Journal of the American Heart Association

ORIGINAL RESEARCH

Associations of Serum Dickkopf-1 and Sclerostin With Cardiovascular Events: Results From the Prospective Bruneck Study

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BACKGROUND: Dickkopf-1 and sclerostin have been implicated in atherosclerosis and vascular calcification. We aimed to quantify the association of their serum levels with incident cardiovascular disease (CVD) in the general population.

METHODS AND RESULTS: Among 706 participants of the prospective, population-based Bruneck Study, mean±SD of serum levels were 44.5±14.7 pmol/L for dickkopf-1 and 47.1±17.5 pmol/L for sclerostin. The primary outcome was a composite CVD end point composed of ischemic or hemorrhagic stroke, transient ischemic attack, myocardial infarction, angina pectoris, peripheral vascular disease, and revascularization procedures. Over a median follow-up duration of 15.6 years, 179 CVD events occurred. For the primary CVD outcome, multivariable-adjusted hazard ratios (HRs) per SD higher level were 1.20 for dickkopf-1 (95% CI, 1.02–1.42; *P*=0.028) and 0.92 for sclerostin (95% CI, 0.78–1.08; *P*=0.286). Secondary outcome analyses revealed that the association of dickkopf-1 was primarily driven by ischemic and hemorrhagic stroke (67 events; HR, 1.37; 95% CI, 1.06–1.78; *P*=0.017), whereas no increase in risk was observed for transient ischemic attack (22 events; HR, 0.87; 95% CI, 0.53–1.44; *P*=0.593), myocardial infarction (45 events; HR, 1.10; 95% CI, 0.78–1.54; *P*=0.598), or for other CVD (45 events; HR, 1.25; 95% CI, 0.88–1.76; *P*=0.209).

CONCLUSIONS: In this prospective, population-based study, elevated baseline levels of dickkopf-1, but not sclerostin, were independently associated with incident cardiovascular events, which was mainly driven by stroke. Our findings support the hypothesis of a role of dickkopf-1 in the pathogenesis of CVD.

Key Words: cardiovascular disease ■ dickkopf-1 ■ population studies ■ prospective cohort study ■ sclerostin

Dickkopf-1 and sclerostin are soluble glycoproteins that regulate bone formation by antagonizing osteogenic Wnt/β-catenin signaling on osteoblast and osteoclast cell lineages.[1] In addition to their role in bone homeostasis, emerging evidence implicates dickkopf-1 and sclerostin in the pathogenesis of cardiovascular disease (CVD). Endothelial- and platelet-derived dickkopf-1 may contribute to formation and destabilization of atherosclerotic lesions by promoting inflammation[2] and endothelial cell (EC) injury[3,4], whereas sclerostin may protect from vascular inflammation and aortic matrix degradation[5].

In cross-sectional analyses, circulating dickkopf-1 and sclerostin levels were elevated in individuals with acute ischemic stroke.[6,7] In addition, levels of dickkopf-1 were found to be higher in coronary heart disease.[2,8] Prospective data on 5165 patients with acute coronary syndrome in the PLATO (Platelet Inhibition and Patient Outcomes) trial indicated that dickkopf-1 levels at admission were positively associated with risk

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CLINICAL PERSPECTIVE

What Is New?

- In this prospective, population-based study, we evaluated the association of serum levels of dickkopf-1 and sclerostin with incident cardiovascular events over a follow-up interval between 2000 and 2016.
- Our study demonstrates an independent association of serum dickkopf-1 levels with future cardiovascular events, which was primarily driven by an increased risk for stroke; this finding supports the hypothesis of a role of this glycoprotein in the pathogenesis of cardiovascular disease.
- In contrast, no statistically significant associations between serum levels of sclerostin and the composite cardiovascular disease end point and its individual components were found.

What Are the Clinical Implications?

- This study highlights the potential of dickkopf-1 as a biomarker for cardiovascular disease risk and, in specific, for stroke risk.

Nonstandard Acronyms and Abbreviations

CVD	cardiovascular disease
EC	endothelial cell
HR	hazard ratio

of cardiovascular death.[9] Furthermore, 2 interventional studies that evaluated the efficacy and safety of the antisclerostin monoclonal antibody romosozumab for osteoporosis treatment have suggested that sclerostin inhibition may be associated with an increased CVD risk.[10,11] However, these findings remain controversial, given that no such increased CVD risk was observed in another trial.[12] Moreover, prospective cohort studies, primarily involving patients with chronic kidney disease and diabetes mellitus, have yielded inconsistent results, with findings ranging from positive to negative associations.[13,14] Apart from findings in 1 earlier study in a selected sample of people at intermediate CVD risk,[15] the relationship of circulating dickkopf-1 and sclerostin with incident CVD in the general population is unknown.

In the present study, we evaluated the relationship between dickkopf-1 and sclerostin and CVD in the prospective, population-based Bruneck Study. Our aims were to: (1) quantify cross-sectional associations between serum levels and baseline patient characteristics and (2) explore the association between serum levels and incident cardiovascular events within a follow-up period extending from 2000 to 2016.

METHODS

Research Data Availability

Study data are not publicly available because of local data protection regulations. Anonymized data are available upon reasonable request to the corresponding author for researchers, accreditation as approved researcher by the local data sharing committee, and signing of a data sharing agreement with the study.

Study Population

The Bruneck Study is a prospective, population-based cohort study with a main focus on the epidemiology, pathophysiology, and prevention of atherosclerosis and CVD.[16–18] The study protocol conformed to the Declaration of Helsinki and was approved by the local ethics committees (Bolzano and Verona). All participants gave their written informed consent before entering the study. The study population was selected in 1990 as an age- and sex-stratified random sample of all inhabitants aged 40 to 79 years (125 per sex each from the fifth to eighth decade of age, n=1000) living in Bruneck, a province of Bolzano in northern Italy. The Bruneck Study population is exclusively of white ethnicity and is unique for its low annual mobility rates within the survey area (0.2% per year). The major advantages of the Bruneck Study are that virtually all subjects living in the Bruneck area were referred to one and the same local hospital, and that the network existing between the local hospital and general practitioners allowed retrieval of practically all medical information on people living in the area. A total of 933 women and men participated in the baseline examination in 1990 (participation rate, 93.3%); 703 of 764 individuals being alive in 2000 participated in the 2000 re-evaluation (participation rate, 92.0%). To be included in the current analysis, people were required to have complete information on clinical variables. The baseline for the current investigation was the visit in the year 2000 (n=691 with available measurements of dickkopf-1 and sclerostin levels); for participants without measurements at this time point, the 2005 visit (n=13) or the 2010 visit (n=2) was used instead. The total number of participants contributing to our baseline population was therefore 706.

Clinical Evaluation

Baseline and follow-up examinations involved a comprehensive physical examination with cardio- and neurological focus and assessments of the participants' medical history, cardiovascular risk factors, and lifestyle behavior, using validated standard procedures and

standardized questionnaires and interviews.[16–19] Systolic blood pressure was obtained with a standard mercury sphygmomanometer after ≥10 minutes of rest in a sitting position (mean of 3 independent measurements). Body mass index was calculated as weight divided by height squared (kg/m^2). Individuals were coded as current smokers or nonsmokers (including former smokers). Average alcohol consumption was quantified in grams of ethanol per day.[19] Bone ultrasonographic data were assessed at the heel bones using quantitative ultrasonographic equipment (SAHARA; Hologic, Inc, Marlborough, MA). Estimated bone mineral density in g/cm^2 was derived as a linear combination of bone ultrasound attenuation and speed of sound (estimated bone mineral density=0.002592×(bone ultrasound attenuation in dB/MHz+speed of sound in m/s−3.687)), as previously described.[20] Glomerular filtration rate was estimated using the Chronic Kidney Disease Epidemiology Collaboration equation.[21] There were no diagnoses of type 1 diabetes mellitus. Type 2 diabetes mellitus was diagnosed according to the American Diabetes Association criteria[22] (ie, when fasting glucose was ≥126 mg/dL [≥7.0 mmol/L]) or when the participants had a clinical diagnosis of diabetes mellitus or received antidiabetic treatment (diet or medication). History of CVD was defined as prior ischemic or hemorrhagic stroke, transient ischemic attack, myocardial infarction, angina pectoris, peripheral vascular disease, or revascularization procedures on carotid, coronary, or peripheral arteries.

Laboratory Methods and Measurement of Serum Dickkopf-1 and Sclerostin

Venous blood samples were collected from antecubital veins after an overnight fasting period and a 12-hour abstinence from smoking, divided into aliquots, and immediately processed or stored at −80°C. Circulating levels of dickkopf-1 and sclerostin in human serum were measured repeatedly in 2000, 2005, and 2010 using commercially available Sandwich-ELISA kits (Catalog: BI-20413 [dickkopf-1] and BI-20492 [sclerostin]; Biomedica Medizinprodukte, Vienna, Austria), according to the manufacturer's protocol. Blinded samples were measured at The Antibody Lab GmbH (Vienna, Austria). Manufacturer-reported lower detection limits were 1.7 pmol/L for dickkopf-1 and 3.2 pmol/L for sclerostin. Intra- and interassay coefficients of variation were both ≤3% for dickkopf-1 and ≤7% and ≤10% for sclerostin. Other laboratory parameters were all quantified by standard methods as described previously.[18,23]

Cardiovascular End Point Definition and Outcome Assessment

The primary outcome was a combined CVD end point, defined as ischemic or hemorrhagic stroke, transient ischemic attack, myocardial infarction, angina pectoris, peripheral vascular disease, or revascularization procedures on carotid, coronary, or peripheral arteries during a follow-up period between 2000 and 2016. Secondary analyses focused on the individual components of the combined CVD end point: (1) stroke (ischemic and hemorrhagic); (2) transient ischemic attack; (3) myocardial infarction; and (4) other CVD (angina pectoris, peripheral vascular disease, and revascularization procedures). Ischemic stroke and transient ischemic attack were classified according to the criteria of the National Survey of Stroke.[24] Myocardial infarction was deemed as confirmed when World Health Organization criteria[25] for definite disease status were met. Stable angina pectoris and symptomatic peripheral vascular disease were diagnosed by a positive response to the Rose questionnaire[26] and a vascular nature of complaints confirmed by standard diagnostic procedures (eg, angiography, exercise ECG, or ankle-brachial pressure index).

Full information on clinical end points occurring during the follow-up period was available for all people, including those who did not participate in later evaluations or died during follow-up (100% follow-up completeness for clinical end points). Ascertainment of fatal and nonfatal cardiovascular events and procedures was based on (1) the participants' self-reported medical history, (2) detailed review of the medical records provided by general practitioners, Bruneck Hospital databases, and death certificates, and (3) the results of clinical and laboratory examinations performed within the scope of the study.

Statistical Analyses

Continuous variables were summarized as means (±SDs) or medians (interquartile ranges) and categorical variables as numbers (percentages). Because of right-skewed distributions, alcohol consumption, triglycerides, and C-reactive protein values were $\log_e$-transformed for analyses.

Correlation between levels of dickkopf-1 and sclerostin was assessed using an age- and sex-adjusted partial correlation coefficient. Within-person variabilities of dickkopf-1 and sclerostin levels over 10 years were quantified using Pearson correlation coefficients in participants with available values at the 2000 and 2010 examinations. Cross-sectional associations of baseline serum levels of dickkopf-1 and sclerostin (dependent variables) with established cardiovascular risk factors, anthropometric, and other variables were assessed using age- and sex-adjusted linear regression models and are presented as mean differences and 95% CIs per 1-SD higher values of continuous variables, or compared to a reference group for categorical variables.

Person-years of follow-up for each participant were accrued until diagnosis of a cardiovascular event, death, or April 2016, whichever came first. Cox proportional hazards regression was used to estimate multivariable-adjusted hazard ratios (HRs) and 95% CIs per 1-SD higher concentration as well as across thirds of dickkopf-1 and sclerostin levels. The first model was adjusted for age and sex; the second model was adjusted for age, sex, systolic blood pressure, body mass index, current smoking (yes, no), alcohol consumption, triglycerides, total cholesterol, high-density lipoprotein cholesterol, C-reactive protein, leukocyte count, platelet count, estimated glomerular filtration rate, current platelet aggregation inhibitor intake (yes, no), type 2 diabetes mellitus (yes, no), and history of CVD (yes, no). Differences in magnitudes of associations in subgroups were investigated by including appropriate multiplicative interaction terms in the multivariable models. *P* values for interaction were calculated using continuous variables, when appropriate. The proportional hazards assumption was tested using Schoenfeld residuals and was met.

We calculated measures of risk discrimination and risk reclassification to assess the incremental predictive value of dickkopf-1 and sclerostin for prediction of the primary CVD end point among participants without a baseline history of CVD.[27,28] A model containing the Pooled Cohort Equation variables (ie, age, sex, smoking, diabetes mellitus, systolic blood pressure, total cholesterol, and high-density lipoprotein cholesterol) was used as a reference.[29] Risk reclassification analyses focused on a 10-year prediction period and used categories of predicted risk of <5.0%, 5.0% to <7.5%, 7.5% to <20.0%, and ≥20.0%.

All statistical tests were 2-sided, and $P \leq 0.05$ was considered as statistically significant. Analyses were not adjusted for multiple comparisons. Data were analyzed using the statistical software Stata (version 15.1; StataCorp LLC, College Station, TX).

RESULTS

Baseline Serum Levels of Dickkopf-1 and Sclerostin and Their Within-Person Variability Over Time

Baseline distributions and 10-year within-person correlations are depicted in Figure 1. Baseline mean±SD of serum levels were 44.5±14.7 pmol/L for dickkopf-1 and 47.1±17.5 pmol/L for sclerostin. Levels of dickkopf-1 and sclerostin at baseline were not correlated with each other (age- and sex-adjusted partial correlation coefficient, r=0.0013; *P*=0.972). Levels of dickkopf-1 measured in the same people in 2000 and 2010 were highly stable over time (10-year Pearson correlation coefficient, r=0.64 [95% CI, 0.58–0.69]), whereas levels of sclerostin were less stable over time (r=0.47 [95% CI, 0.40–0.54]) (both n=471).

Baseline Characteristics and Their Association With Serum Dickkopf-1 and Sclerostin

Baseline demographics, biochemical measurements, and cardiovascular risk factors of the study population (n=706) are presented in the Table. At baseline, the mean age of the study population was 66.3 years (±10.3; range, 49–92), 370 (52.4%) participants were women, and 115 (16.3%) people experienced a prior cardiovascular event.

In cross-sectional analyses, dickkopf-1 levels were positively associated with leukocyte count (age- and sex-adjusted regression coefficient by 1-SD higher value, +2.5 pmol/L [95% CI, +1.4 to +3.6]; *P*<0.001), platelet count (+7.1 pmol/L [95% CI, +6.1 to +8.1]; *P*<0.001), and were somewhat higher in people with a history of ischemic stroke than in those without (+8.1 pmol/L [95% CI, +0.2 to +16.0]; *P*=0.045). Sclerostin levels were positively associated with age (+2.0 pmol/L [95% CI, +0.7 to +3.2]; *P*=0.002) and with estimated bone mineral density (+3.1 pmol/L [95% CI, +1.7 to +4.4]; *P*<0.001), and they were inversely associated with total cholesterol (–1.7 pmol/L [95% CI, –3.0 to –0.5]; *P*=0.008) and estimated glomerular filtration rate (–4.6 pmol/L [95% CI, –6.2 to –3.0]; *P*<0.001). Furthermore, sclerostin levels were lower in female than in male participants (age-adjusted mean difference, –10.3 pmol/L [95% CI, –12.8 to –7.9]; *P*<0.001). There were no statistically significant cross-sectional associations of dickkopf-1 or sclerostin levels with any of the other parameters assessed.

Associations of Serum Dickkopf-1 and Sclerostin With Cardiovascular Events

Over a median follow-up period of 15.6 years, 179 of the 706 participants experienced the composite CVD end point, corresponding to an incidence rate of 22.0 per 1000 patient-years (95% CI, 19.0–25.4). Among these, 49 events occurred in the bottom, 65 in the middle, and 65 in the top third of dickkopf-1 concentration; 58 occurred in the bottom, 54 in the middle, and 67 in the top third of sclerostin concentration.

In Cox regression analysis adjusted for age and sex, the HR per 1-SD higher dickkopf-1 level for the combined CVD end point was 1.15 (95% CI, 1.00–1.33; *P*=0.048). This association remained significant upon multivariable adjustment for potential

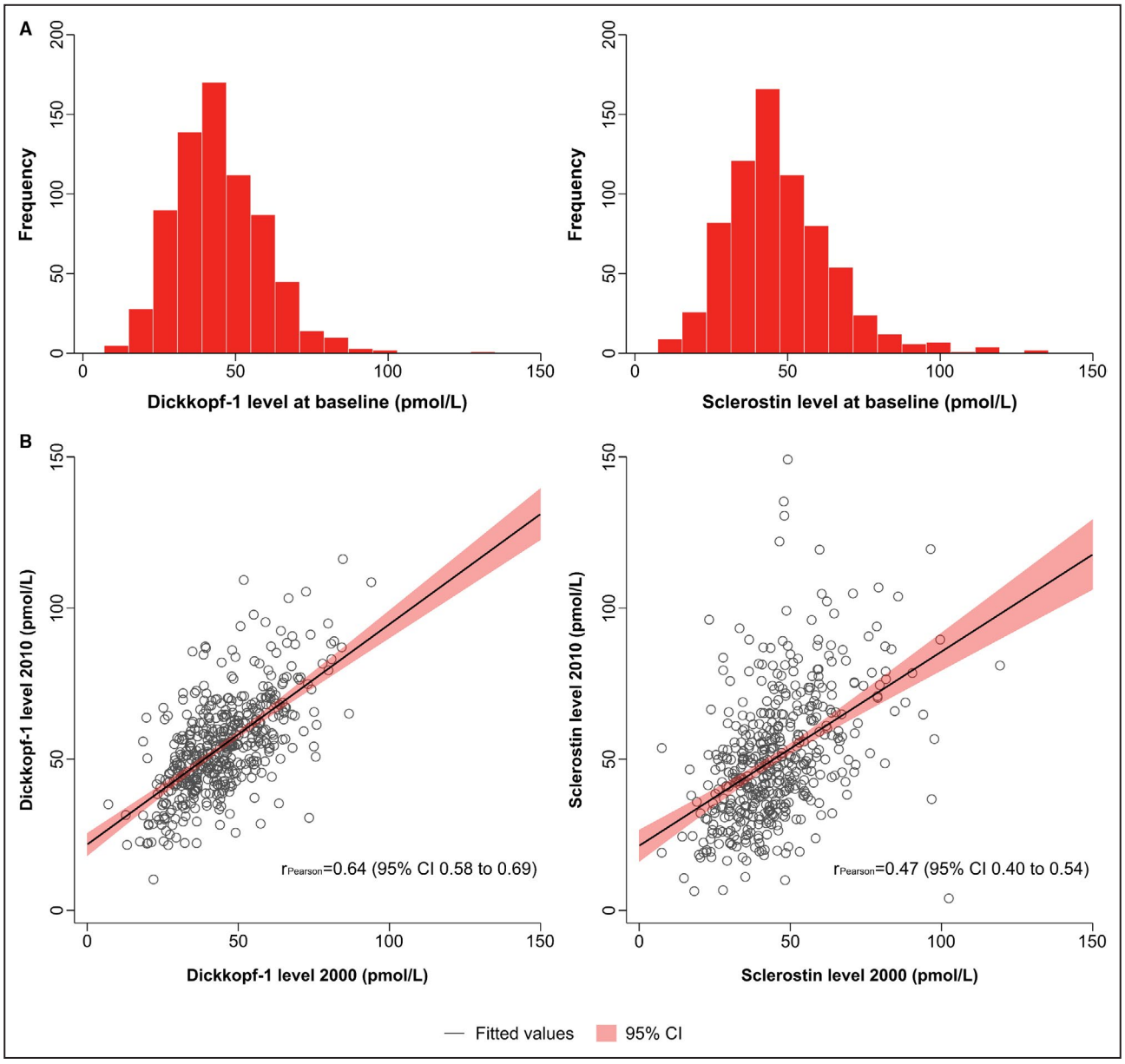

Figure 1. Serum levels of dickkopf-1 and sclerostin.
(**A**) Histogram of levels at baseline (n=706) and (**B**) scatter plot of levels measured in 2000 and 2010 in the same people (n=471) with regression line and 95% CI. r indicates Pearson correlation coefficient.

confounders (HR per 1-SD higher dickkopf-1, 1.20 [95% CI, 1.02–1.42]; *P*=0.028) (Figure 2). When examining the association with individual components of the combined CVD end point, a 1-SD higher value of dickkopf-1 concentration was statistically significantly associated with incident stroke (HR, 1.37 [95% CI, 1.06–1.78]; *P*=0.017) in the multivariable-adjusted model, whereas no statistically significant increase in risk was observed for transient ischemic attack (HR, 0.87 [95% CI, 0.53–1.44]; *P*=0.593), myocardial infarction (HR, 1.10 [95% CI, 0.78–1.54]; *P*=0.598), or for other CVD events (HR, 1.25 [95% CI, 0.88–1.76]; *P*=0.209).

In contrast, no statistically significant association between serum levels of sclerostin and risk for incident CVD was observed. The age- and sex-adjusted HR per 1-SD higher sclerostin for the combined CVD end point was 0.95 (95% CI, 0.81–1.11; *P*=0.507), which remained broadly consistent after multivariable adjustment, with a corresponding HR of 0.92 (95% CI, 0.78–1.08; *P*=0.286). The respective multivariable-adjusted HRs for the individual CVD end points of stroke, transient ischemic attack, myocardial infarction, and other CVD events were 0.97 (95% CI, 0.75–1.26; *P*=0.832), 1.02 (95% CI, 0.66–1.58; *P*=0.917), 0.95 (95% CI, 0.69–1.31; *P*=0.752), and 0.71 (95% CI, 0.49–1.02; *P*=0.063) (Figure 2).

Table. Baseline Characteristics of the Study Population (n=706) and Their Association With Serum Levels of Dickkopf-1 and Sclerostin

Baseline Characteristics	Mean±SD, Median [IQR], or n (%)	Mean Difference (95% CI) in Dickkopf-1 or Sclerostin Per 1-SD Higher Levels of Baseline Characteristics or Compared to Reference Groups*			
		Dickkopf-1		Sclerostin	
		β-Coefficient	*P* Value	β-Coefficient	*P* Value
Dickkopf-1, pmol/L	44.5±14.7	NA	NA	0.0 (–1.2 to 1.3)	0.972
Sclerostin, pmol/L	47.1±17.5	0.0 (–1.1 to 1.2)	0.972	NA	NA
Demographic/physical features					
Age, y	66.3±10.3	0.1 (–1.0 to 1.2)	0.838	2.0 (0.7–3.2)	0.002
Female sex	370 (52.4%)	0.5 (–1.7 to 2.7)	0.668	–10.3 (–12.8 to –7.9)	<0.001
Systolic blood pressure, mm Hg	139.8±18.5	0.7 (–0.4 to 1.9)	0.215	–0.1 (–1.4 to 1.2)	0.932
Body mass index, kg/m^2	25.4±4.0	–1.1 (–2.2 to 0.0)	0.053	0.8 (–0.4 to 2.0)	0.212
Current smoking	122 (17.3%)	–1.4 (–4.3 to 1.6)	0.368	–1.2 (–4.5 to 2.2)	0.488
Alcohol consumption, g/d	12.5 [0.0–50.0]	–0.2 (–1.4 to 1.1)	0.760	–0.6 (–2.1 to 0.8)	0.377
Estimated BMD, g/cm^{2†}	0.5±0.1	–0.3 (–1.5 to 0.9)	0.636	3.1 (1.7–4.4)	<0.001
Blood-based markers					
Triglycerides, mmol/L	1.4 [1.0–1.8]	–1.1 (–2.2 to 0.0)	0.054	–1.2 (–2.4 to 0.0)	0.055
Total cholesterol, mmol/L	6.0±1.1	0.7 (–0.4 to 1.8)	0.215	–1.7 (–3.0 to –0.5)	0.008
Low-density lipoprotein, mmol/L	3.9±1.0	0.7 (–0.4 to 1.8)	0.182	–1.2 (–2.5 to 0.0)	0.059
High-density lipoprotein, mmol/L	1.5±0.4	0.8 (–0.3 to 1.9)	0.151	–0.8 (–2.0 to 0.5)	0.236
C-reactive protein, mg/L	1.8 [0.9–4.1]	–0.5 (–1.6 to 0.6)	0.399	0.5 (–0.8 to 1.7)	0.479
Leukocyte count, 10^9/L	6.2±1.7	2.5 (1.4–3.6)	<0.001	0.1 (–1.1 to 1.4)	0.821
Platelet count, 10^9/L	245.2±57.9	7.1 (6.1–8.1)	<0.001	0.3 (–1.0 to 1.6)	0.654
eGFR, mL/min/1.73 m^2	79.6±14.0	0.2 (–1.2 to 1.6)	0.744	–4.6 (–6.2 to –3.0)	<0.001
Medication intake					
Statin	68 (9.6%)	–1.6 (–5.3 to 2.0)	0.383	–1.2 (–5.4 to 3.0)	0.574
Platelet aggregation inhibitor	107 (15.2%)	0.0 (–3.2 to 3.3)	0.988	1.7 (–2.0 to 5.4)	0.358
History of disease					
Type 2 diabetes mellitus	78 (11.0%)	–2.8 (–6.4 to 0.7)	0.117	3.0 (–1.0 to 7.0)	0.143
Cardiovascular disease‡	115 (16.3%)	–1.5 (–4.8 to 1.8)	0.377	–0.5 (–4.3 to 3.2)	0.774
Coronary heart disease§	70 (9.9%)	–2.8 (–6.7 to 1.0)	0.152	0.3 (–4.1 to 4.7)	0.885
Stroke‖	18 (2.5%)	6.0 (–0.9 to 13.0)	0.090	3.3 (–4.6 to 11.2)	0.418
Ischemic stroke	14 (2.0%)	8.1 (0.2–16.0)	0.045	6.0 (–2.9 to 15.0)	0.187
Hemorrhagic stroke	5 (0.7%)	–4.0 (–16.9 to 9.0)	0.551	5.0 (–9.8 to 19.8)	0.506
Transient ischemic attack	21 (3.0%)	–3.9 (–10.5 to 2.6)	0.239	1.4 (–6.1 to 8.8)	0.721
Peripheral vascular disease	26 (3.7%)	1.1 (–4.9 to 7.0)	0.727	2.1 (–4.6 to 8.9)	0.535
Revascularization procedures¶	22 (3.1%)	–0.1 (–6.4 to 6.2)	0.979	–1.4 (–8.6 to 5.8)	0.705

BMD indicates bone mineral density; eGFR, estimated glomerular filtration rate; IQR, interquartile range; and NA, not applicable.

*β-coefficients were derived from linear regression adjusted for age and sex; alcohol consumption, triglycerides, and C-reactive protein values were $\log_e$-transformed for analyses.

†Available for 692 participants.

‡Defined as previous ischemic or hemorrhagic stroke, transient ischemic attack, myocardial infarction, angina pectoris, peripheral vascular disease, or revascularization procedures.

§Defined as myocardial infarction or angina pectoris.

‖Defined as ischemic or hemorrhagic stroke and includes 1 participant with an unclassified stroke and 2 participants with both ischemic and hemorrhagic stroke.

¶Includes revascularization procedures on carotid (n=4), coronary (n=9), or peripheral (n=6) arteries and multiple vessel beds (n=3).

We further characterized the association between dickkopf-1 and sclerostin and CVD by calculating multivariable-adjusted HRs for thirds (Figure 2). People in the top third of baseline dickkopf-1 concentration had a 1.48-fold higher risk (95% CI, 0.98–2.22; *P*=0.060) for incidence of the composite CVD end point when compared with people with levels in the bottom third. For sclerostin, the HR for comparing people in the top

Biomarker/ outcome	No. of events	Multivariable-adjusted* hazard ratio (95% CI)				P value
		Bottom third	Middle third	Top third	Per 1-SD higher concentration	
Dickkopf-1						
Composite CVD	179	Reference	1.26 (0.85, 1.86)	1.48 (0.98, 2.22)	1.20 (1.02, 1.42)	0.028
Stroke	67	Reference	1.45 (0.75, 2.81)	1.67 (0.84, 3.33)	1.37 (1.06, 1.78)	0.017
Transient ischemic attack	22	Reference	2.74 (0.90, 8.35)	1.04 (0.27, 4.00)	0.87 (0.53, 1.44)	0.593
Myocardial infarction	45	Reference	0.88 (0.40, 1.92)	1.23 (0.56, 2.70)	1.10 (0.78, 1.54)	0.598
Other CVD†	45	Reference	1.02 (0.46, 2.28)	1.69 (0.77, 3.69)	1.25 (0.88, 1.76)	0.209
Sclerostin						
Composite CVD	179	Reference	0.85 (0.58, 1.24)	0.92 (0.62, 1.37)	0.92 (0.78, 1.08)	0.286
Stroke	67	Reference	0.85 (0.46, 1.57)	1.12 (0.60, 2.11)	0.97 (0.75, 1.26)	0.832
Transient ischemic attack	22	Reference	0.50 (0.14, 1.84)	1.04 (0.33, 3.27)	1.02 (0.66, 1.58)	0.917
Myocardial infarction	45	Reference	1.09 (0.49, 2.42)	1.07 (0.46, 2.45)	0.95 (0.69, 1.31)	0.752
Other CVD†	45	Reference	0.80 (0.39, 1.63)	0.59 (0.26, 1.33)	0.71 (0.49, 1.02)	0.063

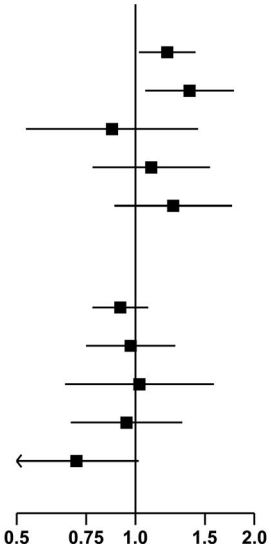

Figure 2. Forest plot of hazard ratios and 95% CIs per 1-SD higher concentration and across thirds of dickkopf-1 and sclerostin levels on the composite cardiovascular disease (CVD) end point and individual CVD end points.
Cutoffs for thirds were 37.4 and 49.0 pmol/L for dickkopf-1 and 38.8 and 51.6 pmol/L for sclerostin. *Adjusted for age, sex, systolic blood pressure, body mass index, smoking status, alcohol consumption, triglycerides, total cholesterol, high-density lipoprotein cholesterol, C-reactive protein, leukocyte count, platelet count, estimated glomerular filtration rate, current platelet aggregation inhibitor intake, type 2 diabetes mellitus, and history of CVD. †Includes angina pectoris (16 events), peripheral vascular disease (18 events), and revascularization procedures (11 events, of which 8 concerned the coronary arteries, 2 the carotid arteries, and 1 the peripheral arteries).

versus the bottom third of serum concentration was 0.92 (95% CI, 0.62–1.37; *P*=0.680) for the combined CVD end point.

In subgroup analyses, we observed no statistically significant effect modification by age, sex, systolic blood pressure, body mass index, smoking status, alcohol consumption, C-reactive protein, leukocyte count, platelet count, estimated glomerular filtration rate, and type 2 diabetes mellitus on the association between dickkopf-1 and sclerostin and the composite CVD end point (all *P* values for interaction >0.05) (Figure 3). There was evidence for a somewhat stronger association of dickkopf-1 with CVD risk in participants with a positive history of CVD than in those without (1.49 [95% CI, 1.16–1.91] versus 1.09 [95% CI, 0.90–1.32]; *P* value for interaction=0.045), but not for sclerostin (0.90 [95% CI, 0.70–1.17] versus 0.92 [95% CI, 0.76–1.12]; *P* value for interaction=0.893).

Added Value of Dickkopf-1 and Sclerostin Assessment for CVD Prediction

We quantified the incremental predictive value of circulating dickkopf-1 and sclerostin levels for risk of the primary CVD end point among participants without a history of CVD at baseline (n=591). The reference model containing Pooled Cohort Equation variables had a C-index of 0.666 (95% CI, 0.620–0.713). Additional assessment of dickkopf-1 yielded a C-index change of 0.003 (95% CI, −0.008 to 0.013; *P*=0.649) and a 10-year net reclassification improvement of 2.7% (95% CI, −4.0% to 9.4%; *P*=0.428). Additional assessment of sclerostin yielded a C-index change of 0.000 (95% CI, −0.003 to 0.003; *P*=0.889) and a 10-year net reclassification improvement of 0.9% (95% CI, −2.8% to 4.5%; *P*=0.648).

DISCUSSION

In this report on data from 706 participants of the prospective, population-based Bruneck Study, we evaluated the relationship of serum dickkopf-1 and sclerostin levels with CVD risk over a median follow-up of 15.6 years. Dickkopf-1 was associated positively with CVD risk, with a HR of 1.20 (95% CI, 1.02–1.42) per 1-SD higher level. This association was primarily driven by stroke and independent of sex, traditional CVD risk factors, and pre-existing CVD. In contrast, there was no statistically significant association of sclerostin with CVD risk. Magnitudes of associations of dickkopf-1 and sclerostin were similar across

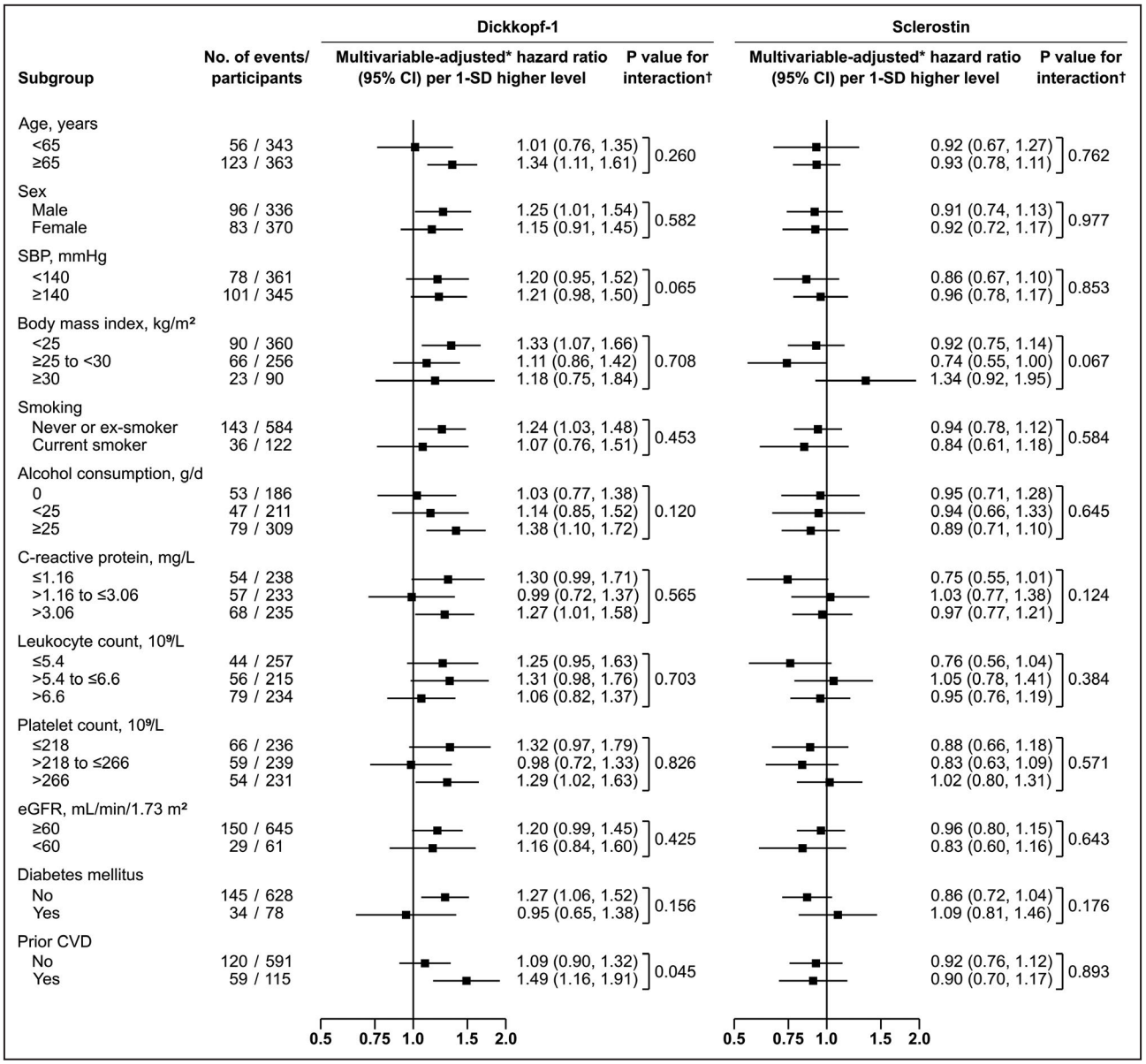

Figure 3. Subgroup analyses of the association between per 1-SD higher concentration of serum levels of dickkopf-1 and sclerostin and the composite cardiovascular disease (CVD) end point.

*Adjusted for age, sex, systolic blood pressure, body mass index, smoking status, alcohol consumption, triglycerides, total cholesterol, high-density lipoprotein cholesterol, C-reactive protein, leukocyte count, platelet count, estimated glomerular filtration rate, current platelet aggregation inhibitor intake, type 2 diabetes mellitus, and history of CVD, unless the variable was used as a subgroup variable. †*P* values for interaction were calculated using continuous variables, when appropriate. eGFR indicates estimated glomerular filtration rate; and SBP, systolic blood pressure.

several subgroups, apart from a somewhat stronger association of dickkopf-1 in participants with pre-existing CVD.

Previous evidence from prospective studies on the role of dickkopf-1 and sclerostin in atherosclerotic CVD is largely limited to high-risk patient populations. In 2 studies of patients with acute coronary syndrome, higher dickkopf-1 at admission predicted major adverse cardiac events[30] and cardiovascular death[9]. In patients with acute ischemic stroke, elevated dickkopf-1 at baseline was linked to higher 1-year risk for the composite of all-cause mortality or major disability, but was unrelated to recurrent stroke and a combined vascular end point.[31] For sclerostin, several studies have been conducted in patients with chronic kidney disease and patients with diabetes mellitus, but they yielded inconsistent results, ranging from positive to negative associations with CVD risk.[13,14] Interpretation of these studies is complicated further by small sample sizes, heterogeneous inclusion criteria, and differences in

outcomes definition, length of follow-up, statistical adjustment, and assay type.[32]

The only prospective study in the general population that previously investigated dickkopf-1 and sclerostin in relation to CVD risk is the SAPHIR (Salzburg Atherosclerosis Prevention Program in Subjects at High Individual Risk) cohort from Salzburg, Austria.[15] In a case-control analysis, baseline circulating levels did not differ between participants with incident CVD (70 cases) and those who did not develop CVD during 10 years of follow-up (157 controls). One explanation for the lack of association could be the exclusion of individuals with pre-existing CVD from the SAPHIR cohort, further endorsed by possible effect modification by baseline CVD in our study. The SAPHIR cohort additionally excluded individuals with conditions conveying elevated CVD risk (eg, uncontrolled hypertension or diabetes mellitus). Another explanation for the lack of association could be that 4 markers of the Wnt signaling pathway were evaluated concomitantly in the same regression model, which could have led to attenuation by collinearity.

Data from interventional studies on antibody-mediated inhibition of sclerostin in osteoporosis treatment—based on the potent inhibitory role of sclerostin on bone formation—have recently raised concern that such inhibition may also increase CVD risk. The humanized monoclonal antisclerostin antibody romosozumab, has been the first agent evaluated in phase III randomized controlled trials, of which 3 (ARCH [Active-Controlled Fracture Study in Postmenopausal Women with Osteoporosis at High Risk of Fracture], BRIDGE [Placebo-Controlled Study Evaluating the Efficacy and Safety of Romosozumab in Treating Men with Osteoporosis], and FRAME [Fracture Study in Postmenopausal Women with Osteoporosis]) reported cardiovascular adverse event data. Both the ARCH[10] and BRIDGE[11] trials, which had enrolled 4093 postmenopausal women and 245 men, respectively, reported higher rates of cardiac ischemic and cerebrovascular events in the romosozumab treatment groups. However, the clinical relevance of this finding remains unclear, given that absolute numbers of CVD events in these trials were low and no such association has been found in the larger FRAME trial[12] as well as in our study.

The mechanisms linking dickkopf-1 and sclerostin with CVD development remain to be fully determined (for an outline of key mechanisms, see Figure 4). Both proteins inhibit the β-catenin-dependent (canonical) Wnt signaling pathway,[1] which directs cellular proliferation and differentiation[33] and regulates bone formation, immune responses[34], multiple processes linked to atherosclerosis[35,36], and vascular calcification. In vascular calcification, a cell-driven process resembling bone morphogenesis[37], canonical Wnt signaling mediates differentiation of progenitor and vascular smooth muscle cells into an osteo/chondrogenic phenotype[38]. In cultured vascular smooth muscle cells, both dickkopf-1[39] and sclerostin[40,41] reduce expression of runt-related transcription factor 2—a downstream target[39] of canonical Wnt signaling and crucial transcription factor for osteogenic differentiation[42]. Whereas both proteins may counteract vascular calcification and modulate arterial stiffening[43] and atherosclerotic plaque stability[44] through this mechanism, their specific effects and clinical sequelae remain to be elucidated.

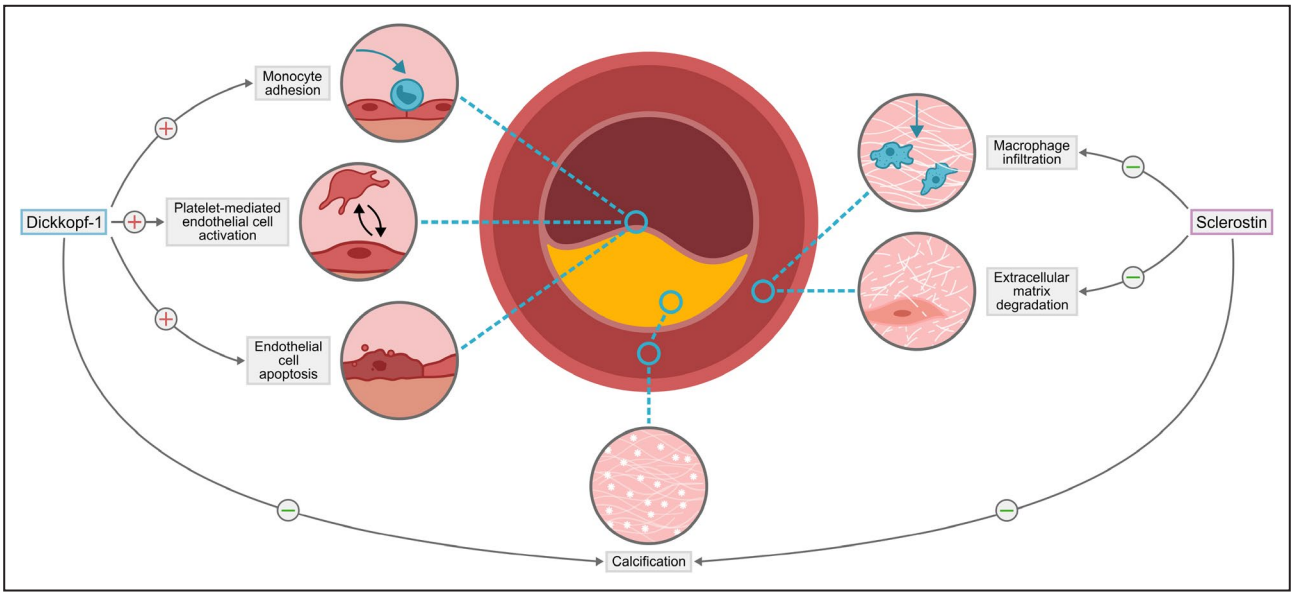

Figure 4. Outline of key mechanisms linking dickkopf-1 and sclerostin with atherosclerotic cardiovascular disease.

As reviewed recently in detail,[45] dickkopf-1 has several unfavorable effects on atherogenesis. In the vasculature, it is predominantly released by ECs[2] and activated platelets and promotes EC apoptosis[3] and platelet-mediated EC activation[2]. In addition, dickkopf-1 may directly regulate expression of several molecules in ECs involved in vascular biology, such as plasminogen activator inhibitor type 1 (thereby inhibiting fibrinolysis) and pentraxin 3 (thereby modulating inflammation).[46] In mice, genetic silencing of dickkopf-1 reduced monocyte adhesion to ECs[4] and atherosclerosis[3,4], whereas its overexpression[3] increased plaque formation and apoptosis. In humans, symptomatic carotid plaques exhibit higher dickkopf-1 expression than nonatherosclerotic arterial tissue, which, in turn, may contribute to plaque destabilization by driving an inflammatory loop within the lesion.[2]

Importantly, when interpreting studies on circulating dickkopf-1, the type of blood specimen has to be considered. Platelets are an important source of dickkopf-1, indicated by a strong positive correlation between serum dickkopf-1 and platelet count in our and other[31] studies. Ueland et al suggested that serum and plasma may reflect, at least in part, distinct pools of dickkopf-1, where plasma levels may reflect readily available bioactive dickkopf-1 and serum levels may reflect the capacity to release dickkopf-1 from platelets during coagulation ex vivo, which may be also relevant in vivo during platelet activation in atherosclerotic disease.[2,9]

Experimental evidence suggests that sclerostin may have a protective role in atherogenesis and aortic wall remodeling. In aortic tissue, sclerostin is expressed in vascular smooth muscle cells.[5] In atherosclerotic plaques obtained from carotid endarterectomy, sclerostin is detectable by immunohistochemical staining in vascular smooth muscle cells as well as in macrophages.[47] In angiotensin II–treated mice, transgenic introduction of human sclerostin or recombinant mouse sclerostin administration inhibited aortic aneurysm formation and attenuated atherosclerosis development.[5] In this sclerostin overexpression model, extracellular matrix degradation and systemic and aortic wall inflammation was decreased, which likely resulted from lower canonical Wnt-signaling–induced expression of osteoprotegerin, osteopontin, and matrix metalloproteinase-9. Compared with dickkopf-1, mechanistic insight into effects of sclerostin on atherogenesis is more limited, calling for future studies in this field.

Strengths of our study include its prospective design, high-quality ascertainment of clinical end points, and length and completeness of follow-up with coverage of incident events up to the year 2016. The Bruneck Study is based on a sex- and age-stratified random sample from the official municipal registry, which allowed us to investigate associations based on a representative sample of the general population. Our study has several limitations as well. Given that our cohort was entirely of white origin and was recruited at 1 site in Central Europe, it is unclear to which extent our findings are generalizable to other ethnic populations and geographical regions. The observational nature of this investigation does not allow to infer causality. Although multivariable models were adjusted for a considerable number of clinical and biochemical variables, there was also a possibility of residual confounding. Circulating levels of dickkopf-1 and sclerostin might not entirely reflect concentrations at tissue level, as recently indicated for sclerostin.[47] Finally, the limited number of CVD events might have restricted the power of our study to detect statistically significant associations.

CONCLUSIONS

In this prospective, population-based study, we observed an independent association between serum levels of dickkopf-1, but not sclerostin, with incident cardiovascular events. This association was mainly driven by an increased risk for incident stroke, suggesting that dickkopf-1 may be of particular use for risk stratification for cerebrovascular disease. Our observational data support the hypothesis of a role of dickkopf-1 in the pathogenesis of CVD, whereas no such evidence was found for sclerostin. Further prospective studies are required to validate our findings.

ARTICLE INFORMATION

Received October 1, 2019; accepted February 24, 2020.

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Acknowledgments

We are very grateful to all participants of the Bruneck Study. This publication is dedicated to the memory of Dr Stefan Brugger.

Author Contributions: J. Willeit, Kiechl, and P. Willeit were involved in the conception and design of the study. Klingenschmid and P. Willeit analyzed data and drafted the manuscript. All other authors revised the article for important intellectual content. P. Willeit is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Sources of Funding

This study was supported by an excellence initiative (Competence Centers for Excellent Technologies [COMET]) of the Austrian Research Promotion Agency FFG ("Research Center of Excellence in Vascular Ageing–Tyrol, VASCage"; K-Project No.: 843536). The Bruneck Study was supported by the Pustertaler Verein zur Prävention von Herz- und Hirngefäßerkrankungen, Gesundheitsbezirk Bruneck, and the Assessorat für Gesundheit, Province of Bolzano, Italy.

Disclosures

None.

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