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Exploring biomarkers associated with deteriorating vascular health using a targeted proteomics chip
The SABPA study

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Abstract

In this observational study, by the use of a multiplex proteomic platform, we aimed to explore associations between 92 targeted proteins involved in cardiovascular disease and/or inflammation, and phenotypes of deteriorating vascular health, with regards to ethnicity.

Proteomic profiling (92 proteins) was carried out in 362 participants from the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study of black and white African school teachers (mean age 44.7 ± 9.9 years, 51.9% women, 44.5% Black Africans, 9.9% with known cardiovascular disease). Three proteins with <15% of samples below detectable limits were excluded from analyses. Associations between multiple proteins and prevalence of hypertension as well as vascular health [Carotid intima-media thickness (cIMT) and pulse wave velocity (PWV)] measures were explored using Bonferroni-corrected regression models.

Bonferroni-corrected significant associations between 89 proteins and vascular health markers were further adjusted for clinically relevant co-variates. Hypertension was associated with growth differentiation factor 15 (GDF-15) and C-X-C motif chemokine 16 (CXCL16). cIMT was associated with carboxypeptidase A1 (CPA1), C-C motif chemokine 15 (CCL15), chitinase-3-like protein 1 (CHI3L1), scavenger receptor cysteine-rich type 1 protein M130 (CD163) and osteoprotegerin, whereas PWV was associated with GDF15, E-selectin, CPA1, fatty acid-binding protein 4 (FABP4), CXCL16, carboxypeptidase B (CPB1), and tissue-type plasminogen

activator. Upon entering ethnicity into the models, the associations between PWV and CPA1, CPB1, GDF-15, FABP4, CXCL16, and between cIMT and CCL-15, remained significant.

Using a multiplex proteomic approach, we linked phenotypes of vascular health with several proteins. Novel associations were found between hypertension, PWV or cIMT and proteins linked to inflammatory response, chemotaxis, coagulation or proteolysis. Further, we could reveal whether the associations were ethnicity-dependent or not.

Abbreviations: cIMT = carotid intima media thickness, PWV = pulse wave velocity.

Keywords: carotid intima media thickness, carotid intima media thickness, ethnicity, hypertension, multiplex proteomics, pulse wave velocity, vascular health

1. Introduction

Cardiovascular diseases (CVD) are the leading global cause of death, causing patients great personal suffering and is a major socioeconomic burden for society. Subclinical detection to improve risk stratification and preventive treatment selection along with the further understanding of the biological processes preceding CVD are urgently called upon. A leading CVD risk factor globally is hypertension, it is prevalence being the highest in Africa and also rising in low- and middle-income countries such as South Africa.[1,2] Atherosclerosis is the main pathophysiological process leading to CVD and hypertension is a major contributing factor to this process. Further, arteriosclerosis that is, arterial stiffness, precedes and predicts incident hypertension.[3] Carotid intima media thickness (cIMT) and pulse wave velocity (PWV), markers of early alterations of arterial morphology, structure and function, capture the early stages of the atherosclerotic and arteriosclerotic processes respectively, and are demonstrated to be strong predictors of future cardiovascular events.[4,5] Studying biomarkers' involvement in relation to different aspects of CVD by proteins' associations with hypertension, cIMT and PWV may further increase understanding of the complex biological processes leading to CVD. Multiplex proteomics platforms provide an excellent tool for investigating associations between multiple, targeted proteins and disease (e.g., CVD).[6,7]

The effects of ethnicity on several biomarkers' reference intervals is known[8,9] yet often overlooked. Further, racial disparities in the prevalence of hypertension is known and cannot be fully explained by traditional risk factors.[10,11] Evaluating targeted biomarkers' associations with cardiovascular traits, adjusted for ethnicity can help increase our understanding of possible differing pathophysiological pathways preceding CVD in different ethnic groups.

Here, we aimed to explore associations between 92 proteins implicated in inflammation/CVD and phenotypes of vascular health such as prevalent hypertension, subclinical atherosclerosis (as measured by cIMT) and arteriosclerosis, (as measured by PWV), with regards to ethnicity.

2. Methods

2.1. Study design and population

This study is part of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) study of 409 black and white African school teachers (hereafter referred to as Blacks and Whites) (aged 20–62), working in the North-West Province, South-Africa. The inclusion of teachers ensured a cohort with similar socio-economic status. The examination was conducted in 2008–2009 and a detailed description of the study population is available elsewhere.[12] Out of 409 subjects, 31 did not have proteomics data, including the 19 HIV-infected subjects in the population. Of the remaining 378, 16 participants were excluded from all analyses because of missing data on any of self-reported smoking status, cIMT, PWV, estimated glomerular filtration rate (eGFR), mean arterial pressure (MAP) and heart rate (HR), resulting in 362 participants having data on all co-variates and thus included in analyses (Fig. 1).

The SABPA study conforms to the principles outlined in the Declaration of Helsinki (World Medical Association General Assembly 2004) and abided by the institutional guidelines and was approved by the Health Research Ethics Committee of the North-West University, South Africa (NWU-0003607S6). All participants provided written informed consent.

2.2. Clinical measurements

With an appropriate cuff placed on the upper left arm, after a 5 to 10-minutes rest and with the patients in the semi-Fowler's position, resting blood pressure was measured twice by a registered nurse or doctor, on the non-dominant arm by applying a suitable cuff using a stethoscope (Littman II S.E. Stethoscope 2205) and a calibrated mercury sphygmomanometer (Riester CE 0124 & 1.3 M TM). Two duplicate measures were taken, with a 3- to 5-minute resting period between each; the second of which was used for statistical analyses. Hypertension was defined as resting systolic blood pressure of ≥ 140 and/or diastolic blood pressure ≥ 90 mm Hg, or use of hypertensive medication.[13]

2.3. Carotid ultrasound

Carotid intima media thickness was obtained using a SonoSite Micromaxx ultrasound system (SonoSite, Bothell, WA) and a 6 to 13 MHz linear array transducer. Images from at least 2 optimal angles of the left and right common carotid artery were obtained,[14] and then digitalized and imported into the Artery Measurement Systems automated software[15,16] for analyses of cIMT. When image quality was satisfactory, a maximal 10 mm segment was chosen for analysis (Supplementary Figure S1, http://links.lww.com/MD/G95). The software automatically calculates cIMT, with the possibility of manual correction if found not appropriate upon visual inspection. The mean of the far wall and near wall measurements were used. Intra-observer variability for the far wall was 0.04 mm between 2 measurements made 4 weeks apart on 10 participants.

2.4. Pulse wave velocity

The carotid-dorsalis pedis PWV was carried out across the carotid-dorsalis pedis region as a measure of arterial stiffness
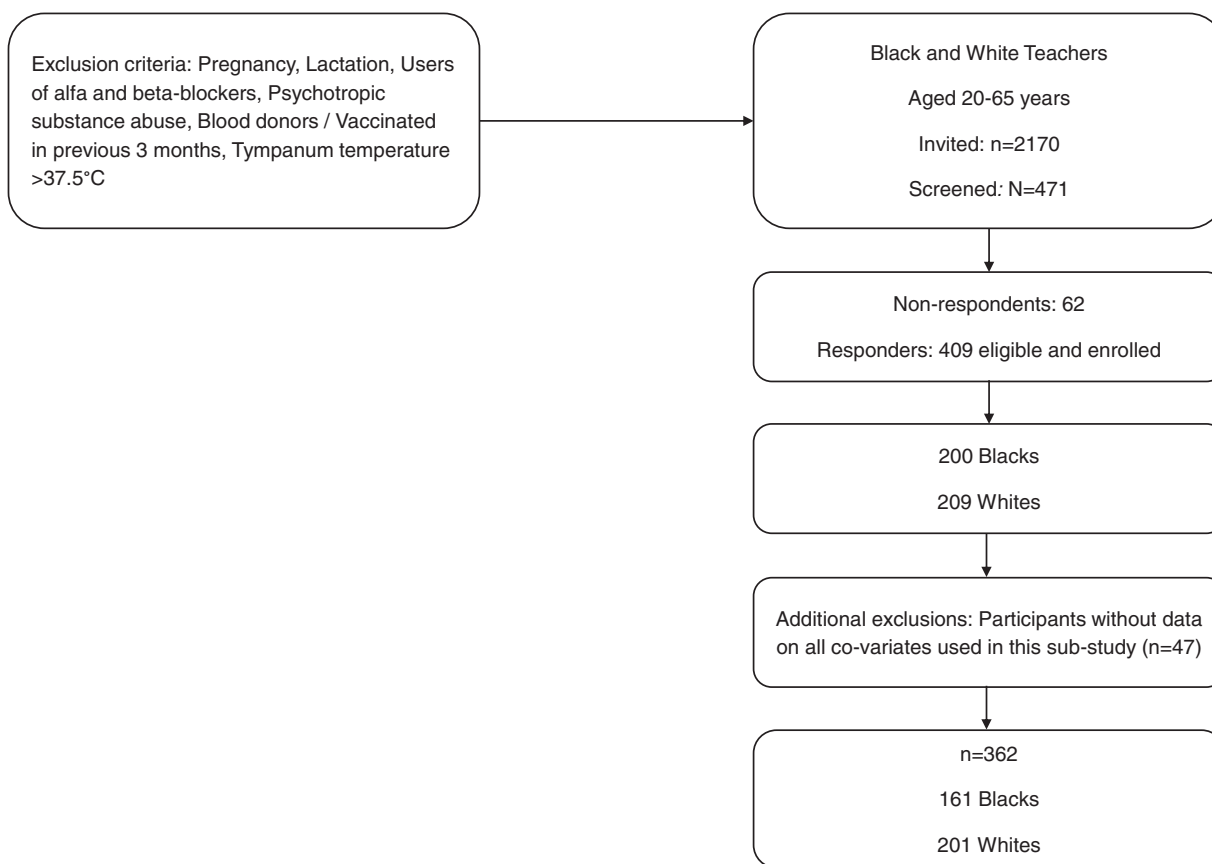
Figure 1. Flowchart outlining inclusion in the study.

with the participant in a supine position (Complior SP device, Artech-Medical, Pantin, France). The distance was determined by subtracting the carotid artery to suprasternal notch distance from the distal measurement (subtraction method). All measurements were taken by the same 2 observers for all participants.[17]

2.5. Questionnaire data and anthropometry

Participants completed a lifestyle questionnaire that included information regarding their habits (ex. smoking), if on a diabetes diet, medical history (ex. CVD or diabetes), medication use. Waist circumference was measured in triplicate to the nearest 0.1 cm with an unstreachable type measure according to the standard method proposed by the International society for the advancement of kinanthropometry. CVD burden was defined as any of the following: history of physician diagnosed heart disease, use of anti-hypertensive medication, anti-diabetic medication, or statins.[18] Diabetes was defined as use of anti-diabetes drug, on a diabetes diet or HbA1c ≥6.5%.

2.6. Biochemical analyses

Sodium fluoride, plasma and serum samples from fasting blood were stored at −80°C. Serum samples for total cholesterol and gamma glutamyl transferase (GGT) were analyzed using 2 sequential multiple analyzers (Konelab 20i; Thermo Scientific, Vantaa, Finland; Unicel DXC 800 - Beckman and Coulter, Germany). The intra- and inter-coefficients of variation for all assays were below 10%.[19] HIV status was measured by a rapid

anti-body test in plasma (First response kit. PMC Medical, Daman, India) and confirmed with the Pareekshak test (BHAT Bio-Tech, Bangalore, India). Estimated glomerular filtration rate (eGFR) was calculated using the modification of diet in renal disease (MDRD) formula: 186*[serum creatinine (μmol/l)88.4]-1.154*[age]-0.203. Consider:*1.210 (if Black) and/or * 0.742 (if female).[20]

2.7. Proteomic profiling

Plasma levels of proteins were analyzed by the Proximity Extension Assay (PEA) technique, PCR-based affinity proteomics technology, using the Proseek Multiplex CVD III 96×96 reagents kit (Olink Bioscience, Uppsala, Sweden). The new PEA technique has opened up for large-scale analyzing of multiple proteins and encompasses high specificity and sensitivity using only small samples of blood.[21] The CVD III panel[22–24] comprises 92 proteins, with either established or suggested associations with CVD and inflammation. Three proteins were below detectable limits in >15% samples (NT-proBNP, pulmonary surfactant-associated protein and spondin-1). Hence, 89 proteins were taken forward to analyses. Across all 92 assays, the mean intra-assay and inter-assay variations were observed to be 7% and 14%, respectively. Validation data and coefficients of variance for all proteins can be found in the online supplemental material (Validation data CVD III) and further technical information about the assays is available on the Olink homepage (http://www.olink.com). A quality control of the technical performance of the assays, as well as the samples, is carried

out during the analyses. The quality control is carried out using 4 internal controls that are spiked into all samples and external controls in every analysis:

1. 2 incubation controls (non-human antigens with matching antibodies);
2. extension control (IgG antibodies conjugated with matching oligo-pair) and
3. detection control (synthetic double-stranded DNA).

For the entire sample plate, standard deviations are calculated for both the detection control and the incubation controls; in order to pass quality control, the standard deviations should be below a predetermined threshold.

2.8. Statistical methods

Proteins are expressed on a log2 scale and presented as normalized protein expression (NPX; arbitrary units), which corresponds to a 2-fold change in protein concentration. Variables that were skewed (serum gamma glutamyl transferase and mean arterial blood pressure) were ln-transformed. Blacks and Whites were compared using one-way ANOVA tests for continuous variables, or χ^2 tests for binary variables. Complete data was available in 362 subjects, and univariate regressions were considered significant if they met the Bonferroni-corrected ($P < .05/89 = 5.6 \times 10^{-4}$) significance threshold for each set of analyses with hypertension, cIMT and PWV as the dependent variables and the 89 proteins as the main independents. Logistic regression analyses were carried out to examine possible associations between hypertension and each one of the 89 proteins in unadjusted models. Bonferroni-corrected significant associations were further adjusted for age and sex (Model 1), and if significant ($P < .05$), additional adjustment was carried out for clinically relevant co-variates (waist circumference, smoking, estimated glomerular filtration rate (eGFR), alcohol use as measured by GGT, prevalent CVD, diabetes and heart rate (Model 2a)). Ethnicity was further entered on top of Model 2a. In order to examine possible associations between each one of the 89 proteins and cIMT, cIMT was log2-transformed and unadjusted linear regression analyses were carried out. Associations that met the Bonferroni-correction were further adjusted for age and sex (Model 1), and if significant ($P < .05$), model 2b was further adjusted for known cardiovascular risk factors as well as factors that may be in the pathway of CVD/inflammation and atherosclerosis (waist circumference, systolic blood pressure, smoking, cholesterol, statin use, diabetes, prevalent CVD and anti-hypertensive treatment).[25] Further, ethnicity was entered on top of Model 2b. Associations between each of the 89 proteins and log2-transformed PWV were carried out unadjusted in linear regression analyses. Associations that met Bonferroni-corrected significance where then adjusted for age and sex (Model 1) and, if significant, further adjusted for waist circumference, smoking, diabetes, use of statins, total cholesterol, anti-hypertensive treatment and mean arterial blood pressure[26] (Model 2c).
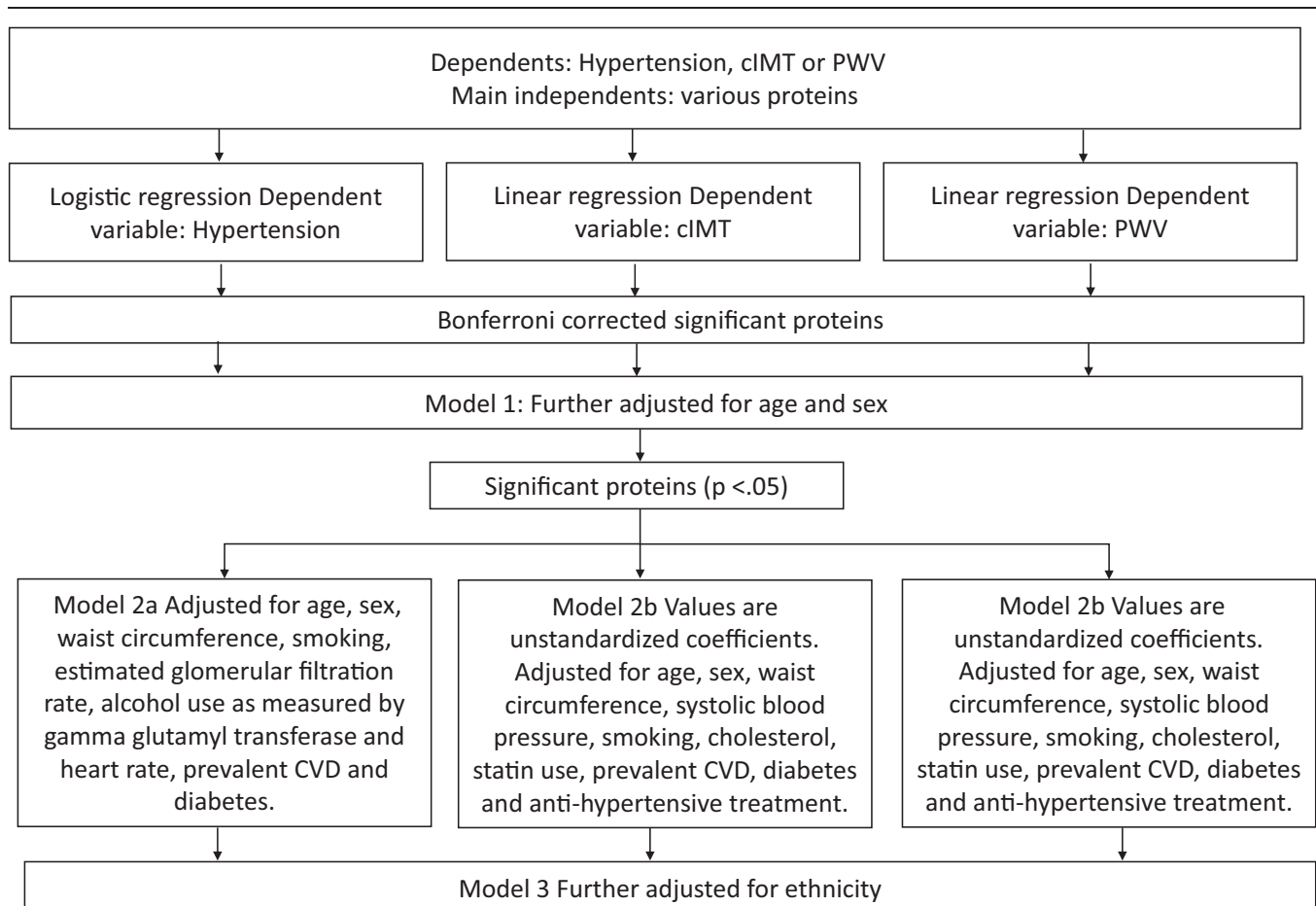

Figure 2. Flowchart illustrating the statistical analysis.

Table 1

Characteristics of the study population.

	Total n = 362	Blacks n = 161	Whites n = 201	P
Age (yrs)	44.8 (±9.9)	44.5 (±8.5)	45.0 (±10.9)	.61
Sex, n female (%)	188 (51.9)	86 (53.4)	102 (50.7)	.61
eGFR	102.8 (±24.0)	113.2 (±27.1)	94.6 (±17.2)	<.001
Total cholesterol (mmol/L)	5.1 (±1.3)	4.6 (±1.1)	5.5 (±1.3)	<.001
Waist circumference (cm)	93.5 (±15.9)	94.0 (±15.5)	93.1 (±16.2)	.63
Lifestyle and medication				
Smoker, n (%)	53 (14.6)	25 (15.5)	28 (13.9)	.67
GGT	27 (16.0–46.8)	40.0 (27.4–68.4)	18.0 (12.0–28.5)	<.001
Use of statins, n (%)	11 (3)	2 (1.2)	9 (4.5)	.08
Use of antihypertensives, n (%)	86 (23.8)	60 (37.3)	26 (12.9)	<.001
Hypertension, n (%)	219 (60.5)	121 (75.2)	98 (48.8)	<.001
Prevalent CVD, n (%)	36 (9.9)	13 (8.1)	23 (11.4)	.29
Diabetes, n (%)	44 (12.2)	27 (16.8)	17 (8.5)	.02
Cardiovascular measurements				
Off SBP (mm Hg)	135.7 (±20.2)	141.0 (±20.6)	131.5 (±15.2)	<.001
Off DBP (mm Hg)	88.7 (±13.5)	93.4 (±13.6)	85.0 (12.1)	<.001
Pulse wave velocity (m/s)	8.3 (7.2 − 9.3)	8.6 (7.4 − 9.6)	8.2 (7.1 − 9.0)	<.001
cIMT (mm)	0.652 (0.57–0.74)	0.665 (0.60–0.75)	0.64 (0.55–0.73)	.001

Values are means ± standard deviation (SD) or median (25–75 interquartile range (IQR)). cIMT = carotid intima media thickness, CVD = cardiovascular disease, eGFR = estimated glomerular filtration rate, GGT = Serum Gamma glutamyl transferase, Off DBP = Office diastolic blood pressure, Off SBP = Office systolic blood pressure, PWV = pulsed wave velocity.

Ethnicity was entered on top of Model 2c (A flowchart illustrating the statistical analysis is given in Fig. 2).

All analyses were performed using SPSS Windows version 26.0.

3. Results

The baseline characteristics of the study population are presented in Table 1. Blacks had overall significantly worse metabolic status overall compared to whites with higher systolic BP ($P < .001$), diastolic BP ($P = <.001$), GGT ($P < .001$) and prevalence diabetes (16.8% vs 8.5%, $P = .02$), with the exception that whites had higher total cholesterol levels ($P < .001$) and poorer kidney function (eGFR) ($P < .001$) than blacks. Regarding the markers of vascular health, PWV ($P < .001$), cIMT ($P = .001$) and

prevalence of hypertension (75.2% vs 48.8% $P = <.001$) were all significantly higher in the Black population.

Names and acronyms of all of the 92 proteins included in the CVD III panel are presented in Supplementary Table S1, http://links.lww.com/MD/G96. Bonferroni-corrected univariate logistic regression analyses of associations between 89 proteins and prevalence of hypertension resulted in 11 significant findings (Supplementary Table S2, http://links.lww.com/MD/G97). Ten proteins remained significant when further adjusted for age and sex (Supplementary Table S3, http://links.lww.com/MD/G98). After further adjustment for waist circumference, smoking, eGFR, GGT, prevalent CVD, diabetes and heart rate, growth differentiation factor 15 (GDF15; odds ratio (OR) 2.06, 95% confidence interval (CI95%) 1.04–4.07, $P = .04$) and C-X-C motif chemokine 16 (CXCL16; OR 2.33, CI95% 1.06–5.11,

Table 2

Associations between proteins and HT, cIMT or PWV.

	HT OR (CI95%) P value		cIMT β (P value)		PWV β (P value)	
Proteins	Model 2a	Model 3	Model 2b	Model 3	Model 2c	Model 3
GDF15	2.06 (1.04–4.07).04	1.96 (0.97–3.96).06	0.032 (.20)	−*	0.086 (.004)	0.080 (.006)
CPA1	−*	−*	0.031 (.048)	0.020 (0.20)	0.050 (.01)	0.041 (.03)
CCL15	−*	−*	0.065 (<.001)	0.045 (.02)	−*	−*
CHI3L1	1.33 (0.98–1.80).07	−*	0.028 (.02)	0.020 (.10)	0.021 (.14)	−*
OPG	−*	−*	0.074 (.01)	0.041 (.17)	−*	−*
SELE	−*	−*	0.033 (.08)	−*	0.049 (.03)	0.038 (.09)
FABP4	0.89 (0.63–1.28).53	−*	0.036 (.05)	−*	0.055 (.01)	0.045 (.04)
CXCL16	2.33 (1.06–5.11).03	1.47 (0.62–3.50).38	−*	−*	0.119 (.001)	0.089 (.04)
tPA	1.12 (0.97–1.28).12	−*	−*	−*	0.014 (.04)	0.010 (.16)
CD163	1.26 (0.76–2.09).37	−*	0.044 (.04)	0.031 (.15)	0.016 (.52)	−*
CPB1	−*	−*	−*	−*	0.043 (.04)	0.045 (.03)

* the associations were not significant in the previous analyses made and were therefore not analysed in this step. CCL15 = C-C motif chemokine 15, CD163 = Scavenger receptor cysteine-rich type 1 protein M130, CHI3L1 = chitinase-3-like protein 1, CPA1 = Carboxypeptidase A1, CPB1 = carboxypeptidase B1, CXCL16 = C-C motif chemokine 16, FABP4 = fatty acid-binding protein 4, GDF15 = growth-differentiation factor 15, OPG = osteoprotegerin, SELE = E-selectin, tPA = Tissue-type plasminogen activator.
All proteins are adjusted for age and sex. Model 2a Values are odds ratios (OR) and 95% confidence intervals (CI95%). Adjusted for age, sex, waist circumference, smoking, estimated glomerular filtration rate, alcohol use as measured by gamma glutamyl transferase and heart rate, prevalent CVD and diabetes. Model 2 b Values are unstandardized coefficients. Adjusted for age, sex, waist circumference, systolic blood pressure, smoking, cholesterol, statin use, prevalent CVD, diabetes and anti-hypertensive treatment. Model 2c Values are unstandardized coefficients. Adjusted for age, sex, waist circumference, mean arterial blood pressure, smoking, use of statins, diabetes and anti-hypertensive treatment. Model 3 is further adjusted for ethnicity.

$P=.03$) remained significantly associated with hypertension (Table 2, Supplementary Table S4, http://links.lww.com/MD/G99).

When ethnicity was entered upon model 2 none of the proteins remained significantly associated with prevalence of hypertension (Table 2).

As for associations between 89 proteins and cIMT, Bonferroni-corrected univariate linear regression analyses resulted in 14 significant findings (Supplementary Table S5, http://links.lww.com/MD/G100). Eleven proteins remained significant when further adjusted for age and sex Supplementary Table S6, http://links.lww.com/MD/G101). After further adjustment for waist circumference, systolic blood pressure, smoking, total cholesterol, statin use, prevalent CVD, diabetes and anti-hypertensive treatment, 5 proteins remained significantly associated with increasing cIMT: carboxypeptidase A (CPA1; β 0.031; $P=.048$), C-C motif chemokine 15 (CCL15; β 0.065, $P<.001$), CHI3L1 (β 0.028, $P=.02$), scavenger receptor cysteine-rich type 1 protein M130 (CD163; β 0.044;$=.04$) and osteoprotegerin (OPG; β 0.074, $P=.01$) (Table 2, Supplementary table S7, http://links.lww.com/MD/G102). When ethnicity was entered upon the adjusted model, only CCL15 remained significantly associated with increasing cIMT (β 0.031, $P=.02$) (Table 2).

Associations between 89 proteins and PWV were carried out in Bonferroni-corrected univariate linear regressions, and resulted in 14 significant findings (Supplementary Table S8, http://links.lww.com/MD/G103). All 14 proteins remained significant when further adjusted for age and sex (Supplementary Table S9, http://links.lww.com/MD/G104). After further adjustment for waist circumference, smoking, diabetes, mean arterial blood pressure, total cholesterol, use of statins and anti-hypertensive treatment, 7 proteins presented significant associations with PWV: GDF15 (β 0.086, $P=.004$), SELE (β 0.049, $P=.03$), CPA1 (β 0.050, $P=.01$), FABP4 (β 0.055, $P=.01$), CXCL16 (β 0.119, $P=.001$), CPB1 (0.043; $P=.04$), and tPA (β 0.020, $P=.03$) (Table 2, Supplementary table S10, http://links.lww.com/MD/G105).

Additional adjustment for ethnicity resulted in 5 significant associations between GDF15 (β 0.080, $P=.006$), CPA1 (β 0.050, $P=.04$), FABP4 (β 0.045, $P=.04$), CXCL16 (β 0.119, $P=.03$) and CPB1 (0.045, $P=.03$), and PWV (Table 2).

4. Discussion

Here, we used a multiplex proteomic panel in a bi-ethnic cohort to explore associations between vascular traits such as hypertension, cIMT and PWV and multiple proteins involved in CVD and inflammation. We could confirm previously demonstrated associations (between PWV and GDF15, FABP4 and SELE[27–29]; between hypertension and GDF-15[30]; and between cIMT and Osteoprotegerin (OPG), CD163 and Chitinase-3-like protein 1 (CHI3L1)[31–33]). CXCL16, involved in inflammatory response, revealed novel associations with hypertension. Four proteins (CXCL16, CPA1, CPB1, and tPA), playing a role in inflammatory response, chemotaxis, coagulation or proteolysis, represent novel associations with increased PWV and 1 protein (CCL15), also with an important role in inflammation through chemotaxis, represents novel associations with cIMT. We could reveal whether the associations were dependent of ethnicity or not which can be relevant to take into consideration, if, for example, developing reference intervals for the various proteins or, if investigating possible ethnic variations in the biological processes preceding CVD.

Although being different phenotypes of vascular health with differing mechanisms, hypertension, PWV and cIMT are all well documented predictors of the development of CVD.[4,5,34] Placing multiplex proteomics in a pre-clinical atherosclerotic, arteriosclerotic and hypertensive context by studying their relation to hypertension, PWV and cIMT, may further increase understanding of the complex biological processes leading to CVD.

4.1. Proteins with novel and ethnicity-independent associations

4.1.1. Carboxypeptidase A1 and B1. CPA1 and CPB1 are biomarkers related to proteolysis and inflammation. Studies on CPA1's association with vascular health/CVD are scarce. Kulasingam et al[35] compared the plasma levels of biomarkers related to cardiovascular disease in the acute phase of ST-elevation myocardial infarction (STEMI), with the protein levels in the stable phase 3 months after STEMI. They found that concentrations of CPA1 were increased at the three-month follow-up, compared to the acute phase. To our knowledge, the positive associations between PWV and CPA1/CPB1 found in this study are novel. Further, when adjusted for ethnicity, the associations remained significant.

4.1.2. Chemokine (C-C motif) ligand 15. CCL15, also named Leukotactin-1 (Lkn-1), is a chemotactic factor involved in leukocyte trafficking and activation, and thus, plays an important role in inflammation.[36] Chemokines recruit leukocytes into the arterial wall and CCL15 has been shown to contribute to plaque destabilization in the progression of atherosclerosis.[37,38] In this light, the positive associations between cIMT and CCL15 demonstrated here are sound. To our knowledge, this is the first time CCL15 has been implicated in subclinical atherosclerosis as measured by cIMT. Furthermore, another finding of this study is that cIMT remained significantly associated with CCL15 when adjusted for ethnicity. Additionally, repeated alcohol exposure induced changes in the CC chemokine family should also be considered as it revealed a link between CCL cytokines and stress.[39] As ethnicity influenced associations between cIMT and CCL15; the higher alcohol consumption in the Black cohort may contribute to their higher mean inflammatory status. However, upon entering cGGT (as a marker of alcohol use) in the model, the association between cIMT and CCL15 remained significant; suggesting the association observed is independent of cGGT. On the other hand, previously black Africans with low grade inflammation demonstrated higher alcohol consumption in relation to coronary artery disease risk and stress.[40]

4.1.3. CXC Ligand 16. CXCL16 is a chemokine expressed at sites of inflammation and has been suggested to play a functional role in atherosclerosis.[41] It has been proposed to have adverse effects on cardiomyocytes in patients with CVD[42] and associated to adverse clinical outcomes in patients with acute coronary syndrome.[43] Here, we found a significant association with CXCL16 and PWV, which remained after adjusting for ethnicity. This is, to the best of our knowledge, a novel finding. Membrane-bound CXCL16 has been suggested to transform vascular smooth muscular cells from contractile to a proliferative form, thereby promoting vascular remodeling,[44] suggesting this could be a reason for CXCL16's correlation to arterial stiffness found in this study. In contrast to Wang et al,[45] we found no association between CXCL16 and cIMT; however, the study

population involved atherosclerotic stroke patients and was older than the population of this study.

4.2. Proteins with novel and ethnicity-dependent associations

4.2.1. CXC Ligand 16. In this study we found the previously described CXCL16 to be significantly associated to hypertension. We believe this is a novel finding and CXCL16's role in hypertension is relatively unknown. In animal models, Xia et al showed that it is a mediator of renal inflammation and propagates hypertensive kidney damage but genetic deletion of CXCL16 did however not affect hypertension.[46] The results suggest that hypertension could cause renal inflammation, rather than the other way around. After adjusting for ethnicity, the association between CXCL16 and hypertension in this study did not remain significant.

4.2.2. Tissue plasminogen activator. tPA is a protein found on the endothelial cells, is a part of the fibrinolytic pathway and has been proven to be an independent risk factor of a first CVD event.[47] Here, we found a significant association between elevated levels of tPA and PWV. This is in contrast to a study by Tuttolomondo et al where they did not see a relationship between tPA and PWV[48] in a population consisting of stroke patients and controls. The association found in this study was no longer significant after adjusting for ethnicity.

4.2.3. Transferrin receptor protein 1. TR is a carrier protein of transferrin, needed for import of iron into cells. In this study we found a significant association between TR and hypertension which we believe is a novel finding, the significance was however lost upon entering ethnicity in the model. In Chinese subjects, Zhu et al[49] did not find an association between soluble transferrin receptor and incident hypertension, however Naito et al reported that heterozygous mice deficient in the transferrin-1 receptor (TfR1) show protection against the development of hypoxia-induced pulmonary hypertension and vascular remodeling.[50]

4.3. Study limitations

The data in the SABPA study were collected at a single regional center, which confines replication of significant findings and limits applicability to other populations. On the other hand, the subjects in our population were of similar age and socio-economic status, and balanced with regards to sex and ethnicity. The cross-sectional associations share the usual limitations of causality and control as seen for all cross-sectional studies. The gold standard for measuring PWV is carotid-femoralis PWV. Here, due to cultural and ethical reasons we used the carotid-dorsalis PWV.

5. Conclusions

In a South African bi-ethnic cohort, we confirmed several proteins' associations with phenotypes of vascular health using a multiplex proteomic approach. Novel associations were found between hypertension, PWV or cIMT and proteins involved in inflammatory response, chemotaxis, coagulation or proteolysis. Further, we could reveal whether the associations were dependent of ethnicity or not.

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