure the comparability between different data sets.[17,18] Identified peptides were assigned to the previously sequenced peptides from the Human Urinary Proteome Database by fragmenting peptides and matching the fragmentation spectra to the protein sequences from the database's International Protein Index, the reference sequence database at National Center for Biotechnology Information, and the UniProt Knowledgebase.[19] In the protein annotation, posttranslational modifications and specific mass spectra were considered. Peptides from different samples were considered the same when the deviations of their molecular weight and the migration time were <100 parts/million and <1 minute, respectively. As high-dimensional data are prone to sparsity, multicollinearity with a high risk of overfitting, peptides were excluded when they were undetectable in >70% of the participants.[20] Thus, of the 21 559 detected urinary peptides, 2336 were eventually analyzed.

Assessment of the Outcomes

The vital status of all the participants was annually ascertained through the Belgian Population Registry in Brussels until December 31, 2016. In cases of death, the causes were indicated with the *International Classification of Diseases, Ninth Revision* (*ICD-9*) codes that were acquired from the Flemish Registry of Death Certificates. Information on nonfatal events was collected via either face-to-face follow-up visits at the examination center with repeated administration of the standard questionnaire that had been used at baseline or a structured telephone interview. Coronary events included sudden death, myocardial infarction, acute coronary syndrome, new-onset angina pectoris, ischemic cardiomyopathy, and coronary revascularization. Cardiac events included all coronary events and heart failure, new-onset atrial fibrillation, life-threatening arrhythmias and high-degree atrioventricular block that required pacemaker implantation, and pulmonary heart disease. Cardiovascular events included cardiac events and were additionally considered aortic aneurysm, atrial embolism and revascularization of peripheral arteries, stroke, and transient ischemic attack. Physicians ascertained the diseases reported on the death certificates, in the questionnaires, and in the telephone interviews against the medical records of general practitioners or hospitals. Participants were censored when the first event within each category occurred.

Statistical Analysis

Statistical analysis was performed with SAS software, version 9.4 (SAS Institute, Cary, NC). Statistical significance was determined with a 2-sided P value of 0.05. Means and proportions were compared via t test or ANOVA test, or Fisher test as appropriate. Because the high dimensionality and multicollinearity of the proteomic data remain unresolved for the conventional linear regression model, the orthogonal projections to latent structures (OPLS) method was used. As a variant of partial least squares method, OPLS is a supervised dimension reduction statistical method. It diminishes the data dimension by projecting the original data into a new space and constructing latent variables (components) to linearly model the dependent variables. Unlike partial least squares, OPLS initially filters the "noise" information that is irrelevant to dependent variables, then builds fewer latent variables. Hence, OPLS models are simpler and easier to interpret.[21,22] With a data matrix comprising of 2336 peptides and PWV in 669 participants, the OPLS analysis was conducted using the SIMCA software, version 14.1 (Umetrics, Sartorius-Stedim, Sweden). Peptide data were scaled to unit variance and log transformed to obtain equal leverage of peptides. The default 7-fold cross-validation and 1000 permutations were used to assess model overfitting. The ANOVA of the cross-validation residuals was used to calculate P value for model significance.[23] The detailed information on the OPLS model can be found in the Supplemental Methods (Data S1). The model outputted PWV-derived urinary proteomic score (PWV-UP), equivalent to predictive values, to integrate the information carried by various urinary peptides. In the OPLS model, the importance of each peptide was assessed by the variable importance in the projection (VIP) to evaluate the association of a peptide with PWV. Peptides with a VIP >1.2 were considered significant for PWV. Besides, the OPLS model also provided the correlation coefficient for a single peptide as an alternative to assess the association between a given peptide and PWV. A figure visualized the VIPs and correlation coefficients of all studied peptides. The peptides in the top left quadrant and top right quadrant of the plot were inversely or positively associated with PWV, respectively.

Multivariable linear regression was used to investigate the association between PWV and PWV-UP, with adjustment for potential clinical confounders. The proportions of the variance in PWV (R^2) explained by PWV-UP, clinical variables, and a model with both of them were calculated. The estimate of a linear association was expressed as a β coefficient that indicated the change in PWV for per SD increment of PWV-UP.

Stepwise linear regression with backward selection was used for the selection of clinical confounders. The potential clinical confounders with a P value of <0.05 were retained in the multivariable linear regression model by the backward selection procedure. The following clinical variables were considered: sex, age, body mass index, heart rate, mean arterial pressure, serum total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, blood glucose, estimated glomerular filtration rate, current smoking, history of diabetes or cardiovascular diseases, hypertension, and being treated for hypertension. The collinearity of linear models was examined. The correlation of PWV-UP and PWV with continuous clinical variables was determined by Pearson correlation. The correlation between a continuous variable and a dichotomous variable was assessed by the point biserial correlation coefficient.

In the categorical analysis, participants were grouped by the median of PWW-UP and PWV, respectively. The crude cumulative incidence of all-cause mortality was estimated across the groups using the Kaplan-Meier method, and the cumulative incidence curves were compared using the log-rank test. The Fine-Gray subdistribution hazard model was used to calculate the crude incidence of cardiovascular events while taking the competing risk of noncardiovascular deaths into account.[24] The difference of cumulative incidence of cardiovascular events between groups was examined by the Gray method. The adjusted 5-year absolute risk was calculated with the baseline PWV-UP and PWV, respectively, with adjustment of sex, age, smoking, mean arterial pressure, body mass index, plasma glucose, total cholesterol, estimated glomerular filtration rate, history of diabetes, and previous cardiovascular events. The prognostic values of PWV-UP and PWV were assessed by multivariable-adjusted Cox proportional hazard regression models. The proportional hazard assumption was examined by the Kolmogorov-type supremum test. Furthermore, a competing risk analysis for cardiovascular events and cardiovascular mortality was performed with cause-specific and subdistribution hazard models.

Pathway Analysis

To interpret the biological function of the urinary proteomics, enrichment pathway analysis was performed with the online Reactome Pathway Database, version 75 (https://reactome.org).[25] Proteins with peptides having a VIP score of ≥1.2 were considered important and, thus, submitted for pathway analysis. P value of the annotated pathways was corrected with false discovery rate. A false discovery rate of <0.05 was considered statistically significant. As a complement to the Reactome Pathway analysis, the biological function of

the significant peptides was also investigated in the Gene Ontology database, which was performed by using the ClueGO plug-in of Cytoscape. Gene Ontology terms were determined by a 2-sided hypergeometric statistical test. P value corrected by Bonferroni step down was set at 0.05, and the κ score threshold of 0.4 was considered as statistically significant.

RESULTS

Characteristics of Participants

Table 1 shows the characteristics of the study population. The age (±SD) of the 669 participants averaged 50.2±15.6 years. Among all the participants, 342 (51.1%) were women, 268 (40.1%) had hypertension, 25 (3.7%) had diabetes, and 39 (5.8%) had a history of cardiovascular diseases. The mean values were 25.8±3.6 kg/m²

Table 1. Characteristics of 669 Participants

Characteristics	Total (n=669)
Participants with characteristic, n (%)	
Women	342 (51.1)
Smoking history	241 (36.0)
Diabetes	25 (3.7)
Cardiovascular diseases	39 (5.8)
Hypertension	268 (40.1)
Treatment of hypertension	150 (22.4)
Statins	83 (12.4)
Antiplatelet drugs	64 (9.6)
Mean±SD or median (IQR) of characteristic	
Age, y	50.2±15.6
Body mass index, kg/m²	25.8±3.6
Waist/hip ratio	0.9±0.1
Heart rate, beats/min	59.2±9.0
Systolic blood pressure, mm Hg	128.6±17.2
Diastolic blood pressure, mm Hg	79.2±9.4
Mean arterial pressure, mm Hg	112.1±13.4
Serum total cholesterol, mmol/L	5.25±0.96
HDL cholesterol, mmol/L	1.44±0.35
LDL cholesterol, mmol/L	3.21±0.85
Blood glucose, mmol/L	4.91±0.70
Serum creatinine, mg/dL	0.92±0.18
eGFR, mL/min per 1.73 m²	85.9±16.9
Urine microalbumin, mg/L	5.5 (4.1–7.5)
Pulse wave velocity, m/s	7.56±2.02

History of smoking refers to inhaling tobacco on a daily basis in the past; hypertension was an office blood pressure of ≥140 mm Hg systolic or ≥90 mm Hg diastolic or use of antihypertensive drugs; diabetes was use of antidiabetic drugs or fasting blood glucose of ≥126 mg/dL; eGFR is estimated using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation. Body mass index was calculated by weight in kilograms divided by height in meters squared. eGFR indicates estimated glomerular filtration rate; HDL, high-density lipoprotein; IQR, interquartile range; and LDL, low-density lipoprotein.

for body mass index, 128.6±17.2/79.2±9.4 mm Hg for systolic/diastolic blood pressure, 59.2±9.0 beats/min for heart rate, 5.25±0.96 mmol/L for total cholesterol, and 3.21±0.85 for low-density lipoprotein cholesterol. cfPWV averaged 7.56±2.02 m/s.

Urinary Proteomic Profile

At baseline, the urinary proteomics data included 2336 peptides that were detectable in ≥30% of the participants (N=669). Of these peptides, 743 were sequenced and annotated to 111 parental proteins. Modeling the 2336 peptides with PWV, the generated PWV-UP model explained 65.0% of the variance in PWV (equivalent to R^2: 0.65; 95% CI, 61.2–68.3; $P<0.0001$; Figure 1). The cross-validation showed that PWV-UP model explained 63.7% of the PWV variance in the leave-out fold, which indicated a low risk of overfitting. This was also confirmed by the 1000 random permutations ($P<0.05$). The distribution of the PWV-UP score is shown in Figure S1, and the average PWV-UP score was 7.59±1.95 m/s.

In the OPLS model, the significance of peptides was reflected by VIPs and correlation coefficients, as shown in the volcano plot (Figure 2). Of the 743 sequenced peptides with parental protein, 276 (37.1%) peptides with a VIP threshold of ≥1.2 from 37 proteins significantly contributed to PWV-UP. The correlation coefficients, VIPs, sequences, mass, and migration time of the 276 peptides were listed in Table S1. The top proteins above the VIP cutoff were 15 different types of collagens and 22 kinds of distinct proteins. Most of them were predominantly extracellular matrix (ECM) proteins, such as collagen I, collagen III, titin, mucin-2, and cadherin 1. Notably, the peptides from collagen I and III accounted for 79.3% of these significant peptides, and most of them were inversely associated with PWV, as shown in Figure 2. Apart from extracellular structural proteins, other top proteins involved in diverse processes, including cell adhesion (titin, mucin, protocadherin-9, and cadherin 1), cell-protein interaction (vesicular integral-membrane protein), lipid metabolism (apolipoprotein A-I and A-VI), vascular calcification (MGP [matrix Gla protein], osteopontin, and collagen 2), coagulation and fibrinolysis (fibrinogen, α-1-antitrypsin, α-1-antichymotrypsin, and plasminogen), and anti-inflammation (annexin A1). The Reactome pathway analysis annotated to 62 biological processes that were mainly related to the ECM turnover, collagen formation and degradation, cell-ECM interaction, cell adhesion, signal transduction, immune-related pathways, platelet activation and hemostasis, inflammation, and lipid metabolism (Table S2). The top 20 pathways are shown in Figure 3. The functional annotations of the significant peptides revealed 137 Gene Ontology terms of biological processes that related to collagen fibril organization, platelet aggregation, lipoprotein oxidation, and immune response (Figure 3 and Table S3).
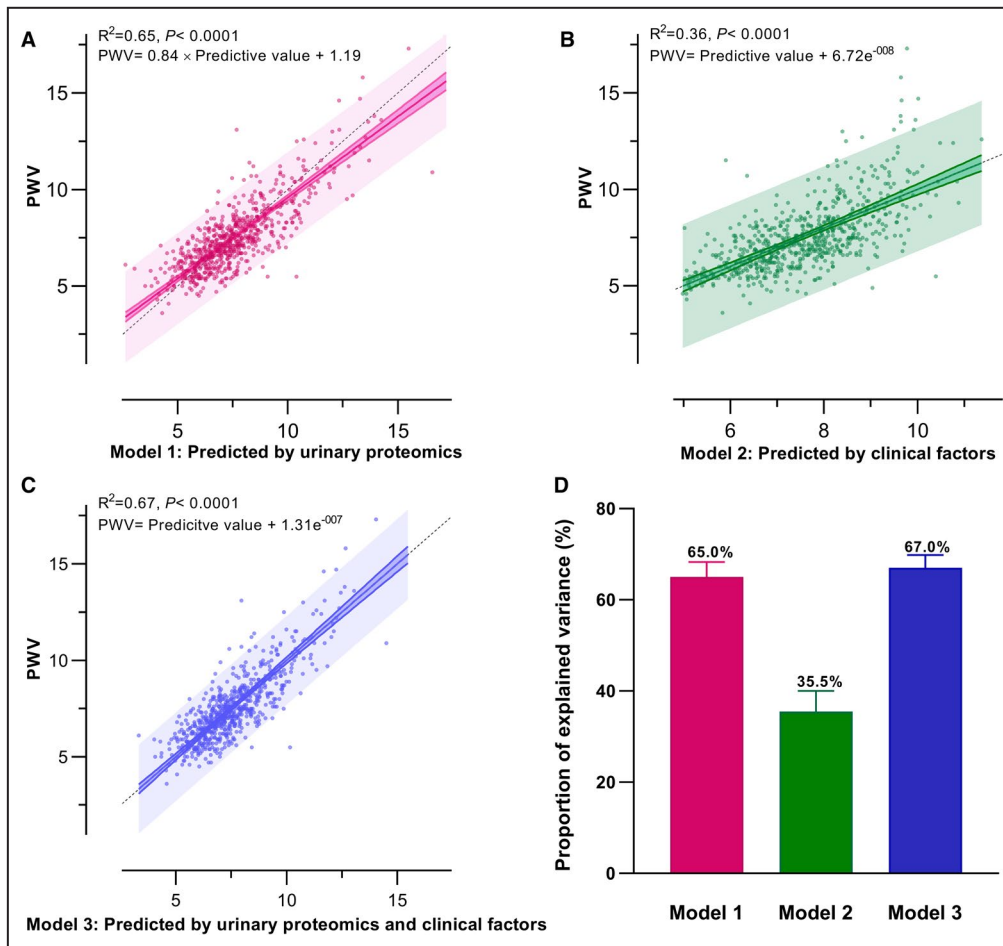
Association With PWV

The adjusted association of PWV with PWV-UP score was assessed by multivariable linear regression. With the backward selection procedure on covariates, the following clinical variables were retained as potential confounders: age, heart rate, mean arterial pressure, blood glucose, and current smoking ($P≤0.006$; Table S4). Sex forced into the model for clinical relevance. With adjustments of these confounders, per 1-SD increment (1.95 m/s) in the PWV-UP was associated with 0.73-m/s increase in PWV (95% CI, 0.67–0.79 m/s; $P<0.0001$). With further adjustment for estimated glomerular filtration rate and urine microalbumin, the β coefficient of PWV-UP (0.73 [95% CI, 0.67–0.79]) barely changed. No multicollinearity was detected through collinearity diagnostics, as the highest score of the variation inflation factor was 2.43. Furthermore, compared with the proportion of variance in PWV explained by PWV-UP (65.0%), the effect of the clinical variables, including urine microalbumin, was 35.2% (95% CI, 29.1%–39.8%; Figure 1). The proportion was slightly improved to 67.0% (equivalent to R^2: 0.67; 95% CI, 62.9–69.7%) when the clinical variables were added into the PWV-UP model.

Correlation With Clinical Variables

The correlations between PWV, PWV-UP, and clinical variables are shown in Table 2. Higher PWV-UP and PWV were positively correlated with age, body mass index, systolic and diastolic blood pressure, mean arterial pressure, total cholesterol, low-density lipoprotein cholesterol, blood glucose, and urine microalbumin, but were inversely correlated with estimated glomerular filtration rate ($P<0.0001$). Although PWV-UP and PWV were modestly correlated with heart rate ($r=−0.02$ and $r=0.05$, respectively), the correlations were in opposite directions. A history of diabetes, cardiovascular disease, or hypertension was correlated with higher PWV-UP and PWV ($P≤0.009$). PWV-UP and PWV had no significant correlation with high-density lipoprotein cholesterol and sex.

Longitudinal Association With Mortality and Cardiovascular Risk

The median follow-up was 9.2 years (5th–95th percentile, 6.1–10.7 years). Of the 669 participants, 36 died, including 10 (27.8%) from cardiovascular deaths and 15 (41.7%) of cancer. The total of 75 cardiovascular events included 51 cardiac events. The detailed information is presented in Table S5. Using Kaplan-Meier survival function estimates and the log-rank

Figure 1. Associations of pulse wave velocity (PWV) with PWV-derived urinary proteomic score (PWV-UP) and clinical variables.
A, Scatterplot of PWV and PWV-UP. The solid line represents the regression line. The band with 2 solid lines indicates the 95% confidence limits of the regression line, and the transparent band refers to the 95% prediction limits of the regression model. The gray dotted line represents the identity line that had a slope of 1. **B**, Scatterplot of PWV and the predicted values of the clinical variables. The clinical variables included age, sex, heart rate, mean arterial pressure, blood glucose, urine microalbumin, and current smoking. **C**, Scatterplot of PWV and the predicted value of PWV-UP and the clinical variables together. **D**, Bar plot illustrating the proportion of explained variance in PWV by 3 models. The error bars denote the upper 95% confidence limit.

test, the cumulative incidence of all-cause mortality and cardiovascular events was significantly higher at the upper half of PWV-UP ($P \leq 0.0002$; Figure 4). A similar result was observed in PWV ($P < 0.0001$; Figure 4). With adjustment of potential confounders, the absolute 10-year risk of all-cause and cardiovascular events increased with the level of baseline PWV-UP and PWV, but the independent effect of PWV was not significant ($P \geq 0.053$; Figure S2). In univariate Cox regression analysis, a higher PWV-UP or PWV was significantly associated with an increased risk of all-cause mortality, cardiovascular mortality, cardiovascular events, cardiac events, coronary events, and stroke ($P \leq 0.025$; Table 3). The association of PWV with all the outcomes lost significance after adjustment for

PWV-UP ($P \geq 0.24$). After adjusting for PWV, PWV-UP was still significantly associated with all outcomes ($P \leq 0.042$), except for stroke ($P = 0.87$). With the adjustment of the potential confounders for per-SD increment of PWV-UP, hazard ratio (HR) was 1.46 (95% CI, 1.08–1.97) for all-cause mortality, 2.04 (95% CI, 1.07–3.87) for cardiovascular mortality, and 1.39 (95% CI, 1.11–1.74) for cardiovascular events ($P \leq 0.031$; Table 3). The adjusted association of PWV-UP with cardiac events, coronary events, and stroke did not reach significance ($P \geq 0.30$). The associations of PWV-UP with all-cause mortality, cardiovascular mortality, and cardiovascular events remained significant when additionally adjusting for urine microalbumin, with the adjusted HRs of 1.46 (95% CI, 1.08–1.97) for
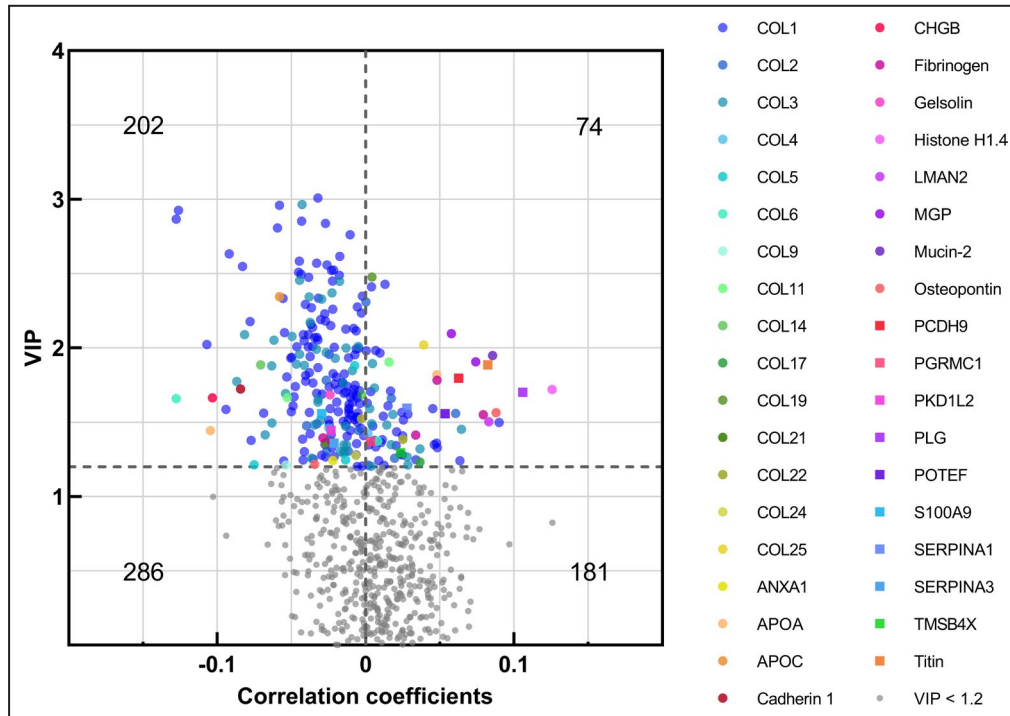
Figure 2. Volcano plot of significant peptides of pulse wave velocity–derived urinary proteomic score (PWV-UP).

Of the 743 peptides with parental protein, 276 peptides (37 proteins) significantly contributed to PWV-UP with a variable importance in the projection (VIP) ≥1.2 as the threshold. Peptides in the top left quadrant (202) and top right quadrant (74) of the plots were inversely or positively associated with pulse wave velocity, respectively. ANXA1 indicates annexin A1; APOA, apolipoprotein A; APOC, apolipoprotein C; CHGB, secretogranin-1; COL, collagen; LMAN2, vesicular integral-membrane protein VIP36; MGP, matrix Gla protein; PCDH, protocadherin-9; PGRMC1, membrane-associated progesterone receptor component; PKD1L2, polycystic kidney disease protein 1-like 2; PLG, plasminogen; POTEF, POTE ankyrin domain family member F; S100A9, protein S100-A9; SERPINA1, α1-antitrypsin; SERPINA3, α1-antichymotrypsin; and TMSB4X, thymosin β4.

all-cause mortality, 1.94 (95% CI, 1.01–3.73) for cardiovascular mortality, and 1.34 (95% CI, 1.06–1.68) for cardiovascular events ($P \leq 0.048$). However, PWV was only significantly associated with cardiovascular events after adjustment of cofounders (HR, 1.22 [95% CI, 1.02–1.47]; $P=0.033$). The adjusted associations of PWV-UP and PWV with cardiovascular events were confirmed as well when considering the competing risk of death for other causes (subdistribution and cause-specific HRs of PWV-UP: 1.21 [95% CI, 1.04–1.40] and 1.39 [95% CI, 1.11–1.74]; subdistribution and cause-specific HRs of PWV: 1.34 [95% CI, 1.08–1.73] and 1.22 [95% CI, 1.02–1.47]). Similarly, cardiovascular mortality remained higher in participants with higher PWV-UP in the competing analysis (subdistribution and cause-specific HRs: 1.89 [95% CI, 1.12–3.17] and 2.04 [95% CI, 1.07–3.87]). All models met the proportional hazard assumption ($P \geq 0.15$). In addition, they are not particularly linked to arterial stiffness because arterial stiffness might not be particularly related to arrhythmias and peripheral vascular diseases.

DISCUSSION

This study developed a PWV-UP score that was significantly associated with aortic stiffness, as measured by cfPWV, independent of clinical confounders. Besides, PWV-UP contributed more to the variation of PWV than multiple clinical variables did. Similar to PWV, PWV-UP was positively correlated with clinical risk factors, such as age, systolic blood pressure, diabetes, and hypertension. Notably, PWV-UP was associated with all-cause mortality, cardiovascular mortality, and cardiovascular events, with the adjustment of potential confounders, whereas PWV was only associated with cardiovascular events. The pathway analysis revealed that peptides included in PWV-UP were involved in multiple biological processes, such as collagen turnover, cell adhesion, inflammation, and lipid metabolism.

Proteomic studies on human arterial stiffness are scarce. Lyck Hanssen et al enrolled 19 patients in their study for coronary artery bypass grafting and measured their cfPWV before the surgery.[10] The patients
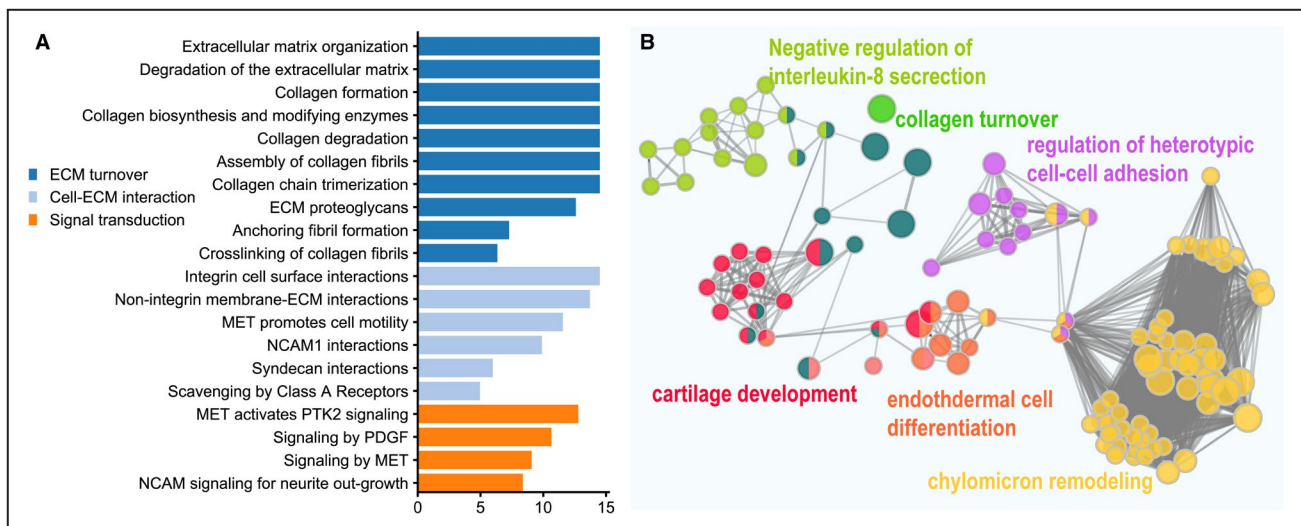
Figure 3. Bioinformatic analysis for the significant proteins in the urinary proteomic profile.
A, Top 20 Reactome pathways annotated by the urinary proteomic profile. The bar length of every pathway indicates the corresponding −log10 (false discovery rate). **B**, The network of enriched Gene Ontology (GO) biological processes. GO terms are clustered and colored by different functional groups. Each dot represents a GO term with adjusted $P<0.05$. Dot size indicates their corresponding adjusted P value, whereas the edge width and transparency suggest the κ score. ECM indicates extracellular matrix; MET, mesenchymal-epithelial transition factor; NCAM1, neural cell adhesion molecule 1; PDGF, platelet-derived growth factor receptor; and PTK, protein-tyrosine kinase.

Table 2. Correlation Between PWV-UP, PWV, and Clinical Variables in 669 Flemish Patients at Baseline

Variable	PWV-UP		PWV	
	r	P value	r	P value
Age	0.61	<0.0001	0.52	<0.0001
Body mass index	0.24	<0.0001	0.21	<0.0001
Heart rate	−0.02	<0.0001	0.05	<0.0001
Systolic blood pressure	0.49	<0.0001	0.48	<0.0001
Diastolic blood pressure	0.16	<0.0001	0.23	<0.0001
Mean arterial pressure	0.46	<0.0001	0.46	<0.0001
Total cholesterol	0.15	0.0001	0.15	0.0001
HDL cholesterol	−0.07	0.071	−0.04	0.26
LDL cholesterol	0.16	<0.0001	0.16	<0.0001
Blood glucose	0.26	<0.0001	0.26	<0.0001
eGFR	−0.44	<0.0001	−0.36	<0.0001
Urine microalbumin	0.19	<0.0001	0.16	<0.0001
Sex (men, women)	−0.03	0.51	−0.07	0.070
Current smoking (0, 1)	−0.001	0.97	0.02	0.52
Diabetes (0, 1)	0.11	0.005	0.10	0.009
Cardiovascular diseases (0, 1)	0.27	<0.0001	0.20	<0.0001
Hypertension (0, 1)	0.45	<0.0001	0.40	<0.0001

The r value was calculated by Pearson correlation for 2 continuous variables, whereas the correlation between a continuous variable and a dichotomous variable was assessed by the point biserial correlation coefficient. eGFR indicates estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PWV, pulse wave velocity; PWV-UP, PWV-derived urinary proteomic score; and r, correlation coefficient.

were classified into the high-PWV group (N=10) and the low-PWV group (N=9), according to the PWV threshold of 10 m/s. The left internal mammary arterial tissue pieces were collected during the surgery and analyzed using liquid chromatography–mass spectrometry. Among 418 proteins, 28 were differentially expressed between groups ($P<0.05$ without multiple testing correction) and mainly consisted of ECM proteins. Part of the tissue proteomic portraits was consistent with our urinary proteomic profile, such as collagen IV, apolipoprotein A-1, and protein S100. Pettersson-Pablo et al performed a plasma proteomic study on 834 healthy young adults (aged 18–26 years).[11] They measured 92 inflammatory proteins with proximity extension assay (OLINK Proteomics, Uppsala, Sweden) and arterial stiffness determined by cfPWV and augmentation index. They used the unsupervised dimension reduction statistical method, principal component analysis to integrate the correlated proteins. However, of the 4 constructed components, only 1 component that consisted of proteins related to hemostasis was significantly associated with PWV, and the model that included all the components and clinical risk factors explained only 5.7% of the variance of PWV. This indicated that the mechanisms underlying arterial stiffness entangle multifactorial molecules, and inflammatory proteins are part of it. Of note, there were several differences between previous studies and the current study. First, the recruited participants had
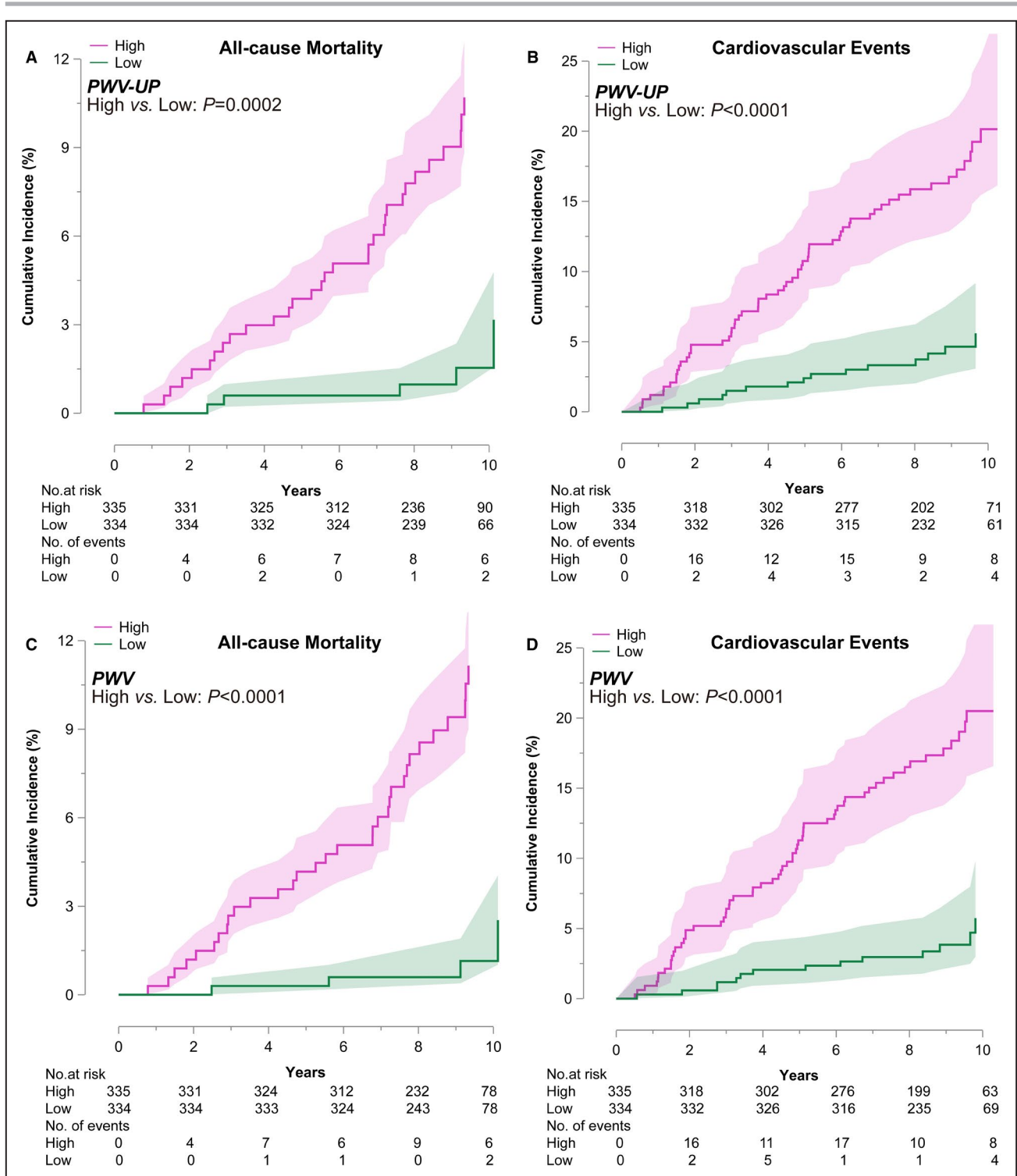
Figure 4. Cumulative incidence of all-cause mortality and cardiovascular events in 669 participants.
The participants were divided into 2 groups according to the median of pulse wave velocity–derived urinary proteomic score (PWV-UP) (**A** and **B**) and the median of pulse wave velocity (PWV) (**C** and **D**). The colored bands represent the SE. *P* values express the significance of the log-rank test or the Gray method for the difference across the groups.

different clinical settings. The participants in previous studies were diagnosed with atherosclerosis or were healthy young adults, whereas our participants were from the general population with a wide age range.

Having a high prevalence of hypertension (40%), our population was exposed to a higher risk of arterial stiffness compared with the young adults. Second, aortic arteries are not easily accessible samples; therefore,

Table 3. Adjusted HRs Associated With Baseline PWV-UP and PWV

End points	Events/at risks	PWV-UP		PWV	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value
All-cause mortality	36/669				
Unadjusted		2.11 (1.74–2.56)	<0.0001	1.49 (1.31–1.69)	<0.0001
Adjusting for PWV or PWV-UP		2.49 (1.80–3.43)	<0.0001	0.86 (0.66–1.12)	0.26
Adjusting for covariates		1.46 (1.08–1.97)	0.014	1.25 (0.97–1.60)	0.085
Cardiovascular mortality	10/669				
Unadjusted		2.74 (1.96–3.84)	<0.0001	1.61 (1.32–1.96)	<0.0001
Adjusting for PWV or PWV-UP		3.53 (2.17–5.75)	<0.0001	0.79 (0.53–1.17)	0.24
Adjusting for covariates		2.04 (1.07–3.87)	0.031	1.35 (0.85–2.15)	0.21
Cardiovascular events	75/669				
Unadjusted		2.03 (1.76–2.35)	<0.0001	1.52 (1.38–1.67)	<0.0001
Adjusting for PWV or PWV-UP		2.12 (1.66–2.70)	<0.0001	0.96 (0.79–1.17)	0.68
Adjusting for covariates		1.39 (1.11–1.74)	0.004	1.22 (1.02–1.47)	0.033
Cardiac events	51/669				
Unadjusted		1.85 (1.55–2.20)	<0.0001	1.40 (1.24–1.59)	<0.0001
Adjusting for PWV or PWV-UP		2.18 (1.60–2.96)	<0.0001	0.85 (0.65–1.12)	0.24
Adjusting for covariates		1.17 (0.87–1.59)	0.30	0.98 (0.72–1.33)	0.88
Coronary events	23/669				
Unadjusted		1.70 (1.28–2.26)	0.0003	1.40 (1.15–1.70)	0.001
Adjusting for PWV or PWV-UP		1.73 (1.02–2.93)	0.042	0.98 (0.64–1.51)	0.94
Adjusting for covariates		0.89 (0.53–1.51)	0.67	0.88 (0.52–1.48)	0.62
Stroke	13/669				
Unadjusted		1.68 (1.15–2.45)	0.007	1.37 (1.04–1.80)	0.025
Adjusting for PWV or PWV-UP		1.82 (0.92–3.63)	0.087	0.92 (0.51–1.66)	0.78
Adjusting for covariates		0.79 (0.42–1.48)	0.45	0.72 (0.38–1.38)	0.32

HRs express the risk per SD increment in PWV-UP (1.95 m/s) or PWV (2.02 m/s). Covariates included baseline characteristics, including sex, age, smoking, diabetes, history of cardiovascular events, mean arterial pressure, body mass index, plasma glucose, total cholesterol, and estimated glomerular filtration rate. HR indicates hazard ratio; PWV, pulse wave velocity; and PWV-UP, PWV-derived urinary proteomic score.

alternative samples were used (muscular artery tissue versus plasma versus urine). As urine and blood samples are easier to collect, the latter 2 studies had a relatively large sample size (N=19 versus N=834 versus N=669). Although we investigated the proteomic patterns in different types of specimens, the findings were complementary to one another. Because the reference value of a normal cfPWV is still controversial, Pettersson-Pablo et al and the current study both modeled continuous PWV with proteomics data to identify potential relevant peptides. Previous study adopted the unsupervised method (principal component analysis), whereas we used the supervised method (OPLS). However, in the previous study, the constructed components were poorly related with PWV. In contrast, PWV-UP was significantly associated with PWV, independent of the confounding effect of the clinical variables. Our study also confirmed that arterial stiffness is poorly correlated with conventional clinical variables.[26] In particular, we found that PWV-UP was remarkably superior to the clinical variables in terms of the explained proportion of PWV variance. This was

probably attributable to the multifunctional peptides included in PWV-UP, as revealed in the pathway analysis. These peptides might be involved in various mechanisms of arterial stiffness.

More important, we demonstrated that PWV-UP was associated with adverse outcomes. Over a follow-up period of 9.2 years, PWV-UP was associated with all-cause mortality, cardiovascular mortality, and cardiovascular events, whereas PWV itself was only associated with cardiovascular events. This appears contradictory because PWV-UP was developed from PWV. The predictive value of PWV-UP was presumably inherited from PWV and constrained within the prediction limit of PWV, but it outperformed PWV. The reasonable explanation for this observation might be that the multifaceted peptides of PWV-UP were also involved in other pathogenesis processes. For instance, MGP, a significant protein in PWV-UP, is a small protein that inhibits vascular calcification. Because vascular calcification is considered one of the main pathogenesis variables of arterial stiffness, circulating inactive MGP has been demonstrated to be associated with

aortic stiffness.[27,28] However, apart from accelerating aortic aging, vascular calcification is also a significant risk factor of mortality and cardiovascular events.[29,30] Consistent with this, Liu et al conducted a prospective study on 2318 Flemish people with 14.1 years of follow-up and discovered that a doubling of the plasma inactive MGP was independently associated with a 15% increased risk of mortality.[31] The association of PWV-UP with adverse outcomes might be partly mediated by the pathological mechanism of arterial stiffness. In fact, previous studies have demonstrated that urinary proteomics-derived classifiers could be independent predictors of cardiovascular events and coronary artery diseases.[7,8] In 791 participants (mean age, 51.2 years; 50.6% women), HF1, a classifier of 85 urinary peptides for left ventricular diastolic dysfunction, was associated with the risk of cardiovascular events after a follow-up period of 6.1 years, instead of systolic blood pressure. Although clearly designed experiments on the role of the various proteins and their interaction with the pathogenesis of arterial stiffness are required, we hypothesized that the association of PWV-UP and adverse outcomes is mediated by these proteins through the enriched pathways, such as fibrosis, inflammation, calcification, and cell-ECM interactions. The constitution of urinary proteomics is not fixed, but can be modified with dynamic pathological processes, attributable to which PWV-UP has the potential to monitor the development of arterial stiffness and provide hints for personalized treatment targets.

The urinary proteome mostly consists of endogenous peptides and low-molecular-weight proteins derived from larger precursor proteins and protein degradation without additional manipulation (eg, proteolytic digestion).[32] These substantial peptides in urine provide a window into interpreting the biological functions of these endogenous peptides, their precursor proteins, and the process of degradation under various situations. Different modified collagen fragments in urine, for example, are considered markers for diabetes and diabetic nephropathy.[33] This desirable information would be buried in the analysis at the protein level to some extent. Besides, the bottom-up proteomics is typically measured at the peptide level, then estimates protein abundance by multiple peptide intensities, and this combining process could also introduce extra error.[34,35] With the advent of deep learning, the dimensionality and multicollinearity extended by the analysis at the peptide level might be an acceptable trade-off for additional information. Furthermore, the definition of a polypeptide of CE-MS was not merely based on the amino acid sequence of the precursor, but considered its mass, the migration time in capillary electrophoresis. Several distinct proteins with different posttranslational modifications might produce peptides with same sequence, but their molecule mass and the migration

time in capillary electrophoresis could be altered with posttranslational modifications.[32] The posttranslational modifications can be informative and useful for some diseases (eg, advanced glycation end products for uremia).[33] Besides, calibrating mass and migration time enables CE-MS to consistently detect the same peptides from different samples.[36] The obtained peptide data from CE-MS have been validated and showed great reproducibility in respect to intra-day variation (0.7%–1.6%) and inter-day variation (1.3%–5.7% for the top ten abundant peptides).

Notably, most of the significant peptides in PWV-UP were fragments of collagen I and III. As primary constituents of ECM, they provide an architectural framework and maintain a high tensile strength for vessels.[37] In contrast, elastic fibers offer elasticity and resilience to arteries and are abundant in elastic arteries, especially in aortic arteries.[38] With vascular aging, the mechanical properties are altered: the elastic fibers are damaged and fragmented, and the collagen fibers accumulated excessively to adapt to the mechanical overloading.[1,39] In the volcano plot, PWV was inversely correlated with most collagen I and III peptides, which implies that arterial stiffness is correlated with fewer degraded collagen products. This is consistent with the unbalanced collagen turnover of arterial stiffness, which is also reflected by the serum collagen I turnover markers. In 80 patients with chronic heart failure, cfPWV was inversely associated with serum level of carboxy-terminal telopeptide of collagen type I (a marker of collagen degradation), but was positively correlated with prometalloproteinase-1, which was a marker of matrix metalloproteinase-1 production.[40] Similar findings were also found in subjects with hypertension.[41,42] Stakos et al measured free amino-terminal propeptides of procollagen type I (a maker of collagen synthesis), carboxy-terminal telopeptide of collagen type I, and matrix metalloproteinase-1 in 72 patients with hypertension and 27 normotensive individuals. They showed that cfPWV was positively associated with the amino-terminal propeptides of procollagen type I/carboxy-terminal telopeptide of collagen type I ratio and matrix metalloproteinase-1.[41] Although matrix metalloproteases are mobilized to degrade the disproportionate collagen fibers, the additional cross-link forged by advanced glycation end products prevents collagen from degrading to a certain extent.[38] The urinary peptides of collagen I and III might be the biomarkers of the dynamic process of unbalanced collagen turnover in arterial stiffness.

In addition to MGP, osteopontin was another inhibitory protein of vascular calcification. Osteopontin was initially identified in osteoblasts to moderate mineralization in bones by inhibiting crystal growth. It is not detectable in the normal vascular walls, but highly expressed in the calcification sites of atherosclerosis

plaque.[43] Similarly, PWV-UP also included collagen II, which is the primary fibrillar protein component in cartilage tissue and generally associated with cartilage and skeletal disorders.[44] However, a previous study that collected 97 aorta samples from human subjects with sudden death reported that the expression of collagen type II was significantly higher around the sites of calcium depositions on the arterial wall and was positively associated with the grade of atherosclerosis.[45] In addition, we also found several lipoproteins in PWV-UP. Apolipoproteins A-I and IV are the primary proteins of the high-density lipoprotein, and are involved in lipid metabolism, reverse cholesterol transport, and protection against atherosclerosis.[46,47] A study investigated the relationship between PWV and the ratio of apolipoprotein B/A-1 in 1252 subjects with metabolic syndrome.[48] It was observed that the ratio significantly increased when PWV increased. Furthermore, PWV-UP also included proteins that are involved in coagulation and platelet activation, such as fibrinogen, α-1-antitrypsin, α-1-antichymotrypsin, and plasminogen. For instance, fibrinogen, a key coagulation factor and acute-phase protein, has been known to associate cardiovascular risks and has been reported to be correlated with aortic stiffness.[49,50] Other studies found that arterial stiffness is also associated with platelet activation and aggregation.[51–53] Consistent with our findings, in previous study, the arterial stiffness index β and intima-media thickness of the carotid artery of 517 participants were significantly associated with the expression level of P-selectin related to platelet adhesion of the activated platelets.[53]

Our findings add to the increasing evidence that arterial stiffness is not only hemodynamic alterations but concerns multifactorial molecules with potentially deleterious influence on mortality. This could explain why arterial stiffness is hardly explained by single clinical risk factors or biomarkers, but PWV-UP that combined >2000 peptides can achieve better. This combination of individual biomarkers also boosted the prognostic performance of arterial stiffness. These findings suggest that PWV may not be sufficient to stratify the risk of mortality related to arterial stiffness, and the PWV-derived proteomic biomarker might be a complementary approach.

Strengths of the current study included a relatively large sample size, standardized PWV measurement and quality control, well-characterized participants, which reduced the confounding effect, a prospective study design, and long-term follow-up. However, our findings also have several potential limitations. As an observational study, potential residual confounding may exist; thus, the association was not subjected to causality inference. However, in this study, we collected a wide range of clinical risk factors, including those from biochemical tests, to eliminate the potential

confounding effects. The specific roles of the PWV-UP-associated proteins in the pathogenesis of arterial stiffness and their effects on adverse outcomes require further validation in experimental studies. Last, the urinary proteomic profiling was developed from the general population from Flemish region. Therefore, the findings should be cautiously generalized to other ethnicities and clinical settings before further verification.

In conclusion, this study showed, for the first time, that the PWV-derived urinary proteomic profile was significantly associated with aortic stiffness in the general population, independent of the conventional clinical risk factors, including age, sex, heart rate, mean arterial pressure, blood glucose, and current smoking. Over a 9-year follow-up period, PWV-UP, but not PWV itself, predicted mortality and cardiovascular mortality, which implies that the peptides included in PWV-UP might be involved in multiple pathophysiological mechanisms and not just limited to arterial stiffness. PWV-UP offers the possibility of using urinary proteomics as a personalized biomarker of arterial stiffness and as a marker of adverse outcomes.

Supplemental Material

Data S1. Supplemental Methods
Tables S1–S5
Figures S1–S2
References 54–61

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Urinary Proteomics

For proteomic analysis, a 0.7 mL aliquot of stored urine was thawed immediately before use and diluted with 0.7 mL of 2 M urea, 10 mM NH_4OH containing 0.02% sodium dodecyl sulphate. To remove higher molecular mass proteins, such as albumin and immunoglobulins, the sample was ultra-filtered, using Centrisart ultracentrifugation filter devices (20 kDa MWCO; Sartorius, Göttingen, Germany) at 3000 relative centrifugal force units until 1.1 mL of filtrate was obtained. This filtrate was then applied onto a PD-10 desalting column (GE Healthcare, Uppsala, Sweden) equilibrated in 0.01% NH_4OH in HPLC-grade in H_2O (Carl Roth GmbH, Karlsruhe, Germany) to decrease matrix effects by removing urea, electrolytes, salts, and to enrich polypeptides. Finally, all samples were lyophilized, stored at 4°C, and suspended in HPLC-grade H_2O shortly before CE-MS analyses.

Capillary electrophoresis coupled to mass spectrometry (CE-MS) was performed, using a P/ACE MDQ capillary electrophoresis system (Beckman Coulter, Fullerton, CA) on-line coupled to a micrOTOF MS (Bruker Daltonics, Bremen, Germany).[54,55] The electrospray ionization device (Agilent Technologies, Palo Alto, CA) was grounded, and the ion spray interface potential was set between -4 and -4.5 kV. Data acquisition and MS acquisition methods were automatically controlled by the CE via contact-close-relays. Spectra were accumulated every 3 seconds over a mass-to-charge ratio (m/z) ranging from 350 to 3000.

Quality control

Accuracy, precision, selectivity, sensitivity, reproducibility and stability of the CE-MS have been previously published.[15, 54] Quality control involves daily CE-MS analysis of a human urine standard.[15] To prevent variability due to carry-over effects from one to the next analysis, capillaries are reconditioned between runs with 1 M NaOH. The coefficient of variance estimated from over 600 human urine standard analyses for over 3 years was 5.8%.[16]

Mass spectrometric data processing

Mass spectral peaks representing identical molecules at different charge states were deconvoluted into single masses, using MosaiquesVisu software.[56] Only signals with a charge > 1 observed in a minimum of three consecutive spectra with a signal-to-noise ratio of at least 4 were considered. Reference signals of 1770 urinary polypeptides were used for CE-time calibration by locally weighted regression. For normalization of analytical and urine dilution variances, signal intensities were normalized relative to 29 ''*housekeeping*'' peptides.[17, 18] The obtained peak lists characterize each polypeptide by its molecular mass, normalized CE migration time and normalized signal intensity. All detected peptides were deposited, matched, and annotated in a Microsoft SQL database, allowing further statistical analysis.[57] For clustering, peptides in different samples were considered identical, if mass deviation was less than 50 ppm. CE migration time was controlled to be below 0.35 minutes after calibration.

Sequencing of polypeptides

CE-MS signals were in silico assigned to the previously sequenced peptides from Human Urinary Proteome Database, version 2.0.[19] Peptides from this database were sequenced, as described elsewhere.[33, 58] Briefly, urinary peptides were fragmented, using different tandem mass-spectrometric techniques with a prior separation step with CE or HPLC. Fragmentation spectra were matched to the protein sequences from up-to-date public databases (IPI, NCBI Reference Sequence Database and Uniprot), using MS/MS search engines MASCOT (Matrix Sciences Ltd., London, UK) and OMSSA (National Center for Biotechnology Information, Bethesda, MD). In matching, we accounted for urinary proteins post-translational modifications, such as hydroxylation of lysine and proline, and specific MS characteristics. Peptide sequences from LC-MS/MS analyses were verified by the comparison of experimental and theoretical CE migration time, which is dependent on the number of basic and neutral polar amino acids.

The orthogonal projections to latent structures (OPLS) model

The proteomic data based on mass spectrometry is characterized by high dimensionality and collinearity, and the data matrix contains undesired 'noises' caused by various confounders.[21] To

extract the effective variation in the data matrix correlated with the dependent variables, the common analytical strategy is projecting the original proteomic data into a new dimensional space, and construct few components to summarize the proteomic data. This dimensional reduction statistical approaches can be classified unsupervised (like principal components analysis) and supervised methods (like the partial least squares) according to if taking the dependent variables into consideration during components construction. In recent decades, the modification of non-linear iterative partial least squares, named orthogonal projections to latent structures (OPLS) has been proposed and extensively used in metabolomics and proteomics studies.[59-61] Comparing with partial least squares, the OPLS remove and separately analyze the unrelated variation (the orthogonal information) from the independent data before modelling with dependent variables.[22] In this way, it could create fewer, more relevant and interpreted predictive components.[22]

In this study, we constructed the OPLS model in the SIMCA software, version 14.1 (Umetrics, Sartorius-Stedim, Sweden). Due to non-normal distribution, all peptides were centered and scaled to mean 0 and standard deviation 1 before analysis. To avoid model overfitting, 7-fold cross-validation was applied to determine how many components created and this procedure was automatically completed with the 'Auto-Fit' in the software. The model significance was tested by analysis of variance (ANOVA) of the cross-validation residuals.[23] The model performance was assessed by the score that equals 1 minus the error sum of squares divided by the sum of the squares of PWV. The score can be interpreted as the percentage of the variation of pulse wave velocity (PWV) explained by the model. Generally, a model is considered having good generalization and a low risk of overfitting when the score in the cross-validation is >0.5. In this study, the final OPLS model included one predictive component and six orthogonal components. According to the values of the urinary peptides, the model generated a predictive value for each participant, named UP score. The contribution of peptides to the PWV prediction was quantified by the variable importance for the projection (VIP), and peptides with a VIP > 1 were considered to be significant. In the study, we plotted the peptides according to VIP and coefficient and selected peptides with VIP >1.2 for pathway analysis.

Table S1. The list of 276 peptides significantly contributed to PWV-UP

Peptide ID	Mass (Da)	CE time (min)	Amino-acid sequence	Parental protein		Coefficient	VIP
				Name	Symbol		
e05560	1508.68	29.43	GSpGSpGPDGKTGPPGp	Collagen alpha-1(I) chain	COL1A1	-0.032	3.008
e20065	4289.92	28.72	ARGNDGARGSDGQpGppGPPGTAGFPGSpGAKGEV GpAGSpGSNGApG	Collagen alpha-1(III) chain	COL3A1	-0.043	2.965
e12851	2407.09	27.69	LDGAKGDAGPAGPKGEpGSpGENGApG	Collagen alpha-1(I) chain	COL1A1	-0.058	2.959
e06520	1617.72	23.27	NSGEpGApGSKGDTGAKG	Collagen alpha-1(I) chain	COL1A1	-0.126	2.925
e09230	1912.84	32.17	NGApGNDGAKGDAGApGApGSQ	Collagen alpha-1(I) chain	COL1A1	-0.128	2.867
e19107	3801.79	33.48	DQGPVGRTGEVGAVGPpGFAGEKGpSGEAGTAGPP GTpGPQG	Collagen alpha-2(I) chain	COL1A2	-0.043	2.853
e03025	1235.55	26.86	GQDGRpGPpGpPG	Collagen alpha-1(I) chain	COL1A1	-0.027	2.837
e12986	2430.1	28.25	ADGQpGAKGEpGDAGAKGDAGPPGPAGP	Collagen alpha-1(I) chain	COL1A1	-0.059	2.808
e16966	3193.43	22.57	PpGESGREGApGAEGSpGRDGSPGAKGDRGETGP	Collagen alpha-1(I) chain	COL1A1	-0.010	2.761
e02933	1225.54	26.43	GppGPDGNKGEpG	Collagen alpha-2(I) chain	COL1A2	-0.092	2.632
e16548	3092.47	31.13	ADGQpGAKGEpGDAGAKGDAGPpGPAGPAGPPGPIG	Collagen alpha-1(I) chain	COL1A1	-0.017	2.615
e09435	1936.88	32.24	GEKGPSGEAGTAGPpGTpGPQG	Collagen alpha-2(I) chain	COL1A2	-0.045	2.584
e19745	4097.86	24.65	SKGESGNKGEpGSAGPQGPpGpSGEEGKRGPNGEA GSAGPPGPpG	Collagen alpha-2(I) chain	COL1A2	-0.033	2.570
e03322	1265.59	27.33	SpGPDGKTGPpGPA	Collagen alpha-1(I) chain	COL1A1	-0.026	2.558
e01213	1025.45	25.92	pGDRGEpGPp	Collagen alpha-1(I) chain	COL1A1	-0.083	2.548
e07678	1737.78	31.06	TGSpGSpGPDGKTGPpGPAG	Collagen alpha-1(I) chain	COL1A1	-0.022	2.524
e04419	1378.61	28.88	ApGDRGEpGPpGPAG	Collagen alpha-1(I) chain	COL1A1	-0.023	2.521
e19885	4169.93	33.6	ERGEQGPAGSpGFQGLpGpAGppGEAGKpGEQGVPG DLGAPGPSG	Collagen alpha-1(I) chain	COL1A1	-0.045	2.508
e04945	1435.66	28.92	SpGSPGPDGKTGPpGP	Collagen alpha-1(I) chain	COL1A1	-0.043	2.495
e17212	3248.55	30.6	RTGEVGAVGPpGFAGEKGPSGEAGTAGpPGTpGPQG	Collagen alpha-2(I) chain	COL1A2	-0.018	2.488

e04820	1422.67	28.3	GVNVpSYPGpPGPPG	Collagen alpha-1(XIX) chain	COL19A1	0.004	2.477
e13065	2446.09	28.29	ADGQpGAKGEpGDAGAKGDAGPpGPAGP	Collagen alpha-1(I) chain	COL1A1	-0.038	2.475
e01935	1115.51	21.6	DGESGRpGRpG	Collagen alpha-1(III) chain	COL3A1	-0.044	2.454
e06441	1609.75	30.21	TGSpGSpGPDGKTGPpGP	Collagen alpha-1(I) chain	COL1A1	-0.022	2.449
e18867	3719.69	32.15	FPGPkGNDGApGKnGERGGPGGPGPQGPPGKNGETGPQGP	Collagen alpha-1(III) chain	COL3A1	-0.017	2.448
e12949	2423.09	27.7	LDGAKGDAGPAGpKGEpGSpGENGApG	Collagen alpha-1(I) chain	COL1A1	0.013	2.428
e08228	1798.78	31.76	GEpGSpGENGApGQMGPRG	Collagen alpha-1(I) chain	COL1A1	0.004	2.411
e17856	3405.56	25.85	ARGNDGARGSDGQPGPpGppGTAGFpGSpGAKGEVGP	Collagen alpha-1(III) chain	COL3A1	-0.023	2.369
e17280	3264.57	25.7	AAGEpGKAGERGVpGPpGAVGPAGKDGEAGAQGPPGP	Collagen alpha-1(I) chain	COL1A1	-0.002	2.348
e19780	4113.85	24.55	SEAEDASLLSFMQGYMKHATKTAKDALSSVQESQVAQQ	Apolipoprotein C-III	APOC3	-0.058	2.345
e04261	1359.61	23.18	DGRNGEKGETGApG	Collagen alpha-1(III) chain	COL3A1	-0.038	2.344
e03890	1321.59	28.38	ApGDRGEpGPpGPA	Collagen alpha-1(I) chain	COL1A1	-0.032	2.334
e18580	3618.69	32.79	FAGpPGADGQPGAKGEpGDAGAKGDAGpPGPAGPAGPPGPIG	Collagen alpha-1(I) chain	COL1A1	-0.056	2.332
e20087	4305.93	28.79	ARGNDGARGSDGQpGpPGPpGTAGFpGSpGAKGEVGpAGSpGSNGApG	Collagen alpha-1(III) chain	COL3A1	-0.030	2.327
e18864	3718.72	32.42	SGPPGRAGEPGLQGPAGPpGEKGEPGDDGpSGAEGPpGPQG	Collagen alpha-1(II) chain	COL2A1	0.000	2.309
e11073	2169.97	26.1	NSGEpGApGSKGDTGAKGEPGpVG	Collagen alpha-1(I) chain	COL1A1	-0.041	2.292
e13825	2584.24	35.08	LTGPIGppGPAGAPGDKGESGPSGPAGPTG	Collagen alpha-1(I) chain	COL1A1	-0.006	2.275
e17626	3343.57	31.67	pPGADGQPGAKGEpGDAGAKGDAGPpGPAGPAGPPGPIG	Collagen alpha-1(I) chain	COL1A1	-0.036	2.270
e17221	3250.4	22.62	GPpGESGREGAPGAEGSpGRDGSPGAKGDRGETGp	Collagen alpha-1(I) chain	COL1A1	-0.022	2.260
e06709	1636.74	30.24	GSpGSpGPDGKTGPpGPAG	Collagen alpha-1(I) chain	COL1A1	-0.003	2.235
e16606	3108.46	31.19	ADGQpGAKGEpGDAGAKGDAGpPGPAGPAGPpGPIG	Collagen alpha-1(I) chain	COL1A1	-0.027	2.229
e11753	2261.99	27.14	GADGQPGAKGEPGDAGAKGDAGppGP	Collagen alpha-1(I) chain	COL1A1	-0.030	2.190
e17596	3337.47	22.75	GPpGESGREGApGAEGSpGRDGSPGAKGDRGETGpA	Collagen alpha-1(I) chain	COL1A1	-0.027	2.181

e16056	2983.32	22.25	GESGREGAPGAEGSpGRDGSPGAKGDRGETGp	Collagen alpha-1(I) chain	COL1A1	-0.078	2.177
e17866	3409.61	31.29	NTGApGSpGVSGPKGDAGQPGEKGSpGAQGPPGAPGPLG	Collagen alpha-1(III) chain	COL3A1	-0.037	2.172
e07513	1721.77	30.87	TGSpGSPGPDGKTGPpGPAG	Collagen alpha-1(I) chain	COL1A1	-0.037	2.158
e03638	1297.58	27.46	SpGSpGPDGKTGPp	Collagen alpha-1(I) chain	COL1A1	-0.023	2.150
e12556	2361.1	20.86	KNGDDGEAGKpGRPGERGpPGPQG	Collagen alpha-1(I) chain	COL1A1	-0.036	2.138
e17288	3266.43	22.62	GPpGESGREGAPGAEGSpGRDGSpGAKGDRGETGp	Collagen alpha-1(I) chain	COL1A1	-0.008	2.131
e05551	1507.73	40.19	VGPpGPPGPpGPpGPPS	Collagen alpha-1(I) chain	COL1A1	-0.013	2.126
e06316	1595.7	30.04	GpPGEAGKpGEQGVpGD	Collagen alpha-1(I) chain	COL1A1	-0.006	2.114
e11325	2205	27.02	ADGQPGAKGEpGDAGAKGDAGPPGp	Collagen alpha-1(I) chain	COL1A1	-0.055	2.102
e00340	884.32	24.72	cDDYRLc	Matrix Gla protein	MGP	0.058	2.096
e02592	1186.53	22.31	DDGEAGKpGRpG	Collagen alpha-1(I) chain	COL1A1	-0.042	2.090
e02169	1141.51	26.28	EpGRDGVpGGpG	Collagen alpha-1(III) chain	COL3A1	-0.082	2.089
e10987	2153.97	25.87	VGEpGpAGSKGESGNKGEPGSAGP	Collagen alpha-2(I) chain	COL1A2	-0.038	2.089
e13707	2564.15	23	GApGQNGEPGGKGERGApGEKGEGGppG	Collagen alpha-1(III) chain	COL3A1	-0.049	2.078
e09358	1927.92	19.53	DkGETGEQGDRGIkGHRG	Collagen alpha-1(I) chain	COL1A1	-0.033	2.070
e03016	1234.56	27.7	ApGDRGEPGPpGP	Collagen alpha-1(I) chain	COL1A1	-0.034	2.068
e03180	1250.56	28	ApGDRGEpGPPGp	Collagen alpha-1(I) chain	COL1A1	-0.032	2.054
e03194	1251.62	22.45	DGVPGKDGPRGPT	Collagen alpha-1(III) chain	COL3A1	-0.062	2.051
e05114	1451.65	29.15	SpGSpGPDGKTGPPGp	Collagen alpha-1(I) chain	COL1A1	-0.041	2.046
e06758	1641.72	25.06	DGQPGAKGEpGDAGAKGD	Collagen alpha-1(I) chain	COL1A1	-0.107	2.023
e09697	1969.85	24.84	PGpPGPHGPPGpmGPHGLpGP	Collagen alpha-1(XXV) chain	COL25A1	0.039	2.020
e07676	1737.78	23.79	NDGAPGKNGERGGpGGpGp	Collagen alpha-1(III) chain	COL3A1	-0.005	2.010
e16708	3133.47	31.24	GADGQPGAKGEpGDAGAKGDAGPpGPAGPAGPPGPIG	Collagen alpha-1(I) chain	COL1A1	-0.036	2.010
e06288	1592.74	19.54	EDGHpGKpGRpGERG	Collagen alpha-2(I) chain	COL1A2	-0.046	2.008

e17585	3334.56	31.03	GDPGKNGDKGHAGLAGARGApGPDGNNGAQGPpGPQG	Collagen alpha-2(I) chain	COL1A2	-0.039	2.006
e16895	3177.44	22.62	pPGESGREGAPGAEGSPGRDGSpGAKGDRGETGP	Collagen alpha-1(I) chain	COL1A1	-0.021	2.004
e17922	3425.61	31.29	NTGApGSPGVSGPKGDAGQpGEKGSpGAQGpPGAPGPLG	Collagen alpha-1(III) chain	COL3A1	-0.013	1.998
e17047	3209.43	22.73	PpGESGREGApGAEGSpGRDGSpGAKGDRGETGP	Collagen alpha-1(I) chain	COL1A1	-0.005	1.998
e11002	2156.97	22.19	AEGSpGRDGSpGAKGDRGETGPA	Collagen alpha-1(I) chain	COL1A1	-0.006	1.993
e06617	1627.7	29.59	MpGSpGGpGSDGKpGpPG	Collagen alpha-1(III) chain	COL3A1	-0.033	1.992
e16771	3149.46	31.28	GADGQpGAKGEpGDAGAKGDAGPPGPAGPAGPpGPIG	Collagen alpha-1(I) chain	COL1A1	0.007	1.982
e11902	2281.98	33.87	ANGApGNDGAKGDAGApGApGSQGApG	Collagen alpha-1(I) chain	COL1A1	0.000	1.981
e13472	2525.2	27.88	LRGGAGpPGPEGGKGAAGPpGPpGAAGTpG	Collagen alpha-1(III) chain	COL3A1	-0.026	1.969
e07275	1697.74	31.08	NGApGNDGAKGDAGApGApG	Collagen alpha-1(I) chain	COL1A1	0.010	1.966
e03938	1326.54	29.12	SpGGpGSDGKpGPpG	Collagen alpha-1(III) chain	COL3A1	-0.045	1.957
e05259	1469.67	23.62	DGQPGAKGEpGDAGAK	Collagen alpha-1(I) chain	COL1A1	-0.025	1.957
e15778	2926.3	22.17	ESGREGAPGAEGSPGRDGSpGAKGDRGETGp	Collagen alpha-1(I) chain	COL1A1	-0.007	1.954
e12310	2332	47.4	GIHSNISVS	Mucin-2	MUC2	0.086	1.948
e11559	2235.03	34.07	GRTGDAGPVGPPGPpGppGpPGPPS	Collagen alpha-1(I) chain	COL1A1	-0.037	1.941
e14445	2687.23	28.98	KDGEAGAQGPPGPAGpAGERGEQGPAGSpG	Collagen alpha-1(I) chain	COL1A1	-0.050	1.937
e17348	3280.45	22.62	ppGESGREGAPGAEGSpGRDGSpGAKGDRGETGPA	Collagen alpha-1(I) chain	COL1A1	-0.012	1.934
e15467	2864.27	20.16	GRDGNpGNDGPpGRDGQpGHKGERGYpG	Collagen alpha-2(I) chain	COL1A2	-0.050	1.932
e14735	2742.25	28.93	KNGETGPQGPPGpTGPGGDKGDTGPpGPQG	Collagen alpha-1(III) chain	COL3A1	-0.020	1.930
e01132	1013.37	25.06	cDDYRLcE	Matrix Gla Protein	MGP	0.074	1.905
e14204	2642.21	27.77	GNEGpSGPPGpAGSPGERGAAGSGGPIGpPG	Collagen alpha-2(XI) chain	COL11A2	0.016	1.904
e10072	2019.95	24.65	GLpGTGGPpGENGKpGEpGPKG	Collagen alpha-1(III) chain	COL3A1	0.005	1.894
e08188	1794.8	24.01	GNDGApGKNGERGGpGGpGP	Collagen alpha-1(III) chain	COL3A1	-0.047	1.891
e13231	2479.06	47.73	NKTPIR	Titin	TTN	0.082	1.885

e15200	2820.31	23.85	GIpGGVGSpGRDGSpGQRGLPGKDGSSGPpG	Collagen alpha-1(XIV) chain	COL14A1	-0.071	1.885
e15237	2825.28	24.45	ERGEAGIpGVpGAKGEDGKDGSpGEpGANG	Collagen alpha-1(III) chain	COL3A1	-0.041	1.883
e07098	1680.74	29.95	SKGDkGEqGpPGPTGPQ	Collagen alpha-1(V) chain	COL5A1	-0.007	1.881
e11765	2264.05	22.67	KGDAGApGApGGKGDAGApGERGpPG	Collagen alpha-1(III) chain	COL3A1	-0.063	1.879
e19752	4098.9	24.55	GEVGPAGpNGFAGpAGAAGQpGAKGERGAKGPKGE NGVVGPTGpVG	Collagen alpha-2(I) chain	COL1A2	-0.032	1.866
e01923	1114.49	25.71	SpGERGETGPp	Collagen alpha-1(III) chain	COL3A1	-0.009	1.847
e03372	1270.55	29.29	DGQPGAKGEpGDAG	Collagen alpha-1(I) chain	COL1A1	-0.038	1.833
e16419	3064.34	20.64	EAGRDGNpGNDGppGRDGQPGHKGERGYpG	Collagen alpha-2(I) chain	COL1A2	-0.022	1.827
e17690	3359.58	31.68	pPGADGQPGAKGEpGDAGAKGDAGppGPAGPAGPP GPIG	Collagen alpha-1(I) chain	COL1A1	-0.014	1.825
e05683	1523.76	22.89	SALEEYTKKLNTQ	Apolipoprotein A-I	APOA1	0.048	1.820
e05433	1492.67	29.28	GSpGSPGPDGKTGPpGP	Collagen alpha-1(I) chain	COL1A1	-0.040	1.815
e11777	2265.98	33.8	ANGApGNDGAKGDAGApGApGSQGAPG	Collagen alpha-1(I) chain	COL1A1	-0.020	1.805
e11213	2188.98	26.86	ADGQPGAKGEpGDAGAKGDAGPPGP	Collagen alpha-1(I) chain	COL1A1	-0.053	1.804
e03248	1257.44	33.56	EATEMcTQEc	Protocadherin-9?	PCDH9	0.063	1.796
e13033	2442.07	34.1	GpAGPPGEKGEPGDDGPSGAEGPpGPQ	Collagen alpha-1(II) chain	COL2A1	-0.026	1.782
e08463	1825.79	20.13	DEAGSEADHEGTHSTKR	Fibrinogen alpha chain	FGA	0.048	1.782
e18839	3706.79	21.98	qGPAGSPGFQGLPGPAGPpGEAGKpGEQGVPGDLGA PGPSGA	Collagen alpha-1(I) chain	COL1A1	-0.040	1.776
e04569	1396.61	28.34	SpGERGETGPpGPAG	Collagen alpha-1(III) chain	COL3A1	-0.087	1.774
e06686	1634.79	29.87	SpGNIGPAGKEGPVGLpG	Collagen alpha-2(I) chain	COL1A2	-0.016	1.767
e11972	2292.02	27.3	ADGQpGAKGEPGDAGAKGDAGppGPA	Collagen alpha-1(I) chain	COL1A1	-0.016	1.764
e02810	1211.54	26.04	GPpGEAGKpGEQG	Collagen alpha-1(I) chain	COL1A1	-0.036	1.764
e17254	3258.47	22.83	ENGKPGEpGpKGDAGApGApGGKGDAGApGERGpPG	Collagen alpha-1(III) chain	COL3A1	-0.015	1.764
e06154	1576.75	19.51	EDGHpGKPGRpGERG	Collagen alpha-2(I) chain	COL1A2	-0.021	1.757
e01758	1096.48	26.17	ApGDRGEpGPp	Collagen alpha-1(I) chain	COL1A1	-0.007	1.749

e18573	3616.72	33.07	DQGPVGRTGEVGAVGpPGFAGEKGPSGEAGTAGPpGTpGP	Collagen alpha-2(I) chain	COL1A2	-0.012	1.741
e10771	2117.95	27.44	DGQPGAKGEPGDAGAKGDAGPpGP	Collagen alpha-1(I) chain	COL1A1	-0.047	1.740
e12366	2339	34	GANGApGNDGAKGDAGApGApGSQGApG	Collagen alpha-1(I) chain	COL1A1	-0.024	1.737
e05568	1509.68	29.89	TGPGGDKGDTGPpGPQG	Collagen alpha-1(III) chain	COL3A1	0.006	1.730
e08736	1858.84	24.34	GGGEEDQDFDLSQLHRG	Cadherin 1	CDH1	-0.084	1.724
e16177	3013.34	22.23	ESGREGApGAEGSPGRDGSpGAKGDRGETGpA	Collagen alpha-1(I) chain	COL1A1	-0.008	1.721
e13673	2559.15	28.1	PpGADGQpGAKGEpGDAGAKGDAGPpGPA	Collagen alpha-1(I) chain	COL1A1	-0.018	1.720
e11826	2272.24	23.91	SETAPAAPAAPAPAEKTPVKKKA	Histone H1.4	H1-4	0.126	1.720
e07807	1750.79	23.87	GpPGpPGKNGDDGEAGKpG	Collagen alpha-1(I) chain	COL1A1	-0.004	1.714
e17890	3416.59	31.96	GPpGADGQPGAKGEpGDAGAKGDAGPPGpAGPAGPPGpIG	Collagen alpha-1(I) chain	COL1A1	0.017	1.709
e04947	1435.7	22.57	EpGKAGERGVPGpPG	Collagen alpha-1(I) chain	COL1A1	0.003	1.706
e17946	3432.59	31.95	PpGPAGFAGPPGADGQPGAKGEpGDAGAKGDAGPPGPAGP	Collagen alpha-1(I) chain	COL1A1	-0.013	1.703
e03952	1328.49	26.78	cEEDEEFTcRA	Plasminogen	PLG	0.106	1.700
e12508	2355.09	22.73	KGNSGEpGAPGSKGDTGAKGEpGpVG	Collagen alpha-1(I) chain	COL1A1	-0.015	1.695
e05671	1522.7	29.55	KpGEQGVpGDLGAPGp	Collagen alpha-1(I) chain	COL1A1	-0.033	1.695
e03768	1310.58	27.1	SpGGPGSDGKpGpPG	Collagen alpha-1(III) chain	COL3A1	-0.054	1.693
e16417	3063.44	30.01	RTGEVGAVGPpGFAGEKGPSGEAGTAGPpGTpGP	Collagen alpha-2(I) chain	COL1A2	-0.009	1.691
e05353	1483.66	22.63	GPpGKNGDDGEAGKpG	Collagen alpha-1(I) chain	COL1A1	-0.029	1.689
e17751	3375.57	31.83	pPGPQGPSGLSIQGMpGmpGEKGEKGDTGLPGPQG	Collagen alpha-1(XIV) chain	COL14A1	-0.002	1.684
e07622	1732.77	28.3	WVGTGASEAEKTGAQEL	Gelsolin	GSN	-0.024	1.683
e02189	1143.51	36.89	GpPGPpGPpGPpG	Collagen alpha-1(I) chain	COL1A1	-0.006	1.681
e12101	2308.01	27.33	ADGQpGAKGEpGDAGAKGDAGPpGpA	Collagen alpha-1(I) chain	COL1A1	-0.048	1.671
e11415	2216.03	33.83	IGPpGPAGApGDKGESGPSGPAGPTG	Collagen alpha-1(I) chain	COL1A1	-0.010	1.668
e11713	2256.97	33.66	PGPPGEKGENGDVGPmGppGPpGP	Collagen alpha-1(XI) chain	COL11A1	-0.053	1.666

e17006	3202.44	30.65	SSQGGSLPSEEKGHPQEESEESNVSMASLGE	Secretogranin-1	CHGB	-0.103	1.665
e05369	1485.68	23.77	DGQpGAKGEpGDAGAK	Collagen alpha-1(I) chain	COL1A1	-0.006	1.664
e16656	3121.43	30.16	FAGpPGADGQpGAKGEQGEAGQKGDAGApGpQGP	Collagen alpha-1(II) chain	COL2A1	0.017	1.660
e05521	1503.65	29.78	GQSRDAEEAISQTI	Collagen alpha-1(VI) chain	COL6A1	-0.128	1.658
e03922	1324.59	28.68	TGPGGDKGDTGPpGP	Collagen alpha-1(III) chain	COL3A1	-0.032	1.654
e13662	2557.17	28.23	KNGETGPQGPPGpTGPGGDKGDTGPpGP	Collagen alpha-1(III) chain	COL3A1	-0.032	1.635
e04169	1350.62	27.21	PpGEAGKpGEQGVP	Collagen alpha-1(I) chain	COL1A1	-0.019	1.634
e16170	3011.39	29.69	LTGSpGSpGpDGKTGPPGPAGQDGRPGPpGppG	Collagen alpha-1(I) chain	COL1A1	-0.009	1.634
e00467	911.44	25.83	DGKTGPpGPA	Collagen alpha-1(I) chain	COL1A1	0.001	1.628
e17419	3295.53	25.47	DRGETGPAGPpGApGAPGAPGPVGpAGKSGDRGETGP	Collagen alpha-1(I) chain	COL1A1	-0.004	1.625
e07388	1708.78	30.2	pPGEAGKpGEQGVpGDLG	Collagen alpha-1(I) chain	COL1A1	-0.011	1.619
e05692	1524.66	30.24	GSpGSpGPDGKTGpPGp	Collagen alpha-1(I) chain	COL1A1	-0.049	1.610
e02795	1209.53	26.33	GPpGPDGNKGEpG	Collagen alpha-2(I) chain	COL1A2	0.015	1.609
e07000	1669.65	46.08	FSNGADL	Alpha-1-antitrypsin	SERPINA1	0.028	1.595
e17825	3400.59	32.15	GPpGADGQPGAKGEPGDAGAKGDAGPPGpAGPAGPpGPIG	Collagen alpha-1(I) chain	COL1A1	-0.011	1.594
e13253	2483.11	27.67	EDGKDGSpGEpGANGLpGAAGERGApG	Collagen alpha-1(III) chain	COL3A1	0.003	1.592
e02286	1154.51	25.72	PpGEAGKpGEQG	Collagen alpha-1(I) chain	COL1A1	0.045	1.591
e05074	1447.7	19.5	DGHpGKpGRPGERG	Collagen alpha-2(I) chain	COL1A2	-0.024	1.591
e13595	2545.13	28.08	GpPGADGQpGAKGEpGDAGAKGDAGPpGP	Collagen alpha-1(I) chain	COL1A1	-0.094	1.586
e11065	2168.97	32.92	GpQGVRGEpGPPGPAGAAGpAGNPG	Collagen alpha-1(I) chain	COL1A1	-0.011	1.583
e09131	1900.87	32.04	ETGPAGRpGEVGpPGPpGPAG	Collagen alpha-1(I) chain	COL1A1	-0.018	1.571
e08740	1859.83	24.47	NSGEpGApGSKGDTGAKGEp	Collagen alpha-1(I) chain	COL1A1	-0.050	1.571
e01399	1050.48	26.93	DGRpGPpGPpG	Collagen alpha-1(I) chain	COL1A1	0.025	1.565
e00214	858.4	23.46	SpGEAGRpG	Collagen alpha-1(I) chain	COL1A1	-0.038	1.565
e05248	1468.63	20.11	DDQSAETHSHKQS	Osteopontin	SPP1	0.088	1.564

ID	Mass	Time	Sequence	Protein	Gene	Δ	Score
e10025	2013.89	25.28	NSGEpGApGSKGDTGAKGEpGP	Collagen alpha-1(I) chain	COL1A1	-0.005	1.563
e04851	1425.66	39.79	GQPGLPGPpGPpGPpG	Collagen alpha-1(I) chain	COL1A1	-0.069	1.561
e11498	2226.98	26.39	GNSGEpGApGSKGDTGAKGEPGPpVG	Collagen alpha-1(I) chain	COL1A1	-0.011	1.560
e12007	2296.95	27.05	pGADGQpGAKGEQGEAGQKGDAGAp	Collagen alpha-1(II) chain	COL2A1	0.061	1.559
e06220	1583.7	23.3	GHPDTLNQGEFKEL	Protein S100-A9	S100A9	-0.030	1.557
e01274	1032.5	21.26	RVAPEEHPV	POTE ankyrin domain family member F	POTEF	0.054	1.557
e20010	4252	28.78	ARGNDGATGAAGpPGPTGPAGPPGFPGAVGAKGEAGpQGpRGSEGpQG	Collagen alpha-1(I) chain	COL1A1	0.029	1.554
e10386	2062.93	26.46	DAGAPGApGGKGDAGApGERGpPG	Collagen alpha-1(III) chain	COL3A1	0.018	1.553
e08185	1793.89	32.41	EEAPSLRPAPPPISGGGY	Fibrinogen beta chain	FGB	0.079	1.550
e11836	2274.03	33.44	GLpGpAGpPGEAGKPGEQGVpGDLG	Collagen alpha-1(I) chain	COL1A1	-0.010	1.548
e06186	1579.71	29.9	SpGSPGpDGKTGPPGpAG	Collagen alpha-1(I) chain	COL1A1	0.012	1.540
e10554	2085.93	21.99	EGSpGRDGSpGAKGDRGETGPA	Collagen alpha-1(I) chain	COL1A1	-0.006	1.534
e11452	2220.98	27.04	ADGQpGAKGEpGDAGAKGDAGPPGp	Collagen alpha-1(I) chain	COL1A1	-0.006	1.533
e05830	1540.74	39.81	GPpGVPGpPGpGGSPGLP	Collagen alpha-1(XXII) chain	COL22A1	-0.003	1.522
e08755	1860.83	21.44	EGSpGRDGSpGAKGDRGET	Collagen alpha-1(I) chain	COL1A1	-0.007	1.519
e13850	2587.19	21.13	PpGKNGDDGEAGKPGRpGERGppGPQ	Collagen alpha-1(I) chain	COL1A1	-0.012	1.519
e10266	2047.92	21.93	NGDDGEAGKpGRpGERGPPGP	Collagen alpha-1(I) chain	COL1A1	0.025	1.515
e13817	2583.2	28.3	AGPpGAPGApGAPGpVGPAGKSGDRGETGP	Collagen alpha-1(I) chain	COL1A1	-0.025	1.513
e18041	3457.61	31.46	NTGAPGSpGVSGpKGDAGQpGEKGSpGAQGppGAPGPLG	Collagen alpha-1(III) chain	COL3A1	-0.030	1.512
e11264	2194.95	33.38	NGApGNDGAKGDAGAPGApGSQGApG	Collagen alpha-1(I) chain	COL1A1	-0.015	1.507
e10453	2071.95	32.61	LDTYPNDETTERVFPYI	Vesicular integral-membrane protein VIP36	LMAN2	0.083	1.503
e02530	1179.52	26.93	pGDRGEpGPpGP	Collagen alpha-1(I) chain	COL1A1	-0.006	1.499
e07132	1684.66	30.88	EpGSpGENGAPGQmGPR	Collagen alpha-1(I) chain	COL1A1	0.090	1.498
e14224	2647.21	23.52	NRGERGSEGSPGHpGQPGPpGPPGApGP	Collagen alpha-1(III) chain	COL3A1	-0.063	1.494

e09856	1989.88	32.53	SNGNPGppGPSGSpGKDGPPGp	Collagen alpha-1(III) chain	COL3A1	0.004	1.490
e07225	1692.79	30.1	pPGEAGKpGEQGVPGDLG	Collagen alpha-1(I) chain	COL1A1	-0.033	1.489
e05423	1491.73	39.89	VGpPGPPGpPGPPGPPS	Collagen alpha-1(I) chain	COL1A1	-0.025	1.486
e17311	3271.52	30.76	NTGApGSpGVSGpKGDAGQpGEKGSPGAQGPPGAPGp	Collagen alpha-1(III) chain	COL3A1	0.014	1.481
e09142	1902.84	31.89	NGNPGppGPSGSpGKDGPpGP	Collagen alpha-1(III) chain	COL3A1	-0.034	1.481
e11013	2158.97	33.2	AGpPGEAGKpGEQGVPGDLGApGP	Collagen alpha-1(I) chain	COL1A1	-0.013	1.480
e11957	2290.04	33.54	PGATGFPGAAGRVGpPGSNGNpGPpG	Collagen alpha-1(II) chain	COL2A1	-0.040	1.476
e11193	2185.99	25.94	NSGEpGApGSKGDTGAkGEpGPVG	Collagen alpha-1(I) chain	COL1A1	0.005	1.474
e06961	1664.74	29.98	GLpGTGGPpGENGKPGEp	Collagen alpha-1(III) chain	COL3A1	-0.013	1.472
e07431	1712.77	31.13	pGFAGNpGPpGLPGMKGD	Collagen alpha-1(IV) chain	COL4A1	-0.025	1.460
e05236	1467.66	29.05	SpGSpGPDGKTGppGP	Collagen alpha-1(I) chain	COL1A1	-0.003	1.458
e10502	2078.93	26.63	DAGApGApGGKGDAGApGERGpPG	Collagen alpha-1(III) chain	COL3A1	0.065	1.452
e15129	2809.2	24.38	ERGEAGIpGVpGAKGEDGKDGSPGEpGANG	Collagen alpha-1(III) chain	COL3A1	-0.023	1.451
e19279	3871.78	27.55	TSPSGQLIPVKNLSENIEIL	Polycystic kidney disease protein 1-like 2	PKD1L2	-0.023	1.446
e12156	2314.01	33.67	GPAGpPGEKGEPGDDGPSGAEGPPGp	Collagen alpha-1(II) chain	COL2A1	-0.005	1.445
e10977	2152.97	21.91	SNNAKEAVEHLQKSELTQQ	Apolipoprotein A-IV	APOA4	-0.105	1.444
e01983	1121.49	27.63	DGRpGPpGPpGA	Collagen alpha-1(I) chain	COL1A1	-0.010	1.443
e06024	1563.7	29.56	SpGSPGPDGKTGpPGPAG	Collagen alpha-1(I) chain	COL1A1	0.021	1.434
e13655	2555.22	28	GMKGDPGLPGVPGFpGmKGpSGVPGSAG	Collagen alpha-5(IV) chain	COL4A5	0.002	1.424
e04647	1405.64	20.15	DGPpGRDGQpGHKG	Collagen alpha-2(I) chain	COL1A2	-0.037	1.422
e04330	1367.64	38.84	pPGpPGPpGpPGPPS	Collagen alpha-1(I) chain	COL1A1	-0.023	1.416
e07586	1729.78	30.72	GPPGPTGPGGDKGDTGPpGP	Collagen alpha-1(III) chain	COL3A1	-0.068	1.416
e06733	1638.73	20.24	AGSEADHEGTHSTKRG	Fibrinogen alpha chain	FGA	0.034	1.413
e02052	1128.49	25.75	ApGEAGRDGNpG	Collagen alpha-2(I) chain	COL1A2	-0.001	1.410
e07944	1765.8	30.98	GpPGEAGKpGEQGVpGDLG	Collagen alpha-1(I) chain	COL1A1	0.025	1.408

e04843	1424.65	39.27	GPpGpPGPpGpPGPPS	Collagen alpha-1(I) chain	COL1A1	-0.012	1.406
e07900	1760.78	30.48	DEAAFFDTASTGKTFPG	Fibrinogen alpha chain	FGA	-0.029	1.394
e13634	2551.15	34.75	GGPpGMTGFPGAAGRTGPpGpSGISGPpG	Collagen alpha-2(I) chain	COL1A2	-0.042	1.386
e16219	3023.39	24.65	ppGAKGQEGAHGAPGAAGNPGAPGHVGAPGPSGpP	Collagen alpha-1(XXII) chain	COL22A1	0.025	1.385
e09408	1933.88	21.64	GDDGEAGKpGRPGERGPPGp	Collagen alpha-1(I) chain	COL1A1	0.011	1.383
e02913	1223.55	27.48	GPGGDKGDTGPpGP	Collagen alpha-1(III) chain	COL3A1	-0.024	1.379
e14876	2767.35	21.71	KEGGKGPRGETGPAGRpGEVGPpGPpGPAG	Collagen alpha-1(I) chain	COL1A1	0.006	1.378
e07578	1728.79	31.15	RTGpPGPSGISGPpGPpGP	Collagen alpha-2(I) chain	COL1A2	-0.077	1.377
e16717	3136.39	24.55	DPGLMGERGEDGpAGNGTEGFPGFPGYpGNR	Collagen alpha-1(VI) chain	COL6A1	0.008	1.372
e08978	1882.8	20.24	DEAGSEADHEGTHSTKRG	Fibrinogen alpha chain	FGA	-0.023	1.369
e06430	1608.73	31.15	SGDSDDDEPPPLPRL	Membrane-associated progesterone receptor component 1	PGRMC1	0.003	1.369
e05743	1531.68	39.25	GLpGPpGSNGNpGPpGP	Collagen alpha-1(III) chain	COL3A1	-0.025	1.368
e11265	2194.97	20.12	NDGPpGRDGQpGHKGERGYpG	Collagen alpha-2(I) chain	COL1A2	0.010	1.363
e14815	2756.29	35.2	pGYPGQpGQDGKPGYQGIAGTpGVpGSPG	Collagen alpha-1(XXI) chain	COL21A1	-0.027	1.361
e11554	2234.92	32.98	GRTGDAGPVGPPGPpGppGpPGPPS	Collagen alpha-1(I) chain	COL1A1	0.048	1.359
e04093	1342.58	28.5	FSISRDYNLND	Alpha-1-antichymotrypsin	SERPINA3	-0.021	1.358
e06176	1578.76	40.28	VGPpGPpGPPGPpGPPSA	Collagen alpha-1(I) chain	COL1A1	-0.006	1.356
e07799	1749.8	30.62	GPpGEAGKpGEQGVPGDLG	Collagen alpha-1(I) chain	COL1A1	-0.027	1.350
e18625	3633.74	27.04	AAGEpGKAGERGVPGppGAVGPAGKDGEAGAQGpPG PAGPAG	Collagen alpha-1(I) chain	COL1A1	0.046	1.349
e11780	2266.03	22.25	QNGEpGGKGERGApGEKGEGGPpG	Collagen alpha-1(III) chain	COL3A1	0.017	1.349
e17353	3281.46	22.78	GPpGpRGNRGERGSEGSPGHPGQpGpPGPPGAPGP	Collagen alpha-1(III) chain	COL3A1	-0.015	1.347
e11536	2233.01	22.45	NGDDGEAGKPGRpGERGPpGPQG	Collagen alpha-1(I) chain	COL1A1	0.002	1.347
e04457	1383.59	27.78	GSpGGpGSDGKpGPpG	Collagen alpha-1(III) chain	COL3A1	-0.013	1.345
e14190	2639.29	21.44	KEGGKGPRGETGPAGRpGEVGPpGPpGP	Collagen alpha-1(I) chain	COL1A1	0.021	1.342

e05682	1523.73	40.32	VGPpGPpGPpGPpGPPS	Collagen alpha-1(I) chain	COL1A1	0.007	1.334
e02667	1194.55	26.82	SpGPDGKTGpPGP	Collagen alpha-1(I) chain	COL1A1	0.048	1.329
e12661	2377.11	20.84	GKNGDDGEAGKPGRpGERGPpGpQ	Collagen alpha-1(I) chain	COL1A1	-0.023	1.328
e14712	2739.19	23.64	AKGEDGKDGSpGEpGANGLpGAAGERGApG	Collagen alpha-1(III) chain	COL3A1	0.037	1.320
e12714	2385.05	33.96	pAGPPGEKGEPGDDGPSGAEGPpGPQ	Collagen alpha-1(II) chain	COL2A1	-0.026	1.306
e17672	3356.52	25.46	pGpAGFAGPPGADGQPGAKGEQGEAGQkGDAGAPGPQ	Collagen alpha-1(II) chain	COL2A1	-0.012	1.305
e13566	2541.18	28.08	KNGETGPQGPPGPTGPGGDKGDTGPpGP	Collagen alpha-1(III) chain	COL3A1	-0.014	1.303
e17949	3434.56	31.78	GEPGPPGPAGFAGPPGADGQPGAKGEPGDAGAKGDAGPpG	Collagen alpha-1(I) chain	COL1A1	-0.041	1.302
e15220	2823.34	29.16	LRGGAGPpGPEGGKGAAGpPGpPGAAGTpGLQG	Collagen alpha-1(III) chain	COL3A1	-0.017	1.302
e20740	4960.48	20.57	sDKPDMAEIEKFDKSKLKKTETQEKNPLPSKETIEQEKQAGES	Thymosin beta-4	TMSB4X	0.024	1.294
e14315	2663.21	23.57	NRGERGSEGSPGHpGQpGPPGpPGApGP	Collagen alpha-1(III) chain	COL3A1	-0.015	1.291
e06309	1594.76	40.28	VGPpGPpGPpGPpGPPSA	Collagen alpha-1(I) chain	COL1A1	0.023	1.286
e11641	2246.02	27.14	GADGQPGAKGEPGDAGAKGDAGPpGP	Collagen alpha-1(I) chain	COL1A1	-0.006	1.280
e01155	1016.45	25.74	ApGDKGESGPS	Collagen alpha-1(I) chain	COL1A1	0.025	1.280
e15679	2907.35	35.85	TGEVGAVGPPGFAGEKGpSGEAGTAGPpGTpGP	Collagen alpha-2(I) chain	COL1A2	-0.029	1.278
e00133	840.41	23.33	KGDTGPpGP	Collagen alpha-1(XXIV) chain	COL24A1	-0.006	1.277
e10141	2030.92	32.65	PpGEAGKPGEQGVpGDLGApGP	Collagen alpha-1(I) chain	COL1A1	-0.021	1.269
e01785	1099.5	21.97	DGESGRPGRpG	Collagen alpha-1(III) chain	COL3A1	0.028	1.267
e05879	1547.67	29.92	SpGSPGPDGKTGPPGPAG	Collagen alpha-1(I) chain	COL1A1	-0.027	1.266
e09966	2004.93	25.02	GARGNDGARGSDGQPGPpGPpG	Collagen alpha-1(III) chain	COL3A1	-0.036	1.257
e02280	1153.52	26.1	ApGEDGRpGPpG	Collagen alpha-1(II) chain	COL2A1	0.020	1.257
e05119	1451.7	22.69	EpGKAGERGVpGPpG	Collagen alpha-1(I) chain	COL1A1	-0.035	1.253
e17213	3248.56	25.78	AAGEPGKAGERGVpGpPGAVGPAGKDGEAGAQGPPGP	Collagen alpha-1(I) chain	COL1A1	0.034	1.247
e10634	2096.91	32.82	GApGNDGAKGDAGApGApGSQGApG	Collagen alpha-1(I) chain	COL1A1	-0.042	1.246

ID	Mass	CE time	Sequence	Protein	Gene	Coefficient	VIP
e11008	2158.02	22.17	pPGEEGKRGPRGDpGTVGPpGP	Collagen alpha-2(V) chain	COL5A2	-0.014	1.245
e04435	1380.63	21.5	AILDETKGDYEK	Annexin A1	ANXA1	-0.022	1.242
e08629	1846.85	32.02	TGPIGpPGPAGApGDKGESGP	Collagen alpha-1(I) chain	COL1A1	0.064	1.241
e10277	2048.94	24.32	nGDDGEAGKpGRPGERGPPGp	Collagen alpha-1(I) chain	COL1A1	0.009	1.240
e08546	1836.79	31.79	NGApGEAGRDGNPGNDGPpG	Collagen alpha-2(I) chain	COL1A2	-0.055	1.239
e04769	1417.64	20.06	pGPpGpKGDQGPpGP	Collagen alpha-1(XVII) chain	COL17A1	0.037	1.232
e05742	1531.67	29.86	DITSHMESEELNGA	Osteopontin	SPP1	-0.034	1.217
e01367	1044.48	20.78	SSGPkGSqGDP	Collagen alpha-2(V) chain	COL5A2	-0.075	1.213
e19425	3944.75	24.39	pGApGVRGFQGQKGSmGDpGLPGPQGLRGDVGDRGPGGAAGP	Collagen alpha-3(IX) chain	COL9A3	-0.054	1.211
e06584	1623.73	24.09	DGApGKNGERGGpGGpGP	Collagen alpha-1(III) chain	COL3A1	0.029	1.211
e17279	3264.53	30.51	RTGEVGAVGPpGFAGEKGpSGEAGTAGpPGTpGPQG	Collagen alpha-2(I) chain	COL1A2	0.012	1.210
e10483	2076.95	21.79	GPpGPpGKNGDDGEAGKpGRpG	Collagen alpha-1(I) chain	COL1A1	0.002	1.210
e13200	2471.16	34.76	TGPIGpPGPAGAPGDKGESGpSGPAGPTG	Collagen alpha-1(I) chain	COL1A1	-0.002	1.204
e13618	2548.16	22.88	GAPGQNGEpGGKGERGApGEKGEGGpPG	Collagen alpha-1(III) chain	COL3A1	0.004	1.203
e11527	2232.01	33.67	IGPpGpAGApGDKGESGPSGPAGPTG	Collagen alpha-1(I) chain	COL1A1	-0.006	1.201

ID, polypeptide identifier (SQL number); CE time, migration time in capillary electrophoresis; VIP: the predictive variable influence on projection. Coefficient refers to the association of the corresponding peptides with pulse wave velocity. The polypeptides were ordered by descending VIP.

Table S2. The annotated biological processes by Reactome pathway analysis

Pathway identifier	Pathway name	FDR	Involved Proteins
R-HSA-1474244	Extracellular matrix organization	3.11E-15	FGB/COL17A1/FGA/COL24A1/COL25A1/COL14A1/COL11A1/COL22A1/COL11A2/ PLG/COL19A1/COL1A1/COL3A1/COL2A1/COL1A2/COL5A1/COL4A1/CDH1/COL6A1/ COL5A2/SPP1/COL21A1/COL4A5/COL9A3
R-HSA-1474228	Degradation of the extracellular matrix	3.11E-15	COL17A1/COL25A1/COL14A1/COL11A1/COL11A2/PLG/COL19A1/COL1A1/COL3A1/ COL2A1/COL1A2/COL5A1/COL4A1/CDH1/COL6A1/COL5A2/SPP1/COL4A5/COL9A3
R-HSA-1474290	Collagen formation	3.11E-15	COL17A1/COL24A1/COL25A1/COL14A1/COL11A1/COL22A1/COL11A2/COL19A1/ COL1A1/COL3A1/COL2A1/COL1A2/COL5A1/COL4A1/COL6A1/COL5A2/COL21A1/ COL4A5/COL9A3
R-HSA-216083	Integrin cell surface interactions	3.11E-15	FGB/FGA/COL1A1/COL3A1/COL2A1/COL1A2/COL5A1/COL4A1/CDH1/COL6A1/ COL5A2/SPP1/COL4A5/COL9A3
R-HSA-1650814	Collagen biosynthesis and modifying enzymes	3.11E-15	COL17A1/COL24A1/COL25A1/COL14A1/COL11A1/COL22A1/COL11A2/COL19A1/ COL1A1/COL3A1/COL2A1/COL1A2/COL5A1/COL4A1/COL6A1/COL5A2/COL21A1/ COL4A5/COL9A3
R-HSA-1442490	Collagen degradation	3.11E-15	COL17A1/COL25A1/COL14A1/COL11A1/COL11A2/COL19A1/COL1A1/COL3A1/ COL2A1/COL1A2/COL5A1/COL4A1/COL6A1/COL5A2/COL4A5/COL9A3
R-HSA-2022090	Assembly of collagen fibrils and other multimeric structures	3.11E-15	COL17A1/COL24A1/COL14A1/COL11A1/COL11A2/COL1A1/COL3A1/COL2A1/ COL1A2/COL5A1/COL4A1/COL6A1/COL5A2/COL4A5/COL9A3
R-HSA-8948216	Collagen chain trimerization	3.11E-15	COL17A1/COL24A1/COL25A1/COL14A1/COL11A1/COL22A1/COL11A2/COL19A1/ COL1A1/COL3A1/COL2A1/COL1A2/COL5A1/COL4A1/COL6A1/COL5A2/COL21A1/

			COL4A5/COL9A3
R-HSA-3000171	Non-integrin membrane-ECM interactions	1.94E-14	COL1A1/COL3A1/COL2A1/COL1A2/COL5A1/COL4A1/COL11A1/COL5A2/ COL11A2/COL4A5
R-HSA-8874081	MET activates PTK2 signaling	1.64E-13	COL1A1/COL3A1/COL2A1/COL1A2/COL24A1/COL5A1/COL11A1/COL5A2/ COL11A2
R-HSA-3000178	ECM proteoglycans	2.53E-13	COL1A1/COL3A1/COL2A1/COL1A2/COL5A1/COL4A1/COL6A1/COL5A2/ COL4A5/COL9A3
R-HSA-8875878	MET promotes cell motility	2.79E-12	COL1A1/COL3A1/COL2A1/COL1A2/COL24A1/COL5A1/COL11A1/COL5A2/ COL11A2
R-HSA-186797	Signaling by PDGF	2.19E-11	COL3A1/COL2A1/COL5A1/COL4A1/COL6A1/COL5A2/SPP1/COL4A5/ COL9A3/PLG
R-HSA-419037	NCAM1 interactions	1.27E-10	COL3A1/COL2A1/COL5A1/COL4A1/COL6A1/COL5A2/COL4A5/COL9A3
R-HSA-6806834	Signaling by MET	8.60E-10	COL1A1/COL3A1/COL2A1/COL1A2/COL24A1/COL5A1/COL11A1/COL5A2/COL11A2
R-HSA-375165	NCAM signaling for neurite out-growth	4.24E-09	COL3A1/COL2A1/COL5A1/COL4A1/COL6A1/COL5A2/COL4A5/COL9A3
R-HSA-2214320	Anchoring fibril formation	5.22E-08	COL1A1/COL1A2/COL4A1/COL4A5
R-HSA-9006934	Signaling by Receptor Tyrosine Kinases	1.06E-07	COL24A1/COL11A1/COL11A2/PLG/COL1A1/COL3A1/COL2A1/COL1A2/COL5A1/ COL4A1/COL6A1/COL5A2/SPP1/COL4A5/COL9A3
R-HSA-2243919	Crosslinking of collagen fibrils	4.52E-07	COL1A1/COL1A2/COL4A1/COL4A5
R-HSA-114608	Platelet degranulation	5.91E-07	FGB/SERPINA3/FGA/SERPINA1/TMSB4X/APOA1/PLG/TTN
R-HSA-76005	Response to elevated platelet cytosolic Ca2+	7.84E-07	FGB/SERPINA3/FGA/SERPINA1/TMSB4X/APOA1/PLG/TTN
R-HSA-3000170	Syndecan interactions	1.04E-06	COL1A1/COL3A1/COL1A2/COL5A1/COL5A2
R-HSA-76002	Platelet activation, signaling and aggregation	1.49E-06	FGB/COL1A1/SERPINA3/FGA/SERPINA1/COL1A2/TMSB4X/APOA1/PLG/TTN
R-HSA-3000480	Scavenging by Class A Receptors	1.10E-05	COL1A1/COL3A1/COL1A2/COL4A1/APOA1

R-HSA-381426	Regulation of Insulin-like Growth Factor transport and uptake by Insulin-like Growth Factor Binding Proteins	8.32E-05	FGA/SERPINA1/SPP1/APOA1/PLG/CHGB
R-HSA-8963888	Chylomicron assembly	1.84E-04	APOC3/APOA1/APOA4
R-HSA-8963901	Chylomicron remodeling	3.26E-04	APOC3/APOA1/APOA4
R-HSA-8963899	Plasma lipoprotein remodeling	4.00E-04	APOC3/APOA1/APOA4
R-HSA-76009	Platelet Aggregation (Plug Formation)	4.00E-04	FGB/COL1A1/FGA/COL1A2
R-HSA-8957275	Post-translational protein phosphorylation	4.35E-04	FGA/SERPINA1/SPP1/APOA1/CHGB
R-HSA-3000157	Laminin interactions	1.43E-03	COL4A1/COL4A5
R-HSA-5686938	Regulation of TLR by endogenous ligand	1.43E-03	FGB/FGA/S100A9
R-HSA-8963898	Plasma lipoprotein assembly	1.43E-03	APOC3/APOA1/APOA4
R-HSA-977225	Amyloid fiber formation	2.20E-03	FGA/GSN/APOA1/APOA4
R-HSA-2173782	Binding and Uptake of Ligands by Scavenger Receptors	2.66E-03	COL1A1/COL3A1/COL1A2/COL4A1/APOA1
R-HSA-174824	Plasma lipoprotein assembly, remodeling, and clearance	3.28E-03	APOC3/APOA1/APOA4
R-HSA-8949275	RUNX3 Regulates Immune Response and Cell Migration	4.16E-03	SPP1
R-HSA-162582	Signal Transduction	4.55E-03	FGB/FGA/ANXA1/COL24A1/COL11A1/COL11A2/APOC3/APOA1/APOA4/PLG/COL1A1/ COL3A1/COL2A1/COL1A2/COL5A1/COL4A1/CDH1/COL6A1/COL5A2/SPP1/COL4A5/ COL9A3/S100A9
R-HSA-430116	GP1b-IX-V activation signalling	4.97E-03	COL1A1/COL1A2
R-HSA-109582	Hemostasis	5.33E-03	FGB/COL1A1/SERPINA3/FGA/SERPINA1/COL1A2/TMSB4X/APOA1/PLG/TTN
R-HSA-75153	Apoptotic execution phase	5.92E-03	H1-4/GSN/CDH1
R-HSA-198933	Immunoregulatory interactions between a Lymphoid and a non-Lymphoid cell	6.44E-03	COL1A1/COL17A1/COL3A1/COL2A1/COL1A2/CDH1

R-HSA-422475	Axon guidance	8.11E-03	COL3A1/COL2A1/COL5A1/COL4A1/COL6A1/COL5A2/COL4A5/COL9A3
R-HSA-75892	Platelet Adhesion to exposed collagen	8.75E-03	COL1A1/COL1A2
R-HSA-9675108	Nervous system development	1.08E-02	COL3A1/COL2A1/COL5A1/COL4A1/COL6A1/COL5A2/COL4A5/COL9A3
R-HSA-354194	GRB2:SOS provides linkage to MAPK signaling for Integrins	1.08E-02	FGB/FGA
R-HSA-372708	p130Cas linkage to MAPK signaling for integrins	1.30E-02	FGB/FGA
R-HSA-975634	Retinoid metabolism and transport	1.39E-02	APOC3/APOA1/APOA4
R-HSA-8964058	HDL remodeling	1.54E-02	APOC3/APOA1
R-HSA-977068	Termination of O-glycan biosynthesis	2.08E-02	MUC2/COL14A1
R-HSA-6806667	Metabolism of fat-soluble vitamins	2.24E-02	APOC3/APOA1/APOA4
R-HSA-8963889	Assembly of active LPL and LIPC lipase complexes	2.38E-02	APOA4
R-HSA-5694530	Cargo concentration in the ER	2.67E-02	SERPINA1/LMAN2
R-HSA-8940973	RUNX2 regulates osteoblast differentiation	2.67E-02	COL1A1
R-HSA-6785807	Interleukin-4 and Interleukin-13 signaling	2.79E-02	ANXA1/COL1A2
R-HSA-111465	Apoptotic cleavage of cellular proteins	2.81E-02	GSN/CDH1
R-HSA-168256	Immune System	2.92E-02	FGB/COL17A1/FGA/SERPINA3/GSN/SERPINA1/ANXA1/COL1A1/COL3A1/ PGRMC1/MUC2/COL2A1/COL1A2/CDH1/S100A9
R-HSA-354192	Integrin signaling	2.95E-02	FGB/FGA
R-HSA-114604	GPVI-mediated activation cascade	3.55E-02	COL1A1/COL1A2
R-HSA-140875	Common Pathway of Fibrin Clot Formation	3.55E-02	FGB/FGA
R-HSA-8941326	RUNX2 regulates bone development	3.55E-02	COL1A1

| R-HSA-9656223 | Signaling by RAF1 mutants | 4.54E-02 | FGB/FGA |

Table S3.The Enriched Gene Ontology (GO) biological processes

GOID	GO Term	P value Corrected with Bonferroni step down	% Associated Genes	Nr. Genes	Associated Genes Found
GO:0030199	collagen fibril organization	1.7172E-13	17.65	9.00	[COL11A1, COL11A2, COL14A1, COL1A1, COL1A2, COL2A1, COL3A1, COL5A1, COL5A2]

GO:0071230	cellular response to amino acid stimulus	1.1266E-06	8.33	6.00	[COL1A1, COL1A2, COL3A1, COL4A1, COL5A2, COL6A1]
GO:0038063	collagen-activated tyrosine kinase receptor signaling pathway	1.3723E-04	27.27	3.00	[COL1A1, COL4A1, COL4A5]
GO:0034371	chylomicron remodeling	1.3723E-04	27.27	3.00	[APOA1, APOA4, APOC3]
GO:0034378	chylomicron assembly	1.8011E-04	25.00	3.00	[APOA1, APOA4, APOC3]
GO:0034372	very-low-density lipoprotein particle remodeling	2.3042E-04	23.08	3.00	[APOA1, APOA4, APOC3]
GO:0033700	phospholipid efflux	2.3042E-04	23.08	3.00	[APOA1, APOA4, APOC3]
GO:0038065	collagen-activated signaling pathway	2.8854E-04	21.43	3.00	[COL1A1, COL4A1, COL4A5]
GO:0010896	regulation of triglyceride catabolic process	2.8854E-04	21.43	3.00	[APOA1, APOA4, APOC3]
GO:0060351	cartilage development involved in endochondral bone morphogenesis	3.1171E-04	8.33	4.00	[COL14A1, COL1A1, COL2A1, COL6A1]
GO:0060351	cartilage development involved in endochondral bone morphogenesis	3.1171E-04	8.33	4.00	[COL14A1, COL1A1, COL2A1, COL6A1]
GO:0034370	triglyceride-rich lipoprotein particle remodeling	3.4950E-04	20.00	3.00	[APOA1, APOA4, APOC3]
GO:0035987	endodermal cell differentiation	3.5671E-04	8.00	4.00	[COL11A1, COL5A1, COL5A2, COL6A1]
GO:0035987	endodermal cell differentiation	3.5671E-04	8.00	4.00	[COL11A1, COL5A1, COL5A2, COL6A1]
GO:0042304	regulation of fatty acid biosynthetic process	4.1148E-04	7.69	4.00	[ANXA1, APOA1, APOA4, APOC3]
GO:0034375	high-density lipoprotein particle remodeling	5.9539E-04	16.67	3.00	[APOA1, APOA4, APOC3]
GO:0043691	reverse cholesterol transport	6.9467E-04	15.79	3.00	[APOA1, APOA4, APOC3]
GO:0001706	endoderm formation	6.9693E-04	6.67	4.00	[COL11A1, COL5A1, COL5A2, COL6A1]
GO:0001706	endoderm formation	6.9693E-04	6.67	4.00	[COL11A1, COL5A1, COL5A2, COL6A1]
GO:0055102	lipase inhibitor activity	7.8961E-04	15.00	3.00	[ANXA1, APOA1, APOC3]
GO:0060192	negative regulation of lipase activity	7.8961E-04	15.00	3.00	[ANXA1, APOA1, APOC3]

GO:1903225	negative regulation of endodermal cell differentiation	9.4231E-04	66.67	2.00	[COL5A1, COL5A2]
GO:0045723	positive regulation of fatty acid biosynthetic process	1.0278E-03	13.64	3.00	[ANXA1, APOA1, APOA4]
GO:0001502	cartilage condensation	1.1597E-03	13.04	3.00	[COL11A1, COL2A1, MGP]
GO:0001502	cartilage condensation	1.1597E-03	13.04	3.00	[COL11A1, COL2A1, MGP]
GO:0051004	regulation of lipoprotein lipase activity	1.2999E-03	12.50	3.00	[APOA1, APOA4, APOC3]
GO:0010903	negative regulation of very-low-density lipoprotein particle remodeling	1.7542E-03	50.00	2.00	[APOA1, APOC3]
GO:0034114	regulation of heterotypic cell-cell adhesion	1.8027E-03	11.11	3.00	[APOA1, FGA, FGB]
GO:0034114	regulation of heterotypic cell-cell adhesion	1.8027E-03	11.11	3.00	[APOA1, FGA, FGB]
GO:0042730	fibrinolysis	2.4439E-03	10.00	3.00	[FGA, FGB, PLG]
GO:1903224	regulation of endodermal cell differentiation	2.7602E-03	40.00	2.00	[COL5A1, COL5A2]
GO:0034369	plasma lipoprotein particle remodeling	2.8637E-03	9.38	3.00	[APOA1, APOA4, APOC3]
GO:0034368	protein-lipid complex remodeling	2.8637E-03	9.38	3.00	[APOA1, APOA4, APOC3]
GO:0034367	protein-containing complex remodeling	3.0833E-03	9.09	3.00	[APOA1, APOA4, APOC3]
GO:0034377	plasma lipoprotein particle assembly	3.3087E-03	8.82	3.00	[APOA1, APOA4, APOC3]
GO:0043152	induction of bacterial agglutination	3.8161E-03	33.33	2.00	[FGA, FGB]
GO:2000542	negative regulation of gastrulation	3.8161E-03	33.33	2.00	[COL5A1, COL5A2]
GO:0010901	regulation of very-low-density lipoprotein particle remodeling	3.8161E-03	33.33	2.00	[APOA1, APOC3]
GO:0019433	triglyceride catabolic process	4.1008E-03	8.11	3.00	[APOA1, APOA4, APOC3]
GO:0065005	protein-lipid complex assembly	4.3505E-03	7.89	3.00	[APOA1, APOA4, APOC3]
GO:0045923	positive regulation of fatty acid metabolic process	4.3505E-03	7.89	3.00	[ANXA1, APOA1, APOA4]
GO:0090207	regulation of triglyceride metabolic process	4.6031E-03	7.69	3.00	[APOA1, APOA4, APOC3]
GO:0035989	tendon development	4.8900E-03	28.57	2.00	[COL11A1, COL5A1]
GO:0035989	tendon development	4.8900E-03	28.57	2.00	[COL11A1, COL5A1]

GO:2000483	negative regulation of interleukin-8 secretion	4.8900E-03	28.57	2.00	[ANXA1, TMSB4X]
GO:0060228	phosphatidylcholine-sterol O-acyltransferase activator activity	4.8900E-03	28.57	2.00	[APOA1, APOA4]
GO:0010470	regulation of gastrulation	5.4983E-03	7.14	3.00	[APOA1, COL5A1, COL5A2]
GO:0010470	regulation of gastrulation	5.4983E-03	7.14	3.00	[APOA1, COL5A1, COL5A2]
GO:0055090	acylglycerol homeostasis	5.4983E-03	7.14	3.00	[APOA1, APOA4, APOC3]
GO:0070328	triglyceride homeostasis	5.4983E-03	7.14	3.00	[APOA1, APOA4, APOC3]
GO:0032489	regulation of Cdc42 protein signal transduction	6.0665E-03	25.00	2.00	[APOA1, APOC3]
GO:1900026	positive regulation of substrate adhesion-dependent cell spreading	6.1755E-03	6.82	3.00	[APOA1, FGA, FGB]
GO:1900026	positive regulation of substrate adhesion-dependent cell spreading	6.1755E-03	6.82	3.00	[APOA1, FGA, FGB]
GO:0002063	chondrocyte development	7.1658E-03	6.38	3.00	[COL11A1, COL14A1, COL6A1]
GO:0002063	chondrocyte development	7.1658E-03	6.38	3.00	[COL11A1, COL14A1, COL6A1]
GO:0002063	chondrocyte development	7.1658E-03	6.38	3.00	[COL11A1, COL14A1, COL6A1]
GO:1904729	regulation of intestinal lipid absorption	7.4083E-03	22.22	2.00	[APOA1, APOA4]
GO:0030300	regulation of intestinal cholesterol absorption	7.4083E-03	22.22	2.00	[APOA1, APOA4]
GO:0010898	positive regulation of triglyceride catabolic process	7.4083E-03	22.22	2.00	[APOA1, APOA4]
GO:0046461	neutral lipid catabolic process	7.7098E-03	6.12	3.00	[APOA1, APOA4, APOC3]
GO:0046464	acylglycerol catabolic process	7.7098E-03	6.12	3.00	[APOA1, APOA4, APOC3]
GO:0050982	detection of mechanical stimulus	7.9732E-03	6.00	3.00	[COL11A1, PKD1L2, TTN]
GO:0033344	cholesterol efflux	7.9732E-03	6.00	3.00	[APOA1, APOA4, APOC3]
GO:0042989	sequestering of actin monomers	8.5354E-03	20.00	2.00	[GSN, TMSB4X]
GO:1904478	regulation of intestinal absorption	1.0127E-02	18.18	2.00	[APOA1, APOA4]
GO:0010873	positive regulation of cholesterol esterification	1.0127E-02	18.18	2.00	[APOA1, APOA4]
GO:0051006	positive regulation of lipoprotein lipase activity	1.0127E-02	18.18	2.00	[APOA1, APOA4]

GO:0043589	skin morphogenesis	1.1788E-02	16.67	2.00	[COL1A1, COL1A2]
GO:0043589	skin morphogenesis	1.1788E-02	16.67	2.00	[COL1A1, COL1A2]
GO:0035376	sterol import	1.1788E-02	16.67	2.00	[APOA1, APOC3]
GO:0061365	positive regulation of triglyceride lipase activity	1.1788E-02	16.67	2.00	[APOA1, APOA4]
GO:0070508	cholesterol import	1.1788E-02	16.67	2.00	[APOA1, APOC3]
GO:0010872	regulation of cholesterol esterification	1.5728E-02	14.29	2.00	[APOA1, APOA4]
GO:0032488	Cdc42 protein signal transduction	1.5728E-02	14.29	2.00	[APOA1, APOC3]
GO:0034116	positive regulation of heterotypic cell-cell adhesion	2.0053E-02	12.50	2.00	[FGA, FGB]
GO:0034384	high-density lipoprotein particle clearance	2.0053E-02	12.50	2.00	[APOA1, APOC3]
GO:0003429	growth plate cartilage chondrocyte morphogenesis	2.1984E-02	11.76	2.00	[COL14A1, COL6A1]
GO:0090171	chondrocyte morphogenesis	2.1984E-02	11.76	2.00	[COL14A1, COL6A1]
GO:0003414	chondrocyte morphogenesis involved in endochondral bone morphogenesis	2.1984E-02	11.76	2.00	[COL14A1, COL6A1]
GO:0034380	high-density lipoprotein particle assembly	2.1984E-02	11.76	2.00	[APOA1, APOA4]
GO:0030299	intestinal cholesterol absorption	2.1984E-02	11.76	2.00	[APOA1, APOA4]
GO:0032717	negative regulation of interleukin-8 production	2.3898E-02	11.11	2.00	[ANXA1, TMSB4X]
GO:0003422	growth plate cartilage morphogenesis	2.3898E-02	11.11	2.00	[COL14A1, COL6A1]
GO:0034433	steroid esterification	2.3898E-02	11.11	2.00	[APOA1, APOA4]
GO:0034434	sterol esterification	2.3898E-02	11.11	2.00	[APOA1, APOA4]
GO:0034435	cholesterol esterification	2.3898E-02	11.11	2.00	[APOA1, APOA4]
GO:0098856	intestinal lipid absorption	2.5781E-02	10.53	2.00	[APOA1, APOA4]
GO:0070206	protein trimerization	2.7617E-02	10.00	2.00	[COL1A2, S100A9]
GO:0070206	protein trimerization	2.7617E-02	10.00	2.00	[COL1A2, S100A9]
GO:0044241	lipid digestion	3.2282E-02	9.09	2.00	[APOA1, APOA4]
GO:0090208	positive regulation of triglyceride metabolic process	3.2282E-02	9.09	2.00	[APOA1, APOA4]

GO:0030277	maintenance of gastrointestinal epithelium	3.3996E-02	8.70	2.00	[MUC2, SERPINA3]
GO:0120020	cholesterol transfer activity	3.3996E-02	8.70	2.00	[APOA1, APOA4]
GO:0031639	plasminogen activation	3.5608E-02	8.33	2.00	[FGA, FGB]
GO:0003433	chondrocyte development involved in endochondral bone morphogenesis	3.5608E-02	8.33	2.00	[COL14A1, COL6A1]
GO:0120015	sterol transfer activity	3.5608E-02	8.33	2.00	[APOA1, APOA4]
GO:0071285	cellular response to lithium ion	3.6758E-02	6.25	1.00	[CDH1]
GO:0045176	apical protein localization	3.6758E-02	6.25	1.00	[POTEF]
GO:0048681	negative regulation of axon regeneration	3.6758E-02	6.25	1.00	[SPP1]
GO:0050910	detection of mechanical stimulus involved in sensory perception of sound	3.6758E-02	6.25	1.00	[COL11A1]
GO:0018158	protein oxidation	3.6758E-02	6.25	1.00	[APOA1]
GO:0018206	peptidyl-methionine modification	3.6758E-02	6.25	1.00	[APOA1]
GO:0018158	protein oxidation	3.6758E-02	6.25	1.00	[APOA1]
GO:0050910	detection of mechanical stimulus involved in sensory perception of sound	3.6758E-02	6.25	1.00	[COL11A1]
GO:0018206	peptidyl-methionine modification	3.6758E-02	6.25	1.00	[APOA1]
GO:0050910	detection of mechanical stimulus involved in sensory perception of sound	3.6758E-02	6.25	1.00	[COL11A1]
GO:0071599	otic vesicle development	3.6758E-02	6.25	1.00	[COL2A1]
GO:0051238	sequestering of metal ion	3.6758E-02	6.25	1.00	[S100A9]
GO:0071801	regulation of podosome assembly	3.6758E-02	6.25	1.00	[GSN]
GO:0050910	detection of mechanical stimulus involved in sensory perception of sound	3.6758E-02	6.25	1.00	[COL11A1]
GO:0018158	protein oxidation	3.6758E-02	6.25	1.00	[APOA1]
GO:0018206	peptidyl-methionine modification	3.6758E-02	6.25	1.00	[APOA1]
GO:0003418	growth plate cartilage chondrocyte differentiation	3.7101E-02	8.00	2.00	[COL14A1, COL6A1]

GO:0045992	negative regulation of embryonic development	4.1477E-02	7.41	2.00	[COL5A1, COL5A2]
GO:2000482	regulation of interleukin-8 secretion	4.2662E-02	7.14	2.00	[ANXA1, TMSB4X]
GO:0060536	cartilage morphogenesis	4.2662E-02	7.14	2.00	[COL14A1, COL6A1]
GO:2000352	negative regulation of endothelial cell apoptotic process	4.2749E-02	6.45	2.00	[FGA, FGB]
GO:0072376	protein activation cascade	4.3002E-02	6.25	2.00	[FGA, FGB]
GO:0072378	blood coagulation, fibrin clot formation	4.3002E-02	6.25	2.00	[FGA, FGB]
GO:0072606	interleukin-8 secretion	4.3020E-02	6.06	2.00	[ANXA1, TMSB4X]
GO:0046457	prostanoid biosynthetic process	4.3020E-02	6.06	2.00	[ANXA1, S100A9]
GO:0001516	prostaglandin biosynthetic process	4.3020E-02	6.06	2.00	[ANXA1, S100A9]
GO:0045940	positive regulation of steroid metabolic process	4.3020E-02	6.06	2.00	[APOA1, APOA4]
GO:0036075	replacement ossification	4.3675E-02	6.90	2.00	[COL1A1, COL2A1]
GO:0001958	endochondral ossification	4.3675E-02	6.90	2.00	[COL1A1, COL2A1]
GO:0036075	replacement ossification	4.3675E-02	6.90	2.00	[COL1A1, COL2A1]
GO:0001958	endochondral ossification	4.3675E-02	6.90	2.00	[COL1A1, COL2A1]
GO:0072377	blood coagulation, common pathway	4.4402E-02	100.00	1.00	[FGA]
GO:1902618	cellular response to fluoride	4.4402E-02	100.00	1.00	[COL1A1]
GO:0070208	protein heterotrimerization	4.4402E-02	100.00	1.00	[COL1A2]
GO:1903906	regulation of plasma membrane raft polarization	4.4402E-02	100.00	1.00	[GSN]
GO:0044858	plasma membrane raft polarization	4.4402E-02	100.00	1.00	[GSN]
GO:0070208	protein heterotrimerization	4.4402E-02	100.00	1.00	[COL1A2]
GO:0010987	negative regulation of high-density lipoprotein particle clearance	4.4402E-02	100.00	1.00	[APOC3]
GO:0010669	epithelial structure maintenance	4.4500E-02	6.67	2.00	[MUC2, SERPINA3]
GO:0003413	chondrocyte differentiation involved in endochondral bone morphogenesis	4.4500E-02	6.67	2.00	[COL14A1, COL6A1]
GO:0050996	positive regulation of lipid catabolic process	4.4500E-02	6.67	2.00	[APOA1, APOA4]

Abbreviation: ANXA1, annexin A1; APOA, apolipoprotein A; APOC, apolipoprotein C; CHGB, secretogranin-1; COL, collagen; FGA, fibrinogen alpha chain; FGB, fibrinogen beta chain; GSN, gelsolin; LMAN2, vesicular integral-membrane protein VIP36; MGP, Matrix Gla protein; MUC, mucin; PCDH, protocadherin-9; PGRMC1, membrane-associated progesterone receptor component; PKD1L2, polycystic kidney disease protein 1-like 2; PLG, plasminogen; POTEF, POTE ankyrin domain family member F; S100A9, protein S100-A9; SERPINA1, α1-antitrypsin; SERPINA3, α1-antichymotrypsin; SPP, signal peptide peptidase; TMSB4X, thymosin β4.

Table S4. Unadjusted and adjusted linear association of PWV with clinical variables

Variables	Univariate models β (95% CI)	P Value	Multivariate model* β (95% CI)	P Value
Age, years	0.51 (0.44 to 0.57)	<0.0001	0.38 (0.31 to 0.45)	<0.0001
Body mass index, kg/m^2	0.21 (0.13 to 0.28)	<0.0001	-	
Heart rate, beats/min	0.05 (-0.03 to 0.12)	0.24	0.09 (0.03 to 0.15)	0.006
Mean arterial pressure, mmHg	0.46 (0.39 to 0.53)	<0.0001	0.26 (0.19 to 0.34)	<0.0001
Total cholesterol, mmol/L	0.15 (0.07 to 0.22)	0.0001	-	
HDL-C, mmol/L	-0.04 (-0.12 to 0.03)	0.263	-	
LDL-C, mmol/L	0.15 (0.08 to 0.23)	<0.0001	-	
Blood glucose, mmol/L	0.26 (0.18 to 0.33)	<0.0001	0.10 (0.04 to 0.17)	0.002
eGFR, ml/min/1.73m2	-0.35 (-0.43 to -0.28)	<0.0001	-	
Sex	-0.07 (-0.15 to 0.01)	0.070	-0.05 (-0.46 to 0.06) †	0.14
Current smoking	0.02 (-0.05 to 0.10)	0.520	0.08 (0.02 to 0.15)	0.009
Diabetes mellitus	0.10 (0.02 to 0.18)	0.009	-	
CV diseases	0.20 (0.12 to 0.27)	<0.0001	-	
Hypertension	0.40 (0.33 to 0.47)	<0.0001	-	
Antihypertensive drugs	0.26 (0.19 to 0.34)	<0.0001	-	
Cholesterol lowering drugs	0.15 (0.07 to 0.22)	0.0002	-	

Diabetes mellitus was use of antidiabetic drugs, fasting blood glucose of ≥126 mg/dL; hypertension was an office blood pressure of ≥140 mmHg systolic or ≥90 mmHg diastolic, or use of antihypertensive drugs; eGFR is estimated using the chronic kidney disease epidemiology collaboration creatinine equation. Body mass index was calculated by weight in kilograms divided by height in meters squared.

β, beta coefficient, referred to the increase of PWV for per standard deviation increment in the continuous variable, while for a categorical variable, it indicates the difference of PWV between categories.

* Model was constructed by backward selection (requiring retained variable with p <0.05) on the listing clinical variables.

† Sex forced into the full model.

Abbreviation: CV diseases, cardiovascular diseases; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, high-density lipoprotein.

Table S5.

Fatal and Nonfatal Cardiovascular Events

| Endpoint	Type	No. of Events
Myocardial infraction	Fatal	0
	Nonfatal	11
Acute coronary syndrome	Nonfatal	3
New-set Angina pectoris	Nonfatal	7
Coronary revascularization	Surgical	1
	Angioplasty	16
Ischemic cardiomyopathy	Nonfatal	7
Heart failure	Fatal	5
	Nonfatal	14
Life-threatening arrhythmias	Fatal	1
	Nonfatal	8
New-set atrial fibrillation	Nonfatal	15
Pulmonary heart disease	Nonfatal	5
Vulvar disorders	Fatal	1
	Nonfatal	5
Stroke	Fatal	2
	Nonfatal	11
Transient ischemic attack	Nonfatal	5
Aortic aneurysm	Fatal	1
	Nonfatal	3
Arterial embolism	Nonfatal	5
Peripheral arterial disease	Nonfatal	17

The median follow-up of 669 participants was 9.2 (5th to 95th percentile, 6.1-10.7) years. One participant could experience multiple nonfatal endpoints, but the first event within each category was considered.

Figure S1. The distribution of the PWV-UP in 669 participants from the general population.
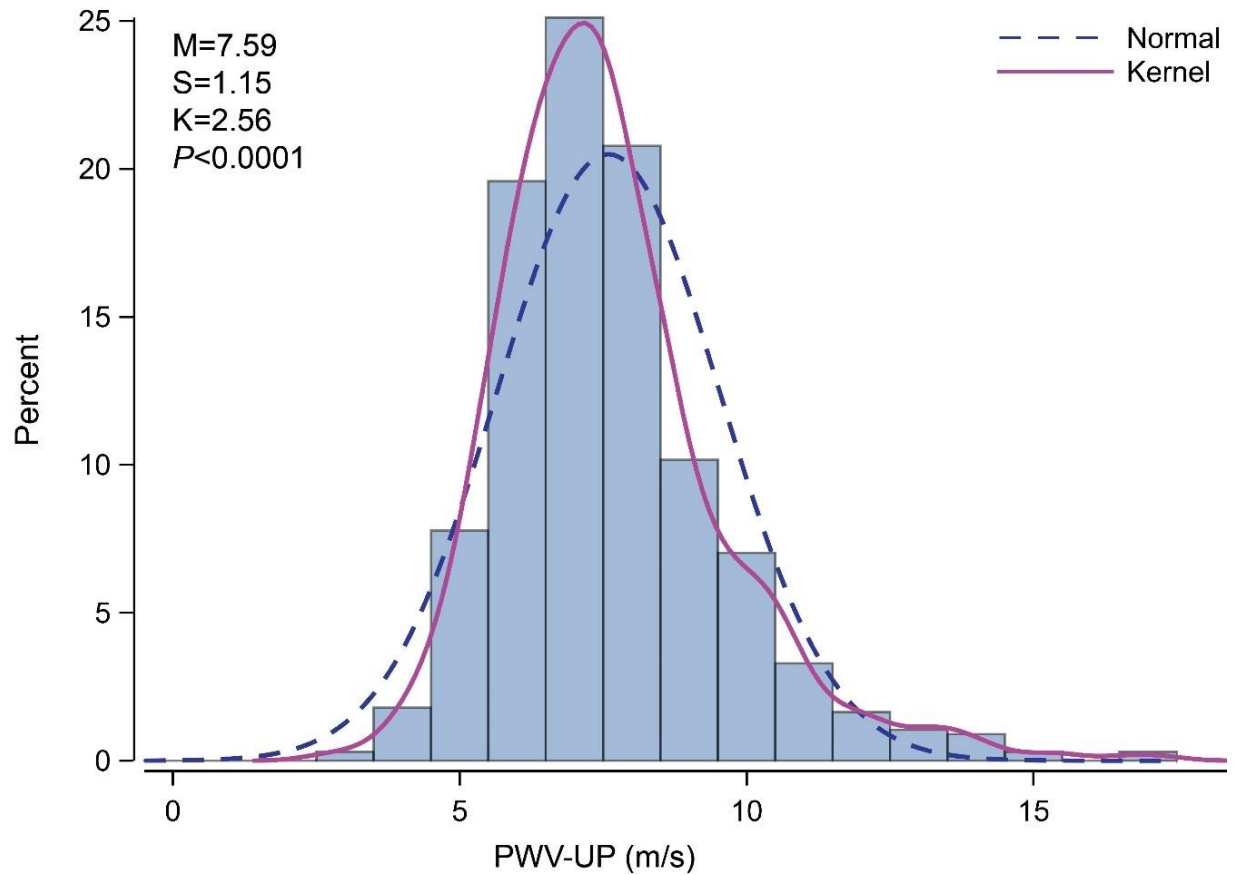
P values are for departure of the observed distribution from normality according to the Shapiro-Wilk statistic. M indicates the mean. Skewness (S) and kurtosis (K) were computed as the third and fourth moments about the mean divided by the cube of the standard deviation.

Figure S2. Ten-year risk of all-cause mortality (a) and cardiovascular events (b) associated with PWV-UP and pulse wave velocity (PWV).
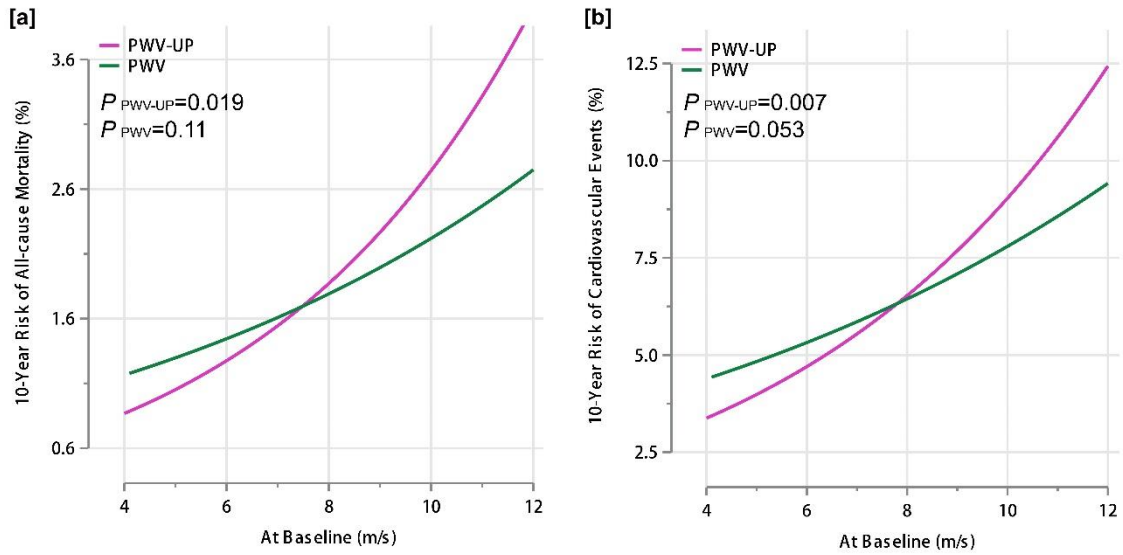
Risk functions were standardized to average values (mean or ratio) of the distributions in the whole study population of sex, age, smoking, diabetes, history of cardiovascular events, mean arterial pressure, body mass index, plasma glucose, total cholesterol, eGFR. *P*-values indicate the independent effect of PWV-UP (P_{PWV-UP}) and PWV (P_{PWV}).