ORIGINAL RESEARCH

Circulating Levels of Proprotein Convertase Subtilisin/Kexin Type 9 and Arterial Stiffness in a Large Population Sample: Data From the Brisighella Heart Study

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***Background*—**Proprotein convertase subtilisin/kexin type 9 (PCSK9) circulating levels are significantly associated with an increased risk of cardiovascular events. This study aimed to evaluate the relationship between circulating levels of PCSK9 and arterial stiffness, an early instrumental biomarker of cardiovascular disease risk, in a large sample of overall healthy participants.

***Methods and Results*—**From the historical cohort of the Brisighella Heart Study, after exclusion of active smokers, participants in secondary prevention for cardiovascular disease, and patients in treatment with statins or vasodilating agents, we selected 227 premenopausal women and 193 age-matched men and 460 postmenopausal women and 416 age-matched men. In these participants, we evaluated the correlation between PCSK9 plasma circulating levels and pulse wave velocity. Postmenopausal women showed higher PCSK9 levels (309.9±84.1 ng/mL) compared with the other groups (P<0.001). Older men had significant higher levels than younger men (283.2±75.6 versus 260.9±80.4 ng/mL; P=0.008). In the whole sample, pulse wave velocity was predicted mainly by age (B=0.116, 95% CI 0.96–0.127, P<0.001), PCSK9 (B=0.014, 95% CI 0.011–0.016, P<0.001), and serum uric acid (B=0.313, 95% CI 0.024–0.391, P=0.026). Physical activity, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and estimated glomerular filtration rate were not associated with pulse wave velocity (P>0.05).By considering the subgroups described, age and PCSK9 levels were mainly associated with pulse wave velocity, which also correlated with serum uric acid in postmenopausal women.

***Conclusions*—**In the Brisighella Heart Study cohort, circulating PCSK9 is significantly related to arterial stiffness, independent of sex and menopausal status in women. **(*J Am Heart Assoc*. 2017;6:e005764. DOI: 10.1161/JAHA.117.005764.)**

Key Words: arterial stiffness • low-density lipoprotein cholesterol • menopause • proprotein convertase subtilisin/kexin type 9 • pulse wave velocity

Proprotein convertase subtilisin/kexin type 9 (PCSK9) is secreted mainly by liver, and its serum concentration depends on its own synthesis, processing, and clearance.[1] The best characterized activity of PCSK9 is the posttranslational regulation of low-density lipoprotein (LDL) receptor expression.[2] In fact, PCSK9 increases serum concentrations of LDL cholesterol (LDL-C) by inducing LDL receptor degradation, increases intestinal triglyceride-rich lipoprotein production and secretion through transcriptional and post-transcriptional mechanisms,[3] and, finally, enhances triglyceride accumulation by targeting the very LDL receptor in the adipose tissue.[4,5] Some experimental studies showed PCSK9 to be significantly expressed in vascular smooth muscle cells as well as in human atherosclerotic plaques[6] and to be

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involved in neointima formation.[7] A 100 ng/mL increase in PCSK9 was related to an increase in total plaque area.[8] Moreover, PCSK9 was associated with carotid intima–media thickness in familial hypercholesterolemic and hypertensive patients.[9,10]

A widely used parameter for an indirect assessment of arterial stiffness is pulse wave velocity (PWV), or rather the propagation speed of the pulse pressure wave, which predicts the adverse cardiovascular events in hypertension and cardiovascular disease (CVD).[10] A positive correlation between arterial stiffness and end-organ damage was clearly established in CVD.[11] Because the plaque components are responsible for arterial stiffness, increment of intima–media thickness, and atherosclerosis,[12] the observation that PCSK9 is expressed in the atherosclerotic plaque[7] could open new avenues of study on the role of PCSK9 on arterial wall remodeling. Nevertheless, to the best of our knowledge, no association has been studied between circulating PCSK9 and arterial stiffness in the general population.

Based on these premises, the present study aimed to evaluate the relationship between circulating levels of PCSK9 and arterial stiffness in a large sample of overall healthy participants.

Methods

Participants

The Brisighella Heart Study is a longitudinal population study on a randomized sample representative of the entire population of Brisighella, a rural northern Italian village. The study has been active since 1972 and is carried out in agreement with the Declaration of Helsinki.[13] The protocol was approved by the institutional ethics board of the University Hospital of Bologna. The complete version of the protocol and the history of the study have been extensively described elsewhere. All participants gave written informed consent to participate.

For the present analysis, we selected from the general database of the Brisighella Heart Study 227 premenopausal women and 193 age-matched men and 460 postmenopausal women and 416 age-matched men, excluding participants who were active smokers, who were known to have carotid atherosclerosis,[14] or who were in secondary prevention for CVD or in treatment with statins or vasodilating agents. Menopause was self-defined by the interviewed patients as the moment when menstruation definitively stopped and was confirmed with their general practitioners' clinical forms. All available routine clinical and laboratory parameters were sampled with standardized methods.[15,16] Estimated glomerular filtration rate (eGFR) was calculated using the CKD-EPI (Chronic Kidney Disease Epidemiology Collaboration) formula.[17] In particular, conventional blood pressure was measured after 10 minutes of rest in the seated position within a half hour of obtaining blood samples and in the arm opposite that used for venesection. These measurements were obtained by a trained nurse using a mercury sphygmomanometer and an appropriately sized cuff according to the European Society of Hypertension guidelines.[18]

PCSK9 Measurement

PCSK9 was blindly measured using a commercial ELISA kit (R&D Systems) with plasma aliquot collected after overnight fasting and stored at −80°C, by as previously described.[19] The minimum detectable dose ranged from 0.030 to 0.219 ng/mL, with a mean concentration of 0.096 ng/mL.

Arterial Stiffness Evaluation

Arterial stiffness parameters were assessed using the Vicorder apparatus (Skidmore Medical Ltd), a validated cuff-based device that estimates central blood pressure using a brachial-to-aortic transfer function.

PWV consists of the measurement of the pulse wave transmission through the arteries and is considered a reliable and early marker of arterial stiffness and a predictor of cardiovascular risk.[20] The theoretical basis of PWV is explained with the equation of Moens–Korteweg,[21] whereas in clinical practice, PWV is calculated as the length between 2 measurement sites divided by the time the pulse wave needs to cover that distance (m/s).[22]

Augmentation index is obtained through the blood pressure waveform analysis. It represents, as well as PWV, a measure of wave reflection and arterial stiffness and a marker of cardiovascular risk.[20] It is calculated as the ratio of the pressure increment caused by the reflected wave (augmented pressure) to the pulse pressure.[23]

Pulse wave analysis, from which the augmentation index is obtained, is recorded simply with a brachial cuff placed at the patient's right arm: The Vicorder apparatus registers the radial pressure and, with a specific algorithm, derives the central blood pressure curve. PWV is calculated with a simultaneous measurement of carotid and femoral blood pressure. A small neck pad containing a photoplethysmographic detector is placed around the neck, and a normal cuff is positioned around the thigh of the patient. The distance between the suprasternal notch and the thigh cuff is measured with a measuring tape. This length represents the distance covered by the pulse wave in its carotid–femoral path and is used by the Vicorder apparatus to establish the PWV value.[24,25] The Vicorder system automatically adjusts the PWV measurement

for heart rate and mean artery pressure, as they are simultaneously recorded.

Statistical Analysis

A full descriptive analysis was performed of all considered variables. The Kolmogorov–Smirnov normality test was performed for the continuous variables. The continuous variables were compared among the different renal function classes by ANOVA followed by the Tukey post hoc test. Nonnormally distributed parameters were then log-transformed before continuing with further analyses. First, we carried out a bivariate correlation for age, LDL-C, high-density lipoprotein cholesterol, serum uric acid (SUA), eGFR, and PWV. Then, we performed a multiple linear regression analysis using PWV as a dependent variable and age, physical activity, LDL-C, high-density lipoprotein cholesterol, PCSK9, SUA, and eGFR as independent variables. The analysis was finally repeated by the predefined participant categories (younger and older men, premenopausal and postmenopausal women). All tests were carried out using SPSS 21.0 for Windows (IBM Corp). A significance level of 0.05 was considered for every test.

All data are available at the research center under the responsibility of Professor Claudio Borghi.

Results

The main anagraphic, anthropometric, hemodynamic, and laboratory characteristics of the subgroup participants are described in Table 1. As expected, there was a specific age and sex distribution of the main CVD risk factors. Among them, LDL-C was significantly higher in postmenopausal versus premenopausal women, whereas similar concentrations were observed between younger and older men. Waist circumference, blood pressure, triglycerides, fasting plasma glucose, and SUA were significantly higher in older participants than in younger ones. High-density lipoprotein cholesterol was significantly lower in men than in women, independent of age. From a clinical point of view, the most relevant ones are the increase of pulse pressure and the decrease of eGFR in older participants.

As reported in Figure, postmenopausal women showed higher PCSK9 levels (309.9±84.0 ng/mL) compared with premenopausal women (269.4±78.8 ng/mL; $P<0.001$) and the other groups of participants ($P<0.001$). Older men had significantly higher levels than younger men (283.2±75.8 versus 260.9±80.4 ng/mL; $P=0.008$).

In the univariate model, circulating PCSK9 levels were related to age ($r=0.180$, $P<0.001$), systolic blood pressure ($r=0.138$, $P<0.001$), pulse pressure ($r=0.143$, $P<0.001$), mean

Table 1. Main Characteristics of the Selected Participants

	Premenopausal Women (n=227)	Younger Men (n=193)	Postmenopausal Women (n=460)	Older Men (n=416)
Age, y	41.0±8.4	40.3±7.7	66.8±10.6*	67.25±9.8*
WC, cm	80.9±15.9	92.5±11.1	90.3±15.4*	98.5±14.5*†
Heart rate, bpm	67.3±12.2†‡	61.6±11.5†‡	65.8±11.9†‡	61.5±11.4†‡
SBP, mm Hg	127.4±14.5	131.3±13.1†	148.0±13.5*	145.7±11.9*
DBP, mm Hg	68.8±8.6	72.4±9.5†	73.4±10.1*	76.9±9.5*
Pulse pressure, mm Hg	58.5±10.9	58.9±9.8	74.6±10.4*	68.7±10.8*†
MAP, mm Hg	94.3±10.9	95.9±11.2	104.7±13.9*	104.9±11.9*
TC, mg/dL	208.1±36.8	209.2±36.3	227.3±40.2	215.8±40.2
Triglycerides, mg/dL	95.9±62.4	118.2±76.8†	119.5±57.9*	131.0±78.7*
HDL-C, mg/dL	55.4±5.4	48.4±4.5†	54.3±5.5	48.0±4.7†
LDL-C, mg/dL	132.9±25.8	136.4±24.4	148.6±28.8*	135.6±30.5
Lipoprotein(a), mg/dL	17.4±15.6	19.3±17.6	26.3±22.0*	20.4±18.1†
FPG, mg/dL	87.2±17.1	90.5±9.8	95.1±16.7*	101.9±15.9*†
SUA, mg/dL	4.3±1.0	5.7±1.1†	4.9±1.2*	6.0±1.2†
eGFR, mL/min	79.1±13.5	85.9±12.8†	63.5±14.1*	68.7±13.4*†

DBP indicates diastolic blood pressure; eGFR, estimated glomerular filtration rate (CKD-EPI [Chronic Kidney Disease Epidemiology Collaboration] equation); FPG, fasting plasma glucose; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MAP, mean arterial pressure; SBP, systolic blood pressure; SUA, serum uric acid; TC, total cholesterol; WC, waist circumference.

*$P<0.05$ vs same sex, younger category.

†$P<0.05$ vs same age class, other sex.

‡$P<0.001$ vs other age class, other sex.

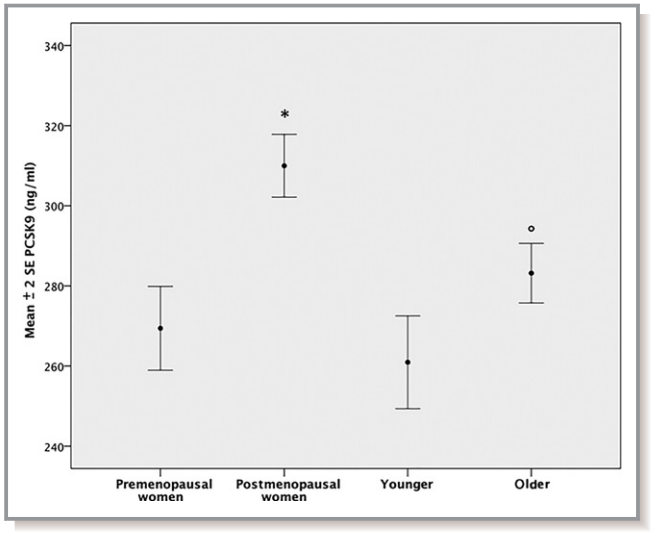

Figure. Serum proprotein convertase subtilisin/kexin type 9 (PCSK9) level (ng/mL; mean, 95% CI) in the study population. *P<0.001 vs all other groups; P=0.008 for older men vs younger men.

arterial pressure (r=0.116, P<0.001), total cholesterol (r=0.159, P<0.001), triglycerides (r=0.178, P<0.001), LDL-C (r=0.089, P=0.001), SUA (r=−0.060, P=0.030), fasting plasma glucose (r=0.107, P<0.001), lipoprotein(a) (r=0.100, P<0.001), eGFR (r=−0.101, P<0.001), and carotid–femoral PWV (r=0.302, P<0.002).

The multiple linear regression model carried out on the whole population sample showed that PWV was predicted mainly by age (B=0.114, 95% CI 0.92–0.129, P<0.001), PCSK9 (B=0.027, 95% CI 0.021–0.033, P<0.001), and SUA (B=0.301 95% CI 0.022–0.388, P=0.029). Physical activity, LDL-C, high-density lipoprotein cholesterol, and eGFR were not significantly associated with PWV (P>0.05).

The significant predictors of PWV in the different subgroups were reported in Table 2. Age and PCSK9 were the main factors associated with PWV, which also correlated with SUA but only in postmenopausal women.

Discussion

The present study, carried out in a large sample of overall healthy participants not in treatment with vasoactive medications, showed that circulating PCSK9 tends to increase with age regardless of sex and was significantly more elevated in women independent of menopausal condition. These effects agree with previous findings reporting that PCSK9 levels increase in women reaching menopause but not in older men.[26] Whether age could affect the PCSK9 levels remains an open question because PCSK9 concentrations are partly related to growth hormone serum levels, as reported by Persson et al.[27]

In our population sample, as emerged from the univariate analysis, the circulating levels of PCSK9 were related to a large number of CVD risk factors, namely, age, systolic blood pressure, pulse pressure, mean arterial pressure, LDL-C, triglycerides, SUA, fasting plasma glucose, lipoprotein(a), eGFR, and carotid–femoral PWV. Most of these correlations are in line with previous findings showing how PCSK9 levels positively correlate with LDL-C, with non–statin-treated patients,[28] with lipoprotein(a),[29] and with atherogenic lipoproteins in patients with high cardiovascular risk.[30]

Table 2. Significant Predictors of Pulse Wave Velocity in the Different Subgroups

	Premenopausal Women				Postmenopausal Women			
		95% CI				95% CI		
Predictor	B	Lower Limit	Upper Limit	*P* Value	B	Lower Limit	Upper Limit	*P* Value
Age	0.144	0.98	0.213	<0.001	0.225	0.135	0.316	0.003
PCSK9	0.021	0.008	0.031	0.002	0.036	0.026	0.045	<0.001
SUA				>0.05	0.496	0.088	0.901	0.017
	Younger Men				Older Men			
		95% CI				95% CI		
Predictor	B	Lower Limit	Upper Limit	*P* Value	B	Lower Limit	Upper Limit	*P* Value
Age	0.162	0.084	0.290	0.002	0.258	0.165	0.342	<0.001
PCSK9	0.029	0.018	0.046	<0.001	0.028	0.015	0.039	<0.001
SUA				>0.05				>0.05

Independent variables: age, physical activity, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, PCSK9, SUA, and estimated glomerular filtration rate (CKD-EPI [Chronic Kidney Disease Epidemiology Collaboration] equation). PCSK9 indicates proprotein convertase subtilisin/kexin type 9; SUA, serum uric acid.

Circulating levels of PCSK9 and vascular aging (evaluated in term of carotid intima–media thickness) were recently studied with conflicting results in hypertensive patients.[10] In contrast, circulating PCSK9 was found to be an independent predictor of carotid arteriosclerosis in asymptomatic adults.[31]

Our findings showed that PCSK9 is significantly associated with arterial stiffness (estimated in term of carotid–femoral PWV) regardless of sex and, in women, menopausal condition. The strength of this association was very high from a statistical point of view but relatively weak in absolute terms. However, because small differences in arterial stiffness seem to be associated with clinically significant differences in CVD risk, our observation should not be underestimated.[12] Moreover, PCSK9 is measurable with a standardized method, and its circulating level is related to its tissue concentration.[19]

A relatively large number of clinical studies clearly documented the feasibility of using monoclonal antibodies against PCSK9, alone or in combination with statins, to achieve very low LDL-C levels.[32] Waiting for long-term trial results on morbidity and mortality data, the recent findings from the GLAGOV (GLobal Assessment of Plaque reGression With a PCSK9 antibOdy as Measured by intraVascular Ultrasound) study demonstrated the efficacy of evolocumab on reducing progression of atherosclerosis as measured by intravascular ultrasound.[33] Additional evidence suggested a direct role of PCSK9 on atherosclerotic plaque formation independent of the LDL-C lowering effect. In particular, the ATHEROREMO-IVUS study showed that higher serum PCSK9 levels are linearly associated with a higher necrotic core fraction in coronary atherosclerosis, regardless of serum LDL-C.[34] In line with this evidence, a significant association between serum PCSK9 levels and intima–media thickening was reported in hypertensive patients and persisted after adjustment for blood lipids.[10] Furthermore, Werner et al demonstrated in a prospective cohort study that elevated PCSK9 serum concentrations were associated with cardiovascular events in patients with stable coronary artery disease, despite a well-controlled LDL-C concentration.[35] Consequently, serum PCSK9 levels seem to predict early atherosclerosis and potentially involve plaque development and composition. By using an experimental model of carotid restenosis, our research group recently demonstrated that PCSK9$^{-/-}$ mice are partially protected from neointimal formation, further supporting the positive effect of PCSK9 on intimal thickening.[7]

At the same time, SUA, which is the final end product of purine catabolism, is considered a CVD risk factor[36] and, in healthy persons, an early marker of vascular stiffness.[37] The association between SUA and PWV was previously studied in the Brisighella Heart Study cohort.[14,16] Nevertheless, it is interesting to note in the present analysis that SUA did not affect the correlation between PCSK9 and PWV.

The main limitation of this study was the relatively small size of the single predefined groups; however, this was representative of the participants' distribution in the Brisighella Heart Study cohort. Moreover, the considered sample included more participants than a large part of the previously published paper investigating the association between circulating PCSK9 and arterial aging. Another limitation is the selection of the participants, which could have introduced a bias; however, a large number of the participants that we excluded from the analysis had characteristics that could affect the measurement of PWV and, consequently, the reliability of our observations. A further limitation regards the evaluation of menopausal age, which was based on patient self-reporting of menstruation cessation and not on specific laboratory parameters; however, data were confirmed by comparison with the information included in the general practitioner clinical forms.

Based on the assumptions that PCSK9 levels are associated with increased risk of total cardiovascular events[38] and that variants in genes encoding *PCSK9*, leading to decreased LDL-C levels, are protective for the risk of CVD events,[39] our results support the hypothesis that an increment in circulating PCSK9 levels could be associated with arterial stiffness independent of atherogenic lipoproteins levels and a possible early marker of cardiovascular risk in overall healthy persons.

In conclusion, in an overall healthy population sample, circulating PCSK9 seems to be significantly related to arterial stiffness independent of sex and, in women, menopausal status.

Appendix

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Disclosures

None.

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