Atherosclerosis 239 (2015) 218–223

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Association of circulating sclerostin with vascular calcification in Afro-Caribbean men

Allison L. Kuipers [a, *], Iva Miljkovic [a], J. Jeffery Carr [b], James G. Terry [b], Cara S. Nestlerode [a], Yaorong Ge [c], Clareann H. Bunker [a], Alan L. Patrick [d], Joseph M. Zmuda [a, e]

[a] Department of Epidemiology, University of Pittsburgh, Pittsburgh, PA, USA
[b] Department of Radiology, Vanderbilt University Medical Center, Nashville, TN, USA
[c] Department of Software and Information Sciences, University of North Carolina, Charlotte, NC, USA
[d] Tobago Health Studies Office, Scarborough, Tobago, Trinidad and Tobago
[e] Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA, USA

ARTICLE INFO

Article history:
Received 6 October 2014
Received in revised form
2 January 2015
Accepted 10 January 2015
Available online 17 January 2015

Keywords:
Sclerostin
Coronary artery calcification
Aortic artery calcification
African ancestry

ABSTRACT

Objective: Sclerostin, a Wingless (Wnt) pathway antagonist, is an established regulator of bone mineralization in humans but its potential importance in the regulation of vascular calcification is less clear. Therefore, our objective was to assess the relationship of serum sclerostin levels with coronary and aortic artery calcification (CAC and AAC, respectively) in Afro-Caribbean men on the island of Tobago.
Methods: Serum sclerostin levels and computed tomography of CAC and AAC were measured in 191 men (age mean(SD): 62.9(8.0)years) recruited without regard to health status. Multivariable logistic regression models were used to assess the cross-sectional association of sclerostin with prevalent arterial calcification.
Results: Mean(SD) sclerostin was 45.2 pmol/L (15.6 pmol/L). After adjusting for risk factors including age, physical and lifestyle characteristics, comorbidities, lipoproteins and kidney function, 1 SD greater sclerostin level was associated with a 1.61-times (95%CI 1.02–2.53) greater odds of having CAC. Sclerostin was not associated with AAC in any model.
Conclusions: This is the first study to show that, among Afro-Caribbean men, greater serum sclerostin concentrations were associated with prevalence and extent of CAC. Further studies are needed to better define the role of the Wnt signaling pathway in arterial calcification in humans.

1. Introduction

With aging, calcifications develop within the vasculature and in the presence of vascular disease progression, calcification arise within the medial vessel wall and/or as calcified plaques [1]. The presence and amount of arterial calcification predicts cardiovascular disease (CVD) events and mortality [2–4]. Emerging evidence indicates that the Wingless (Wnt) plays a role in vascular biology including vascular calcification [5–7], angiogenesis [8–10], and atherosclerosis [11–13]. Wnt signaling occurs when the Wnt ligand binds to co-receptors, Frizzled and Low-density Lipoprotein Receptor related Protein, which induces β-catenin translocation to the nucleus to regulate the transcription of Wnt target genes. The Wnt pathway is involved in many aspects of biology including cell survival, stem cell development and cell differentiation, including bone and vascular lineages [14].

Sclerostin, one of the most studied circulating Wnt inhibitors in humans, is a secreted glycoprotein that acts as a Wnt antagonist [15,16]. Although sclerostin is an established regulator of bone mineralization [17], its potential role in vascular biology and arterial health is less clear. Sclerostin has been detected in the human aorta [18] and is up-regulated in calcifying vascular smooth muscle cells [19,20] and calcified valvular plaques [21]. The relationship of human serum sclerostin and vascular calcification, to our knowledge, has been investigated in four previous studies with two showing a direct correlation with calcification [22,23] and two reporting an inverse correlation [24,25]. These inconsistent results may be due, in part, to the measurement of calcification since three of the studies [22–24] only used vertebral x-ray scans to measure

* Corresponding author. Department of Epidemiology, University of Pittsburgh, 130 DeSoto St, A543 Crabtree Hall, Pittsburgh, PA 15261, USA.
E-mail address: kuipers@pitt.edu (A.L. Kuipers).

http://dx.doi.org/10.1016/j.atherosclerosis.2015.01.010
0021-9150/

aortic artery calcification and did not assess the more clinically relevant [26] coronary arteries. The previous studies also included vastly different population samples such as only diabetic patients [23,25], chronic kidney disease patients [24] or post-menopausal women [22]. Therefore, in the current study we assessed the association of serum sclerostin with vascular calcification measures from computed tomography imaging of the clinically relevant coronary and aortic arteries in a community-dwelling sample of 191 adult Afro-Caribbean men.

2. Materials and methods

2.1. Study sample

The computed tomography (CT) scans were obtained as an ancillary study of the Tobago Bone Health Study (TBHS), a population-based prospective study of 2652 community-dwelling men aged 40 years and older, residing on the Caribbean island of Tobago [27]. Participants for the TBHS were recruited without regard to health status and men were eligible if they were ambulatory, not terminally ill and without a bilateral hip replacement. Men from Tobago are of homogeneous African ancestry with low European admixture (<6%) [28]. The CT sample consisted of 304 men who were recruited consecutively during a follow-up visit of the TBHS from 2011 to 2012. During this ancillary visit, participants underwent a chest and abdominal CT, an extensive clinical exam and health history assessment, and collection and biobanking of specimens including fasting serum samples. Serum measures of sclerostin (SOST) were obtained in a random sample of 191 men who serve as the basis of the current analysis. The men with sclerostin measured were more likely to be diabetic but less likely to be hypertensive than men from the larger cohort who did not have sclerostin measured (both P-values<0.05; data not shown). Other factors, such as age, demographics, physical and lifestyle characteristics, biochemical markers, and vascular calcification measures, did not differ between groups (data not shown). Written informed consent was obtained from each participant using forms and procedures approved by the University of Pittsburgh Institutional Review Board, the U.S. Surgeon General's Human Use Review Board, and the Tobago Division of Health and Social Services Institutional Review Board.

2.2. Serum sclerostin and other biochemical assays

Blood samples were collected from participants in the morning after an overnight fast. Serum was separated and stored at −80 °C until time of assay. Serum sclerostin levels were measured according to the manufacturer's protocol using a validated sandwich enzyme-linked immunosorbent assay (ELISA) (Biomedica Gruppe, Vienna, Austria) and standardized across plates. Intra- and inter-assay coefficients of variation were 5% and 3%, respectively.

Fasting serum glucose was measured using a coupled enzymatic reaction similar to the procedure described by Bondar and Mead [29]. Low-density lipoprotein cholesterol (LDL-c) was calculated by the Friedewald equation. High-density lipoprotein cholesterol (HDL-c) was determined using the selective heparin/manganese chloride precipitation method. Triglycerides were determined enzymatically using the procedure of Bucolo and David [30]. Serum creatinine was quantitatively determined by the VITROS CREA Slide method. The Modification of Diet in Renal Disease Study formula was used to estimate glomerular filtration rate (eGFR) as: eGFR [mL min^{-1} (1.73 m^2)$^{-1}$] = 175 × (serum creatinine [mg/dL]$^{-1.154}$ × age [years]$^{-0.203}$[× 0.742, if female] [× 1.212, if African American] [31].

2.3. Arterial calcification

Arterial calcification was assessed by central computed tomography using a dual slice, high-speed NX/I scanner, 120 KVp, 290 mA and gantry speed 0.7 s (GE Medical Systems, Waukesha, WI). The scans were obtained using the axial, two-slice scan mode (2i) and a segmented (a.k.a "half-scan") reconstruction to provide an effective temporal resolution of approximately 350 msec for each 3 mm thick slice without cardiac gating. Coronary artery calcification (CAC) values were obtained from cross-sectional slices through the chest from the carina through the entire inferior aspect of the heart and measurements made by vessel for each of the major epicardial coronary arteries. For the abdominal scan series, a helical scan mode (120 KVp, 250 mA, 3 mm slice thickness and pitch of 1.5:1 was utilized since the higher temporal resolution for the coronary arteries was not required. For participants with body weight greater than 200 lbs, the mA was increased to 300. Aortic artery calcification (AAC) values were obtained from cross-sectional slices through the abdomen from L3 to S1 and included the summation of calcification in the abdominal aorta and common iliac arteries. Measurements were performed by experienced analysts using an FDA approved computer workstation and software (Calcium, Aquarius workstation, TeraRecon San Mateo, CA) that accounts for slice thickness and scan field of view. The Agatston method [32] method was used to report scores of calcified plaque using a 130 HU threshold, minimum lesion size of >1 mm^2 and a display field of view of 350 mm designed to be comparable to other population based studies that have measured CAC. In this report, presence of CAC was defined by an Agatston score of >10 to further reduce false positive classification. The lead reader at the Wake Forest University CT Reading Center read all CT scans for the present study. This reader also led the Coronary Artery Risk Development In young Adults (CARDIA) Study CT analyses, and a careful blinded re-read of 153 CARDIA scans found intra-reader technical error of 6.6% [32].

2.4. Other characteristics

Demographic, health history and anthropomorphic characteristics were assessed by trained staff using interview and clinical exams. Body weight was measured to the nearest 0.1 kg on a balance beam scale and standing height was measured to the nearest 0.1 cm using a wall-mounted stadiometer, both without participants wearing shoes. Body mass index (BMI) was calculated as weight in kilograms divided by standing height in meters squared. Total body fat was measured using dual X-ray absorptiometry. Smoking status was classified both as either current or not, or ever or not wherein participants reporting smoking <100 cigarettes in their lifetime were considered never-smokers. Alcohol consumption is very limited in this cohort sample and was, therefore, coded as consuming >3 drinks per week (yes/no) to identify individuals with greater than average cohort alcohol intake. As walking is the predominate form of physical activity on the island, physical activity was dichotomized into participants reporting >60 min of walking for exercise per week vs. not. Grip strength was calculated as the average grip strength of four trials (two left handed and two right handed) as measured using a dynamometer (Preston Grip Dynamometer, JA Preston Corp.).

Diabetes was defined as a fasting serum glucose level ≥126 mg/dl, current self-reported use of diabetes medication or an affirmative response to the question "has a doctor ever told you that you have diabetes?" Blood pressure was measured three times while seated and the average of the 2nd and 3rd reading were used in this analysis. Hypertension was defined as a systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure (DBP) ≥90 mmHg,