Proprotein convertase subtilisin/kexin type 9 in patients with systemic sclerosis

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ABSTRACT

Objective. *Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a serine protease that regulates cholesterol metabolism through low-density lipoprotein receptor degradation, and which has been linked to cardiovascular risk. The purpose of the present study was to examine whether PCSK9 serum levels are disrupted in patients with systemic sclerosis (SS) compared to controls, and if PCSK9 is related to disease-related data and the subclinical atherosclerosis that occurs in these patients.*

Methods. *Cross-sectional study that encompassed 146 individuals; 73 patients with SS and 73 age- and sex-matched controls. PCSK9, lipoproteins serum concentrations, and standard lipid profiles were assessed in patients and controls. Carotid intima-media thickness (cIMT) and the presence of carotid plaques were evaluated in SS patients. A multivariable analysis, adjusted for traditional cardiovascular risk factors, was performed to evaluate the differences in PCSK9 between patients and controls, the association of SS-related manifestations with PCSK9 levels, and if PCSK9 was associated with subclinical carotid atherosclerosis in SS patients.*

Results. *After multivariable analysis, PCSK9 was downregulated in SS patients compared to controls (beta coefficient -78 (95%CI -106 – -50) ng/ml, p=0.000) and skin thickness was associated with higher serum levels of PCSK9 (beta coef. 22 (7–37) units, p=0.005). PCSK9 was significantly and positively associated with cIMT (beta coef. 0.65 (0.06–1.24) ng/ml, p=0.031) in SS patients after multivariable adjustment.*

Conclusion. *PCSK9 serum concentration is downregulated in SS patients compared to controls and is directly associated with disease severity subrogated parameters. PCSK9 was independently related to cIMT in SS patients.*

Introduction

Systemic sclerosis (SS) is a chronic multisystemic disease in which underlying vasculopathy plays a major role in the progressive fibrosis of the skin and internal organs (1). It is well established that SS patients suffer a greater cardiovascular (CV) risk that controls (2, 3) although the mechanisms by which this happens are not yet well defined. While the prevalence of traditional CV risk factors does not seem to be higher in SS (4-6), the use of medications such as NSAIDs and oral glucocorticoids seems to contribute, at least in part, to this increased risk (3). Studies assessing subclinical atherosclerosis showed that SS patients had significantly increased carotid intima-media thickness (cIMT), indicating a greater atherosclerotic burden compared to controls (7, 8). More-over, in a recent report by Tountas *et al*. (9), speckle-tracking echocardiography of the right ventricle was found to be impaired in SS patients. This was influenced by peripheral microvascular abnormalities, although it occurred in the absence of macrovascular damage. Respect to lipid panel, SS has been linked to disrupted lipid profiles, although reports have been contradictory in this regard (10-12). The mechanisms responsible for this modification to the lipid profile and its relevance in the development of atherosclerosis in patients with SS remain unknown.

Proprotein convertase subtilisin kexin 9 (PCSK9), a serine protease, plays an important role in low-density lipoprotein (LDL) metabolism via its binding to hepatic LDL receptors and its targeting of them for degradation (13). This process reduces the capacity of the liver to bind and remove LDL-cholesterol, resulting in increased LDL-cholesterol serum levels (14). PCSK9 serum levels have been linked to cardiovascular

events (15). New therapies blocking the interaction between PCSK9 and LDL receptors by the use of a monoclonal antibody that binds PCSK9 have been successful in lowering LDL-cholesterol levels in patients with hypercholesterolaemia and in reduce the incidence of CV events (15, 16). Additionally, PCSK9 has been found to be downregulated in rheumatoid arthritis (17) and systemic lupus erythematosus patients (in press).

Information on the role of PCSK9 in patients with SS is lacking. For this reason, we conducted a study to assess whether PCSK9 is disrupted in patients with SS. Based on carotid ultrasounds, we additionally set out to establish whether PCSK9 is linked to an increased atherosclerosis burden in SS patients.

Patients and methods

Study participants

This was a cross-sectional study that included 73 patients with SS and 73 age- and sex-matched controls. All patients were 18 years old or older and fulfilled with the American College of Rheumatology criteria for the classification of SS (18). They had been diagnosed by rheumatologists and were periodically followed-up at rheumatology outpatient clinics. For the purpose of inclusion in the present study, SS disease duration needed to be ≥1 year. Since glucocorticoids are often used in the management of SS, patients taking prednisone were not excluded. None of the patients had established CV disease. The controls were community-based, recruited by general practitioners in primary health centres. Controls with a history of any inflammatory rheumatic diseases were excluded, as well as those with a history of CV disease. Moreover, patients and controls were excluded if they had a history of cancer or any other chronic disease, evidence of an active infection or a glomerular filtration rate <60 ml/min/1.73 m2. The study protocol was approved by the Institutional Review Committee at Hospital Universitario de Canarias and all subjects provided informed written consent (CARESHUC Study, Approval Number 2016_86).

Assessments and data collection

Surveys in SS patients and controls were performed to assess CV risk factors and medication. Subjects completed a questionnaire and underwent a physical examination to determine anthropometric measurements and blood pressure. Medical records were reviewed to ascertain specific diagnoses and medications. Hypertension was defined as a systolic or a diastolic blood pressure higher than, respectively, 140 and 90 mmHg. Disease duration for SS was defined as the time since the onset of the first SS-related symptom other than Raynaud's phenomenon. SS subtypes, limited and diffuse, were determined based on the distribution of skin thickness. The modified Rodnan Skin Score (*mRSS*) was used to assess skin thickening (19). This score has been commonly used as an outcome measure in clinical trials. It rates the severity of these features from 0 (normal) to 3 (most severe) in 17 distinct areas of the body and shows an acceptable degree of intra-rater variability. Oesophageal involvement was defined as any sign of dysmotility evident by manometry. Articular involvement was determined by clinical evidence of joint swelling, deformity, contractures, and tendon friction rubs or radiographic evidence of joint space narrowing or erosion. Interstitial lung disease (ILD) was defined as signs of fibrosis on radiograph, high-resolution computed tomography, or by abnormal pulmonary function tests.

Lipids and PCSK9 assessments

Fasting serum samples were collected and frozen at -80°C until analysis of circulating lipids. Human PCSK9 was measured using an ELISA kit (R&D Duoset). Intra- and inter-assay coefficients of variation were 4.9% and 6.3%, respectively. Cholesterol, triglycerides, and HDL-cholesterol were measured using the enzymatic colorimetric assay (Roche). Lipoprotein (a) and lipoproteins were assessed using a quantitative immunoturbidimetric assay (Roche). Cholesterol ranged from 0.08 to 20.7 mmol/l (intra-assay coefficient of variation of 0.3%); triglycerides ranged from 4 to 1.000 mg/dl (intra-assay coefficient of variation of 1.8%); and HDL cholesterol ranged from 3 to 120 mg/dl (intra-assay variation coefficient of 0.9%). The atherogenic index was calculated using the total cholesterol/HDL-C ratio according to the Castelli formula. LDL cholesterol was calculated using the Friedewald formula. A standard technique was used to measure high-sensitivity C-reactive protein (CRP).

Carotid ultrasound assessment

A carotid ultrasound examination was performed to assess cIMT in the common carotid artery and to identify focal plaques in the extracranial carotid tree in patients with SS (20). A commercially available scanner, the Esaote Mylab 70 (Genoa, Italy), equipped with a 7-12 MHz linear transducer and an automated software-guided radiofrequency technique, Quality Intima Media Thickness in real-time (QIMT, Esaote, Maastricht, Holland), was used for this purpose. As previously reported (21), based on the Mannheim consensus, plaque criteria in the accessible extracranial carotid tree (common carotid artery, bulb and internal carotid artery) were defined as follows: a focal protrusion in the lumen measuring at least cIMT >1.5 mm; a protrusion at least 50% greater than the surrounding cIMT; or arterial lumen encroaching >0.5 mm (19).

Statistical analysis

Demographic and clinical characteristics were compared between SS patients and controls using χ^2 tests for categorical variables or a Student's t-test for continuous variables (data expressed as percentages and mean ± standard deviation- SD-). For non-continuous variables, either a Mann-Whitney U-test was performed, or a logarithmic transformation was made, and data were expressed as median and interquartile ranges (IQR). Differences between patients and controls regarding their lipid profiles was assessed through multivariable regression analysis. Confounding variables in this analysis were those with a statistical *p* value lower than 0.20 in those differences between patients and controls in traditional CV risk factors. Univariable linear regression analyses were performed to establish the relation of demographics,

traditional CV risk factors, lipid profile and SS-related data with PCSK9 serum levels. Similarly, the relations between traditional CV risk factors, lipid profile, and SS related data *vis-à-vis* carotid plaque and cIMT were studied through multivariable lineal regression analysis assuming the same criteria for confounding variables. All analyses used a 5% two-sided significance level and were performed using SPSS software, v. 25 (IBM, Chicago, IL, USA). A *p*-value <0.05 was considered statistically significant.

Results

Demographic, laboratory and disease-related data

A total of 146 participants, 73 patients with SS and 73 age- and sex-matched controls, were included in this study. Demographic and disease-related characteristics of the participants are shown in Table I. There were no differences between patients and controls regarding body mass index and the presence of hypertension, current smoking, or obesity. Only the presence of diabetes type 2 tended to be higher in controls, although statistical significance was not reached.

Disease duration was 9 (IQR 4–15) years in SS patients. The *mRSS* score was 3 (IQR 1–6), and the presence of digital ulcers and calcinosis was reported, respectively, in 11% and 10% of the patients. At the time the study was conducted, about one-fifth of patients (21%) were taking prednisone with a median dose of 5 (IQR 5–10)) mg/day. Additionally, 47 (64%) patients were found to be positive for anti-centromere, and 14% were positive for anti-Scl70. Disease-modifying anti-rheumatic drug (DMARD) use was reported in 14% of the patients, including 5% azathioprine, 3% hydroxychloroquine and 3% methotrexate. Other features related to the disease are shown in Table I.

Table I. Demographics of systemic sclerosis patients and controls.

	Controls (n=73)	SS patients (n=73)	*p*
Demographics			
Female, n (%)	68 (93)	68 (93)	1.00
Age, years	56 ± 17	59 ± 11	0.20
BMI, mg/cm^2	30 ± 4	28 ± 5	0.15
Waist circumference, cm	97 ± 6	96 ± 14	0.33
Systolic pressure, mmHg	127 ± 15	132 ± 19	0.052
Diastolic pressure, mmHg	82 ± 7	80 ± 12	0.28
Comorbidities			
Hypertension, n (%)	34 (47)	27 (37)	0.24
Current smoking, n (%)	17 (23)	14 (19)	0.57
Diabetes, n (%)	17 (23)	8 (11)	0.052
Body mass index >30, n (%)	24 (33)	22 (30)	0.72
Statins, n (%)	26 (36)	25 (34)	0.91
Systemic sclerosis-related data			
CRP, mg/dl	2.03 (1-30-3.53)	2.20 (1.31-4.14)	0.52
SS type			
Limited		50 (68)	
Diffuse		23 (32)	
Disease duration, years		9 (4-15)	
Modified Rodnan Skin Score, units		3 (1-6)	
Raynaud phenomenon, n (%)		62 (85)	
Digital ulcers, n (%)		8 (11)	
Calcinosis, n (%)		7 (10)	
Arthritis, n (%)		15 (21)	
Gastric reflux, n (%)		29 (40)	
Pathological oesophageal manometry, n (%)		40 (55)	
Interstitial lung disease		16 (22)	
Pulmonary hypertension		16 (22)	
Anti-centromere antibody positivity		47 (64)	
Anti-Scl70 antibody		10 (14)	
Current prednisone, n (%)		15 (21)	
Prednisone, mg/day		5 (5-10)	
DMARDs, n (%)		10 (14)	
Azathioprine, n (%)		4 (5)	
Methotrexate, n (%)		2 (3)	
Hydroxychloroquine, n (%)		2 (3)	
Carotid ultrasound			
cIMT, carotid intima media thickness, mm		705 ± 148	
Carotid plaque, n (%)		34 (47)	

Data represent means ± SD or median (IQR) when data were not normally distributed.
BMI: body mass index; cIMT, carotid intima media thickness; CRP: C reactive protein; DMARD: disease-modifying antirheumatic drug; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

Multivariable analysis of the differences in lipid profiles between SS patients and controls

No differences were found in the lipid profiles between patients and controls in the univariable and multivariate analyses (Table II). The mean PCSK9 serum levels were significantly lower in SS patients compared to controls (268±96 *vs*. 187±60, ng/ml, *p*=0.000) when this univariate analysis was performed. Interestingly, after adjusting for BMI, systolic pressure and diabetes mellitus PCSK9 remained downregulated in SS patients (beta coefficient -78 (95%CI -106 – -50) ng/ml, *p*=0.000).

Relation of demographics, lipid profile, and disease-related data with PCSK9 in SS patients and controls

Traditional CV risk factors others than lipids were not associated with PCSK9 serum levels in either controls or patients (Table III). In this sense, only the use of statins was positive and significantly related to PCSK9 in patients and controls. Regarding lipid profiles, PCSK9 was negatively associated with total cholesterol (beta coefficient -0.64 (95%CI -1.19 – -0.09) ng/ml, *p*=0.023) and LDL-cholesterol (beta coef. -0.85 (95%CI -1.47 – -0.23) ng/ml, *p*=0.008), albeit only in controls. These relations were not found in SS patients. Regarding disease-related data, some subrogated parameters of disease severity like the *mRSS*, and the presence of Raynaud's phenomenon and interstitial lung disease, were significantly and positively associated with PCSK9 serum levels. In contrast, the presence

Table II. Multivariable analysis of the differences in lipid profile between systemic sclerosis patients and controls.

			Univariable model	Multivariable model *	
	Controls (n=73)	SS patients (N=73)	*p*	beta coef. (95% CI),	*p*
Lipid profile					
Cholesterol, mg/dl	189 ± 40	199 ± 38	0.12	10 (-4-23)	0.16
Triglycerides, mg/dl	144 ± 64	151 ± 80	0.62	12 (-13-36)	0.35
HDL cholesterol, mg/dl	50 ± 14	50 ± 13	0.99	-1 (-5-3)	0.74
LDL cholesterol, mg/dl	109 ± 35	119 ± 34	0.10	8 (-3-20)	0.16
LDL:HDL cholesterol ratio	2.30 ± 0.89	2.60 ± 1.44	0.14	0.34 (-0.08-0.75)	0.11
Non-HDL cholesterol, mg/dl	138 ± 38	149 ± 39	0.12	10 (-3-24)	0.13
Lipoprotein (a), mg/dl	45 (23-85)	23 (11-67)	0.12	-13 (-37-11)	0.28
Apolipoprotein A1, mg/dl	167 ± 34	170 ± 32	0.52	3 (-8-15)	0.58
Apolipoprotein B, mg/dl	96 ± 23	100 ± 25	0.45	3 (-6-12)	0.48
Apo B:Apo A ratio	0.60 ± 0.19	0.62 ± 0.28	0.61	0.02 (-0.06-0.11)	0.59
Atherogenic index	3.94 ± 1.10	4.27 ± 1.64	0.17	0.39 (-0.09-0.88)	0.11
PCSK9, ng/ml	268 ± 96	187 ± 60	**0.000**	**-78 (-106- -50)**	**0.000**

Data represent means ± standard deviation or median (interquartile range) when data were not normally distributed. Beta coefficients are expressed without decimals except for those with a value <1.
*Adjusted for body mass index, diabetes and systolic pressure.
HDL: high-density lipoprotein; LDL: low-density lipoprotein; PCSK9: Proprotein Convertase Subtilisin/Kexin Type 9.

of anti-centromere antibody was negatively associated with PCSK9 (Table III). Nail fold capillaroscopy findings were not related to CEC in SS patients (data not shown).

PSCK9 association with cIMT and the presence of carotid plaque

Age, systolic blood pressure, and the present of hypertension were significantly and positively associated with both cIMT and carotid plaque in SS patients. None of the lipid profile-related molecules were associated with cIMT or carotid plaque to any significant degree apart from PCSK9. In this sense, PCSK9 was univariately associated with cIMT (Pearson's correlation coefficient 0.322, p=0.010), though not with the presence of carotid plaque. When the cIMT relation was analysed after adjusting for confounding factors, its significance was maintained (beta coef. 0.65 (0.06–1.24), p=0.031).

Discussion

To the best of our knowledge, this is the first study in which PCSK9 has been assessed in SS patients. PCSK9 is independently downregulated in SS patients compared to controls, but positively associated with characteristics of the disease that are related to severity or damage, such as the extension of skin involvement and the presence of Raynaud's phenomenon and interstitial lung disease. Additionally, PCSK9 serum levels were independently related to cIMT in SS patients.

Studies on lipid profiles in SS are scarce and the trend of the potential disturbance in lipid parameters of these patients has been contradictory among different series. For example, Lippi *et al*. found that SS patients displayed statistically and significantly higher serum levels of Lipoprotein (a), though other lipid parameters remained within normal ranges (11). Others have found only a partial presence of inflammatory dyslipidaemia in SS, characterised by lower levels of total cholesterol and LDL-cholesterol (10, 12). In our study, patients with SS did not present a different lipid profile compared to controls even after multivariable analysis. We believe that the fact that neither CRP serum levels nor the presence of CV comorbidities differed between patients and controls explains the absence of inflammatory lipid profiles in SS patients found in our study. Nonetheless, PCSK9 serum levels were downregulated in SS patients. We think this supports our finding that the differences in PCSK9 cannot be attributed to other effects the disease may exert over the lipid profile. Our results are also in agreement with other reports on the role of PCSK9 in inflammatory diseases. In this sense, a previous study by our group that encompassed 326 patients with rheumatoid arthritis and 194 age- and sex-matched controls bears this out, since PCSK9 was found to be downregulated in rheumatoid arthritis after multivariable analysis (17).

The exact mechanisms of PCSK9 downregulation in SS patients found in our study, as well as in other inflammatory diseases like rheumatoid arthritis, have not yet been identified. However, we hypothesise that PSCK9 levels could be downregulated as a compensatory mechanism in the context of heightened cardiovascular disease risk or to reduce the accelerated CV risk associated with these diseases. Although higher levels of plasma PCSK9 are independently associated with major systemic inflammatory markers in patients with acute coronary syndrome and coronary artery disease (22), we believe that PCSK9 could be downregulated in chronic inflammatory states. This would be consistent with the increased metabolic clearance of total cholesterol and LDL-cholesterol that accompanies chronic inflammatory diseases.

Interestingly, correlations between PCSK9 and total or LDL-cholesterol that have been observed in healthy populations (23) were present in controls but lost in SS patients. Although the explanation for this finding is not readily apparent, we believe that inflammatory dyslipidaemia may disrupt the normal relationships of lipid molecules. In addition, because this disturbance is found in lipid patterns, associations with lipid-related molecules likely become aberrant to what occurs in healthy sub-

Table III. Associations of demographics, lipid profiles, and disease-related data with PCSK9 in SS patients and controls.

	PCSK9 ng/ml, beta coef. (CI95%), *p*	
	Controls (n=73)	Patients (n=73)
Age, years	0.24 (-1.09-1.57), 0.72	**2 (0-3), 0.016**
Female	52 (-36-140), 0.24	26 (-30-82), 0.36
Body mass index, kg/m^2	1 (-4-7), 0.65	2 (-1-4), 0.26
Abdominal circumference, cm	-1 (-4-3), 0.61	1 (-0-2), 0.17
Systolic blood pressure, mmHg	0.63 (-0.82-2.09), 0.39	0.16 (-0.66-0.98), 0.71
Diastolic blood pressure, mmHg	0.12 (-3.07-3.30), 0.94	-0.57 (-1.74-0.60), 0.34
Cardiovascular co-morbidities		
Smoking	-24 (-77-28), 0.34	12 (-24-49), 0.50
Diabetes	15 (-38-68), 0.58	37 (-11-85), 0.13
Hypertension	25 (-20-69), 0.28	24 (-5-54), 0.10
Body mass index >30	28 (-20-75), 0.25	17 (-15-49), 0.30
Statins	**61 (16-105), 0.009**	**52 (24-81), 0.001**
Analytical and lipid profiles		
CRP, mg/dl	**4 (1-6), 0.008**	4 (-1-9), 0.16
Cholesterol, mg/dl	**-0.64 (-1.19- -0.09), 0.023**	-0.11 (-0.51-0.30), 0.59
Triglycerides, mg/dl	0.31 (-0.04-0.65), 0.085	0.15 (-0.04-0.34), 0.13
HDL cholesterol, mg/dl	-1 (-3-0), 0.14	-0.38 (-1.56-0.79), 0.52
LDL cholesterol, mg/dl	**-0.85 (-1.47- -0.23), 0.008**	0.26 (-0.71-0.19), 0.25
LDL:HDL cholesterol ratio	-16 (-42-9), 0.19	-2 (-13-8), 0.64
Non-HDL cholesterol, mg/dl	-0.54 (-1.13-0.04), 0.067	-0.06 (-0.45-0.33), 0.77
Lipoprotein (a), mg/dl	0.10 (-0.21-0.41), 0.53	-0.11 (-0.35-0.14), 0.39
Apolipoprotein A1, mg/dl	-0.16 (-0.82-0.51), 0.64	0.14 (-0.35-0.62), 0.58
Apolipoprotein B, mg/dl	-0.74 (-1.59-0.11), 0.085	-0.19 (-0.81-0.43), 0.54
Apo B:Apo A ratio	-74 (-193-45), 0.22	-27 (-81-27), 0.33
Atherogenic index	-4 (-26-15), 0.62	-023 (-9.12-9.58), 0.96
SS-related data		
log Disease duration, years		0.84 (-1.00-2.68), 0.36
log Modified Rodnan Skin Score, units		**22 (7-37), 0.005**
Raynaud phenomenon		**46 (5-88), 0.030**
Digital ulcers		-44 (-88-1), 0.055
Calcinosis		-28 (-76-20), 0.24
Arthritis		28 (-8-65), 0.13
Gastric reflux		-4 (-34-25), 0.77
Pathological oesophageal manometry		1 (-33-36), 0.93
Interstitial lung disease		**49 (15-83), 0.006**
Pulmonary hypertension		30 (-7-67), 0.11
Anti-centromere antibody positivity		**-46 (-75- -17), 0.002**
Anti-Scl70 antibody positivity		36 (-1-76), 0.12
Current prednisone		1 (-36-37), 0.98
Prednisone, mg/day		1 (-8-10), 0.77
DMARDs		-11 (-53-30), 0.59
Methotrexate		-2 (-89-85), 0.96
Hydroxychloroquine		-60 (-142-30), 0.20

Data represent means±SD or median (interquartile range) when data were not normally distributed. Beta coefficients are expressed without decimals except for those with a value <1.
ANA: antinuclear antibodies; BMI: body mass index; CRP: C-reactive protein; DMARD: disease-modifying anti-rheumatic drug; HDL: high-density lipoprotein; LDL: low-density lipoprotein.

jects. Whether the downregulation of PCSK9 in SS stems from other effects the disease exerts over the lipid profile, or if SS pathophysiological pathways primarily affect PCSK9, warrants further study. Similarly, the use of statins was associated with higher PCSK9 serum levels in patients and controls. This issue has been previously described in a recent meta-analysis in which statin therapy was shown to increase plasma PCSK9 concentrations, an effect that has been correlated to the magnitude of reduction that statins exert over plasma LDL-cholesterol (24).

Remarkably, although PCSK9 serum levels were downregulated in SS, some disease features like the skin thickness score and the presence of Raynaud's phenomenon and interstitial lung disease, were positively associated with PCSK9. Several studies have demonstrated that patients with diffuse cutaneous SS have higher mortality rates than those with limited cutaneous SS, with skin and pulmonary involvement being the most frequently observed risk factors associated with this increased mortality (25). CV mortality in SS has also been associated with more severe forms of the disease (26). For this reason, we believe that although PCSK9 may be diminished in patients with SS due to the global effects of the disease, it is also reasonable to postulate that a certain subgroup of patients with more severe manifestations of the disease had elevated levels of PCSK9 which, in turn, would put them at risk for a greater number of CV events and mortality. We believe that our findings could reflect a different pathogenic mechanism underlying atherosclerosis in those patients with diffuse and limited SS. Supporting this notion, in a previous study that assessed arterial stiffness in an SS cohort, patients with anti-centromere showed a higher aortic augmentation index and lower pulse pressure amplification than those with anti–Scl-70 antibodies (27).

In our study, PCSK9 was associated with cIMT, but not with the presence of carotid plaque, an outcome we cannot readily explain. It is well established that carotid plaque and cIMT are biologically and genetically distinct entities, representing different phenotypes of atherosclerosis (28). cIMT is thought to represent a mainly hypertensive medial hypertrophy, while carotid plaque is more strongly associated with traditional risk factors and coronary artery disease than is cIMT. Although extensive evidence suggests that there is a significant correlation between PCSK9 levels and future CV risk, scarce data exist about the relationship between PCSK9 levels and vascular biomarkers such as cIMT. For example, in a recent report on the relationship between PCSK9 and cIMT in 256 subjects, a positive correlation between the two vis-à-vis hypertensiveness was found in the univariate analyses, whereas such an association was absent in normotensive subjects (29). In contrast, in another study involving 295 asymptomatic subjects, serum PCSK9 remained an independent predictor of cIMT after

Table IV. Cardiovascular risk factors and lipid profile association with cIMT and carotid plaque.

	cIMT, mm		Carotid plaque	
	Pearson's r	*p*	Pearson's r	*p*
Age, years	**0.391**	**0.001**	**0.391**	**0.000**
Female	0.003	0.98	0.008	0.95
Body mass index, kg/m^2	-0.136	0.28	0.124	0.32
Abdominal circumference, cm	-0.136	0.28	-0.043	0.73
Systolic blood pressure, mmHg	**0.327**	**0.007**	0.230	0.064
Diastolic blood pressure, mmHg	0.108	0.39	0.084	0.50
Cardiovascular co-morbidities				
Smoking	-0.191	0.13	0.099	0.43
Diabetes	0.040	0.75	0.137	0.27
Hypertension	**0.255**	**0.039**	**0.391**	**0.001**
Body mass index >30, n (%)	-0.081	0.52	0.112	0.37
Analytical and lipid profiles				
Cholesterol, mg/dl	0.111	0.40	0.013	0.92
Triglycerides, mg/dl	0.127	0.33	0.190	0.15
HDL cholesterol, mg/dl	0.003	0.98	0.119	0.36
LDL cholesterol, mg/dl	0.076	0.56	-0.144	0.27
LDL:HDL cholesterol ratio	0.033	0.80	-0.196	0.13
Non-HDL cholesterol, mg/dl	0.103	0.43	-0.028	0.83
Lipoprotein (a), mg/dl	0.092	0.48	0.065	0.62
Apolipoprotein A1, mg/dl	0.050	0.70	0.161	0.22
Apolipoprotein B, mg/dl	0.101	0.44	-0.011	0.93
Apo B:Apo A ratio	0.080	0.54	-0.109	0.40
Atherogenic index	0.047	0.72	-0.136	0.30
PCSK9, ng/ml	**0.322**	**0.010**	0.205	0.11
Multivariate relation of PCSK9 with subclinical atherosclerosis*				
	beta coef. (95%CI)		odds ratio (95%CI)	
PCSK9, ng/ml	**0.65 (0.06-1.24), 0.031**		1.00 (0.99-1.01), 0.58	

*Adjusted for age, systolic blood pressure, and smoking.
cIMT: carotid intima media thickness; HDL: high-density lipoprotein; LDL: low-density lipoprotein; PCSK9: Proprotein Convertase Subtilisin/Kexin Type 9.

multivariate regression analysis (30). In addition, one study reported a positive association between serum PCSK9 levels and cIMT in hypertensive patients (31), suggesting that serum PCSK9 may play a role in the early pathogenesis of atherosclerosis. These studies did not assess carotid plaque, thus no conclusions regarding a link between PCSK9 and carotid plaque can be reached. We believe that the fact that PCSK9 was associated with cIMT in SS patients points to the validity of this molecule as a potential future target in the treatment of atherosclerosis in such patients.

Moreover, we believe our finding that downregulated PCSK9 serum levels in SS patients are related to subclinical atherosclerosis is consistent with what is known as the "lipid paradox" in inflammatory states (32). This means that contradictory reductions in lipid molecules are still associated with the risk of cardiovascular disease, whereby lower total cholesterol and LDL-cholesterol levels are linked to increased cardiovascular risk (33). This pattern is mirrored in sepsis and other inflammatory states, suggesting systemic inflammation has the general effect of lowering circulating lipid levels (34). Although the existence of the "lipid paradox" in SS is controversial, in our study the fact that PCSK9 was found to be downregulated, albeit positively related to cIMT, could be in accordance with current knowledge on the relationship between dyslipidaemia and inflammatory states. We acknowledge the limitation that carotid assessments were not carried out in healthy control subjects. Although PCSK9 has been widely associated with CV events in the general population, the availability of carotid assessments in control subjects would have facilitated the study of differential effects or statistical links between these two populations.

In conclusion, PCSK9 is downregulated in SS patients. Some disease features related to severity and damage were positively associated with PCSK9. The fact that PCSK9 was independently associated with subclinical atherosclerosis in SS remains a challenging finding and suggests that the possibility of PSCK9 as a target pathobiological pathway for atherosclerosis in SS warrants further study. More prospective studies are needed to assess the prognostic, as well as the therapeutic, implications of these novel findings.

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