Supplementary Data

RELATIONSHIP BETWEEN CIRCULATING PCSK9 AND MARKERS OF SUBCLINICAL ATHEROSCLEROSIS – THE IMPROVE STUDY

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Supplementary Table S1. Baseline characteristics of the IMPROVE study cohort stratified by quintiles of plasma PCSK9 levels (ng/mL).

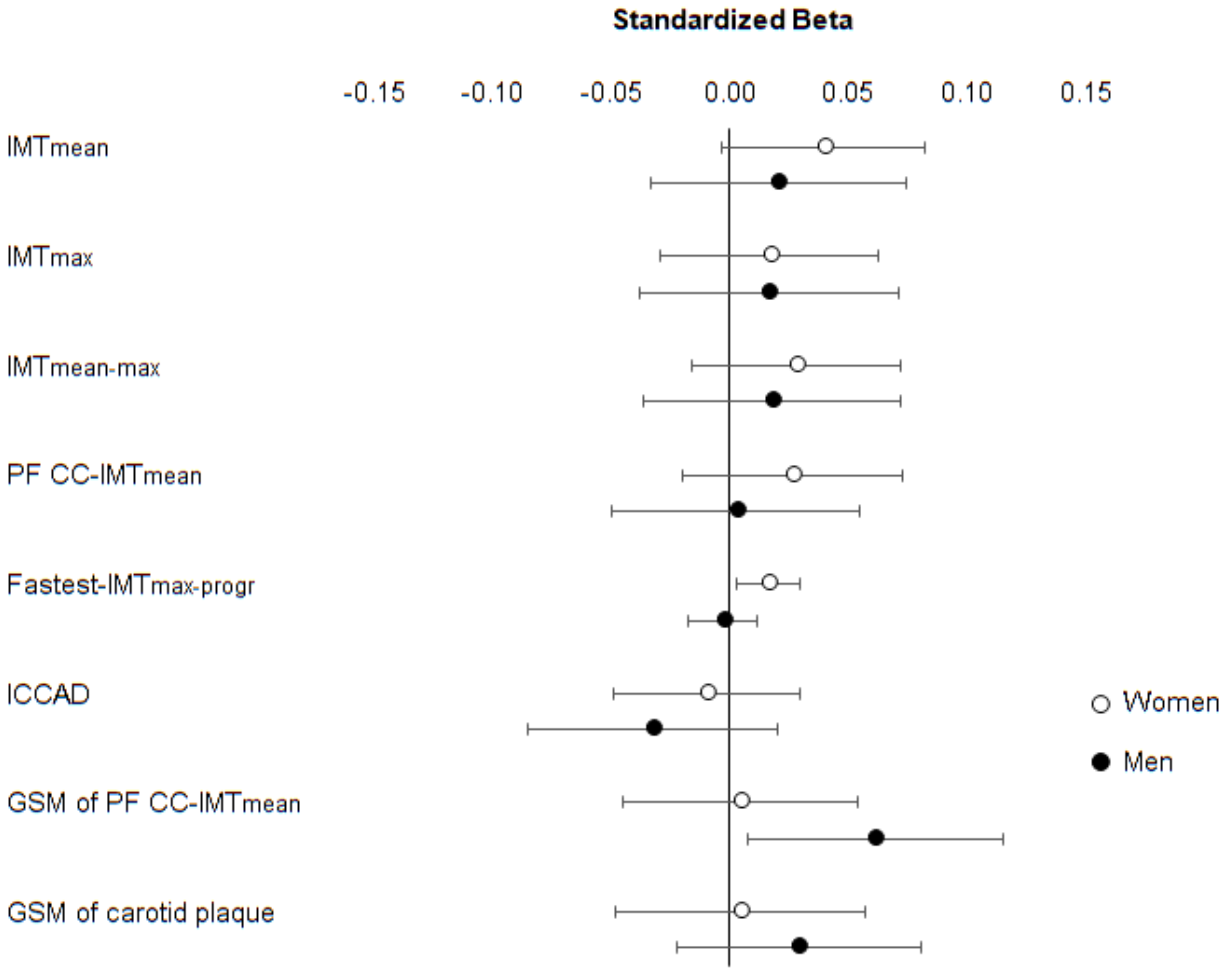
	1st quintile (n=734)	**2nd quintile (n=735)**	**3rd quintile (n=735)**	**4th quintile (n=735)**	**5th quintile (n=734)**	
PCSK9 (ng/mL)	183 (155; 203)	251 (238; 264)	300 (287; 312)	356 (340; 373)	449 (416; 503)	**Pvalue**
Latitude						
Perugia, n (%)	25 (3.4)	70 (9.5)	114 (15.5)	159 (21.6)	173 (23.6)	
Milan, n (%)	82 (11.2)	85 (11.6)	91 (12.4)	89 (12.1)	205 (27.9)	
Paris, n (%)	35 (4.8)	91 (12.4)	109 (14.8)	136 (18.5)	125 (17.0)	**<0.0001**
Groningen, n (%)	51 (6.9)	133 (18.1)	147 (20.0)	109 (14.8)	75 (10.2)	
Stockholm, n (%)	107 (14.6)	134 (18.2)	119 (16.2)	111 (15.1)	60 (8.2)	
Kuopio, n (%)	434 (59.1)	222 (30.2)	155 (21.1)	131 (17.8)	96 (13.1)	
Other anthropometric variables						
Height (m)	1.70 ± 0.10	1.69 ± 0.10	1.67 ± 0.10	1.66 ± 0.10	1.65 ± 0.10	**<0.0001**
Weight (Kg)	80.2 ± 14.6	78.5 ± 15.6	76.5 ± 15.0	74.9 ± 15.4	73.3 ± 14.4	**<0.0001**
Waist (mm)	96.1 ± 12.5	94.7 ± 12.7	94.4 ± 12.5	92.9 ± 12.7	92.1 ± 12.0	**<0.0001**
Hip (mm)	102.4 ± 8.4	103.0 ± 9.7	102.7 ± 10.2	102.4 ± 10.0	101.2 ± 9.4	**0.01**
Smoking habits						
Current smokers, n (%)	117 (15.9)	110 (15.0)	95 (12.9)	108 (14.7)	114 (15.5)	
Former smokers, n (%)	272 (37.1)	290 (39.5)	274 (37.3)	261 (35.5)	263 (35.8)	0.57
Never smokers, n (%)	345 (47.0)	335 (45.6)	366 (49.8)	366 (49.8)	357 (48.6)	

Pack-years	0.7 (0.0; 18.0)	1.5 (0.0; 18.5)	0.0 (0.0; 19.0)	0.0 (0.0; 18.0)	0.6 (0.0; 17.3)	0.57
Physical activity						
Low, n (%)	91 (12.4)	119 (16.2)	154 (21.1)	159 (21.6)	205 (28.0)	
Medium, n (%)	276 (37.6)	352 (48.0)	345 (47.3)	335 (45.6)	320 (43.7)	**<0.0001**
High, n (%)	367 (50.0)	262 (35.7)	231 (31.6)	241 (32.8)	207 (28.3)	
Family history of						
Coronary Heart Disease, n (%)	501 (71.1)	469 (67.5)	456 (64.0)	448 (62.4)	418 (58.6)	**<0.0001**
CerebroVascular Disease, n (%)	277 (37.7)	263 (35.8)	263 (35.8)	250 (34.0)	262 (35.7)	0.69
Peripheral Vascular Disease, n (%)	99 (13.5)	91 (12.4)	64 (8.7)	91 (12.4)	94 (12.8)	**0.045**
Hyperlipidemia, n (%)	237 (32.3)	279 (38.0)	311 (42.3)	335 (45.6)	401 (54.6)	**<0.0001**
Hypertension, n (%)	459 (62.5)	405 (55.1)	420 (57.1)	426 (58.0)	449 (61.2)	**0.03**
Diabetes, n (%)	301 (41.0)	260 (35.4)	253 (34.4)	270 (36.7)	221 (30.1)	**0.001**
Other biochemical variables						
Leucocytes (WBC) (x 10^9/L)	6.02 ± 1.6	6.16 ± 1.7	6.12 ± 1.6	6.19 ± 1.6	6.21 ± 2.3	0.053
Neutrophils (%)	56.85 ± 9.7	58.02 ± 9.1	57.21 ± 8.9	57.75 ± 9.0	55.98 ± 8.8	0.058
Lymphocytes (%)	33.57 ± 8.6	32.28 ± 8.1	33.00 ± 8.2	32.67 ± 8.2	33.95 ± 8.4	0.24
Monocytes (%)	6.82 ± 2.2	6.74 ± 2.2	6.67 ± 2.1	6.49 ± 2.1	7.00 ± 2.2	0.50
Eosinophils (%)	3.16 ± 2.2	3.03 ± 2.0	3.06 ± 2.1	2.94 ± 1.9	2.96 ± 1.9	0.13
Basophils (%)	0.4 (0.0; 0.7)	0.4 (0.0; 0.7)	0.5 (0.2; 0.8)	0.5 (0.2; 0.7)	0.5 (0.3; 0.8)	**0.02**

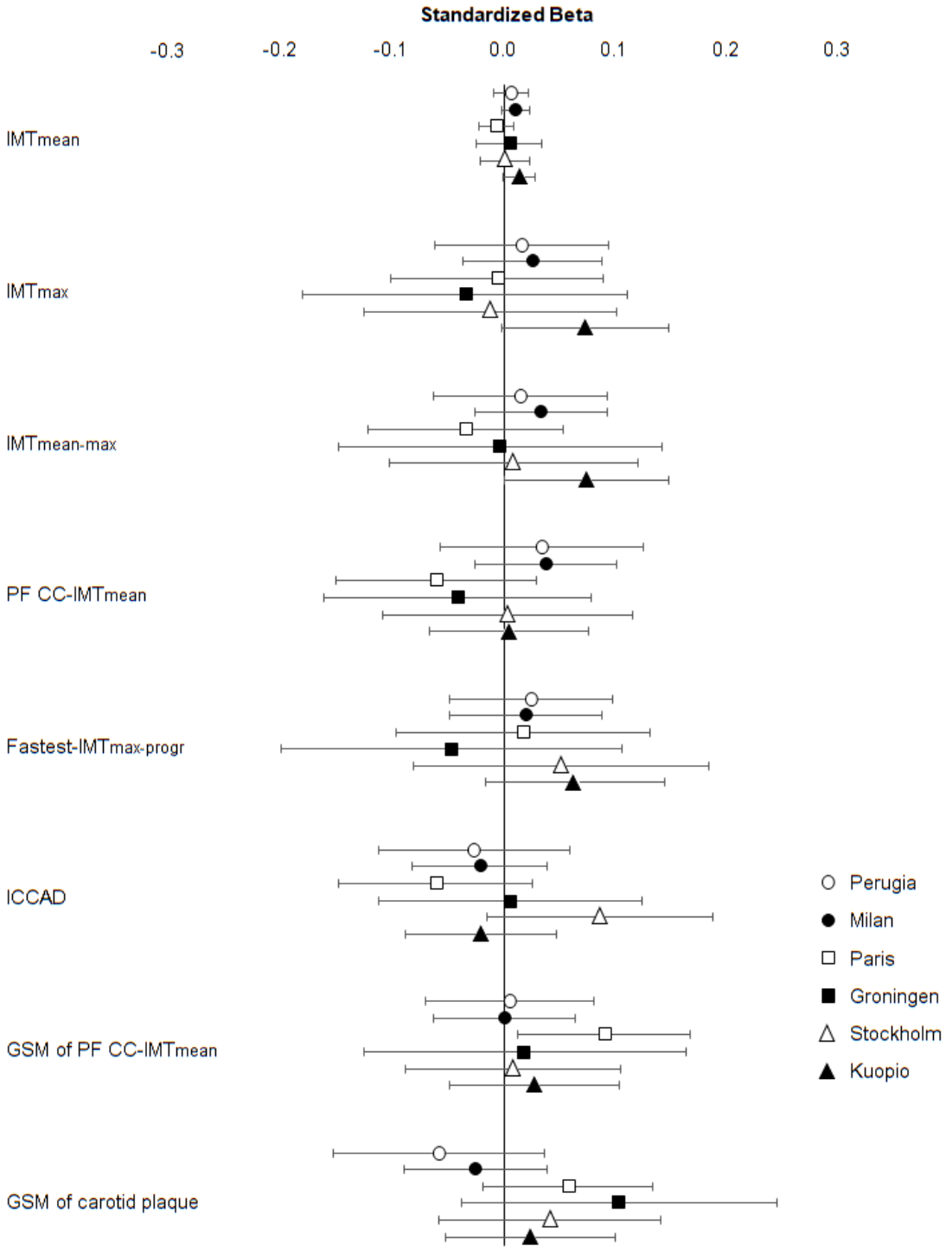
Erythrocytes (RBC) (x 10^{12}/L)	4.69 ± 0.4	4.66 ± 0.4	4.65 ± 0.4	4.65 ± 0.4	4.63 ± 0.4	**0.01**
Haemoglobin (g/dL)	14.45 ± 1.2	14.26 ± 1.2	14.14 ± 1.1	14.11 ± 1.1	13.99 ± 1.1	**<0.0001**
Haematocrit (%)	42.58 ± 3.4	42.19 ± 3.7	42.18 ± 4.4	42.12 ± 3.6	41.95 ± 3.2	**0.002**
MCV (fl)	90.98 ± 4.5	90.44 ± 4.5	90.51 ± 5.2	90.75 ± 5.1	90.73 ± 4.4	0.74
MCH (pg)	30.92 ± 1.8	30.64 ± 1.7	30.54 ± 1.7	30.48 ± 1.7	30.28 ± 1.7	**<0.0001**
MCHC (g/dL)	33.98 ± 0.9	33.88 ± 1.1	33.69 ± 1.1	33.54 ± 1.1	33.36 ± 1.0	**<0.0001**
Platelets (x 10^{9}/L)	225.5 ± 56.0	236.4 ± 56.9	237.6 ± 57.2	241.1 ± 58.1	246.5 ± 55.0	**<0.0001**
hs-CRP (mg/L)	1.7 (0.7; 3.4)	2.1 (0.8; 3.7)	1.9 (0.7; 3.8)	2.0 (0.8; 3.8)	1.8 (0.8; 3.3)	0.60
Food items						
Wine, n (%)	278 (7.59)	273 (7.45)	295 (8.05)	287 (7.83)	363 (9.91)	**<0.0001**
Beer, n (%)	177 (4.83)	140 (3.82)	124 (3.38)	110 (3)	93 (2.54)	**<0.0001**
Spirits, n (%)	158 (4.31)	124 (3.38)	96 (2.62)	90 (2.46)	72 (1.96)	**<0.0001**
Fruit, n (%)	679 (18.51)	679 (18.51)	699 (19.06)	695 (18.95)	698 (19.03)	0.06
Milk, n (%)	590 (16.12)	566 (15.46)	580 (15.84)	553 (15.11)	550 (15.02)	0.06
Coffee, n (%)	663 (18.1)	664 (18.13)	658 (17.96)	661 (18.05)	656 (17.91)	0.98
Tea, n (%)	326 (8.9)	345 (9.42)	271 (7.4)	267 (7.29)	236 (6.44)	**<0.0001**
Meat, n (%)	719 (19.65)	716 (19.57)	722 (19.73)	721 (19.7)	720 (19.68)	0.50
Fish, n (%)	669 (18.28)	655 (17.9)	658 (17.98)	674 (18.42)	674 (18.42)	0.19
Eggs, n (%)	619 (16.92)	577 (15.77)	553 (15.11)	571 (15.61)	515 (14.07)	**<0.0001**

Pharmacological therapies						
Statins, n (%)	169 (23)	234 (32)	279 (38)	369 (50)	423 (58)	**<0.0001**
Fibrates, n (%)	19 (2.6)	25 (3.4)	50 (6.8)	53 (7.2)	136 (19)	**<0.0001**
Fish oil, n (%)	24 (3.3)	20 (2.7)	20 (2.7)	21 (2.9)	40 (5.4)	**0.02**
Other lipid-lowering drug, n (%)	3 (0.4)	9 (1.2)	2 (0.3)	4 (0.5)	5 (0.7)	0.23
Beta-blockers, n (%)	194 (26)	165 (22)	183 (25)	168 (23)	162 (22)	0.23
Calcium antagonists, n (%)	122 (17)	111 (15)	136 (19)	109 (15)	119 (16)	0.33
ACE inhibitors, n (%)	160 (22)	128 (17)	131 (18)	154 (21)	145 (20)	0.15
ARB, n (%)	134 (18)	98 (13)	110 (15)	110 (15)	108 (15)	0.11
Diuretics, n (%)	160 (22)	159 (22)	168 (23)	181 (25)	184 (25)	0.39
Anti-platelet agents, n (%)	150 (20)	106 (14)	114 (16)	110 (15)	134 (18)	**0.01**
Insulin, n (%)	40 (5.4)	33 (4.5)	26 (3.5)	17 (2.3)	24 (3.3)	**0.02**
Estrogen supplement, n (%)	52 (7.1)	47 (6.4)	46 (6.3)	52 (7.1)	29 (4.0)	0.08

PCSK9, proprotein convertase subtilisin/kexin type 9; WBC, white blood cells; RBC, red blood cells; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; hs-CRP, high-sensitivity C-reactive protein; ACE, angiotensin-converting enzyme; ARB, angiotensin-2 receptor blockers. Data are n (percentage) or mean ± SD, except for PCSK9, pack-years, basophils, and hs-CRP, which are summarized as median (1^{st} and 3^{rd} quartiles). Group differences were assessed by Student's t-test for the numerical variables, by $\chi 2$-test or Fisher for the categorical ones, and by Kruskal-Wallis for pack-years, basophils, and hs-CRP. Estrogen supplementation was calculated only in women. The P_{values} refer to the trends across PCSK9 quintiles. $P_{values} < 0.05$ were considered statistically significant.

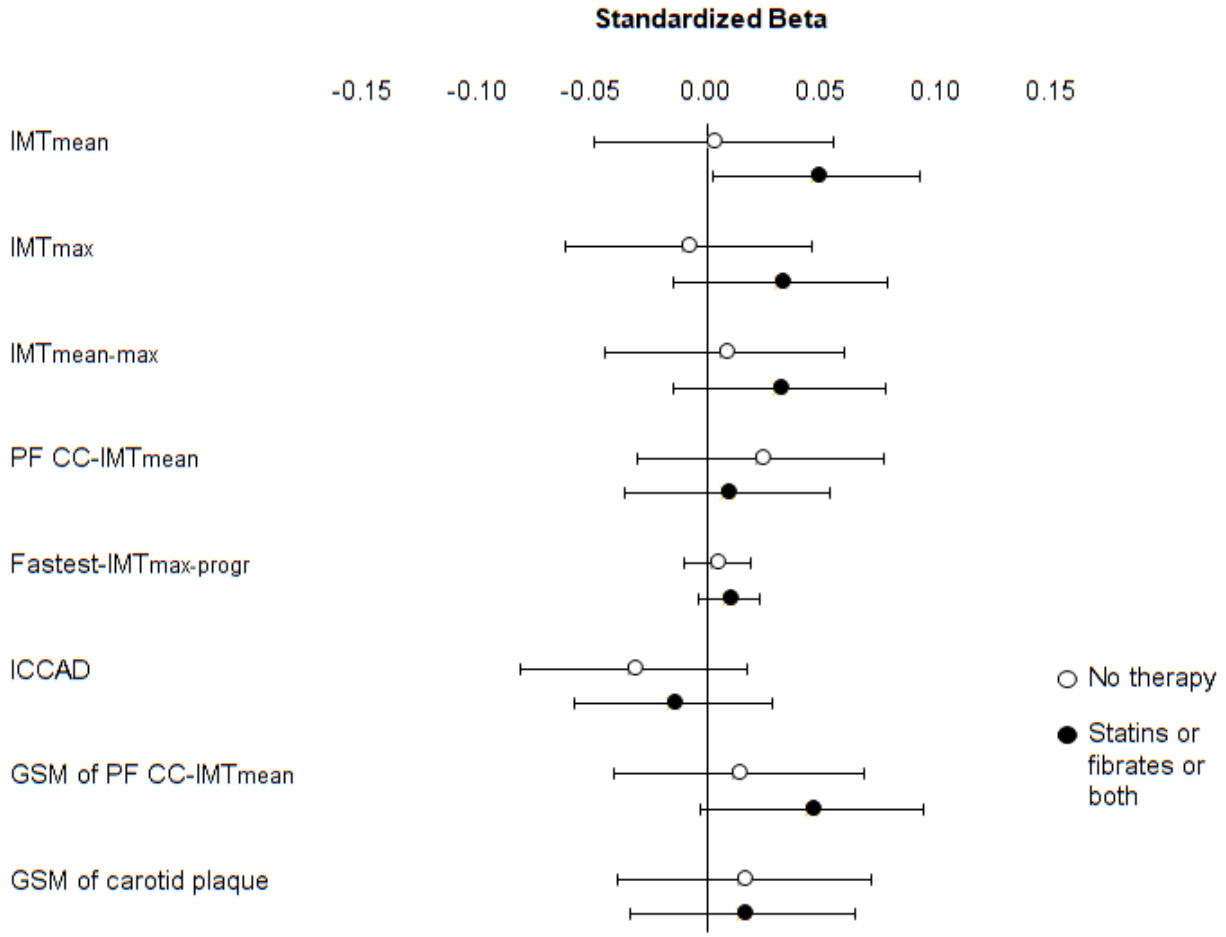


Supplementary Figure S1 Multivariable relationships between plasma PCSK9 levels and carotid IMT phenotypes, ICCAD and echolucency (Grey Scale Median; GSM) after stratification by sex. PCSK9, proprotein convertase subtilisin/kexin type 9; IMT_{mean}, average of mean of intima-media thickness in left and right carotid arteries; IMT_{max}, highest value of maximum of intima-media thickness in left and right carotid arteries; $IMT_{mean-max}$, mean of maximum intima-media thickness in left and right carotid arteries; PF CC-IMT_{mean}, IMT_{mean} measured in the 2nd cm of common carotids in plaque-free areas; Fastest-$IMT_{max-progr}$, the 15-month progression of IMT_{max} detected in the whole carotid tree regardless of location; ICCAD, average of the inter-adventitia diameter measurements carried out in plaque-free areas of the 2nd cm of left and right common carotid arteries; GSM, grey scale median of pixels distribution of the region of interest (cIMT or plaque).



Supplementary Figure S2 Multivariable relationships between plasma PCSK9 levels and carotid IMT phenotypes, ICCAD and echolucency (Grey Scale Median; GSM) after stratification by latitude.

PCSK9, proprotein convertase subtilisin/kexin type 9; IMT_{mean}, average of mean of intima-media thickness in left and right carotid arteries; IMT_{max}, highest value of maximum of intima-media thickness in left and right carotid arteries; $IMT_{mean-max}$, mean of maximum intima-media thickness in left and right carotid arteries; PF CC-IMT_{mean}, IMT_{mean} measured in the 2nd cm of common carotids in plaque-free areas; Fastest-$IMT_{max-progr}$, the 15-month progression of IMT_{max} detected in the whole carotid tree regardless of location; ICCAD, average of the inter-adventitia diameter measurements carried out in plaque-free areas of the 2nd cm of left and right common carotid arteries; GSM, grey scale median of pixels distribution of the region of interest (cIMT or plaque).



Supplementary Figure S3 Multivariable relationships between plasma PCSK9 levels and carotid IMT phenotypes, ICCAD and echolucency (Grey Scale Median; GSM) after stratification by pharmacological treatment.

PCSK9, proprotein convertase subtilisin/kexin type 9; IMT_{mean}, average of mean of intima-media thickness in left and right carotid arteries; IMT_{max}, highest value of maximum of intima-media thickness in left and right carotid arteries; $IMT_{mean-max}$, mean of maximum intima-media thickness in left and right carotid arteries; PF CC-IMT_{mean}, IMT_{mean} measured in the 2nd cm of common carotids in plaque-free areas; Fastest-$IMT_{max-progr}$, the 15-month progression of IMT_{max} detected in the whole carotid tree regardless of location; ICCAD, average of the inter-adventitia diameter measurements carried out in plaque-free areas of the 2nd cm of left and right common carotid arteries; GSM, grey scale median of pixels distribution of the region of interest (cIMT or plaque).