Supplementary File

Annex 1

Full list of eligibility, inclusion, exclusion, and exit criteria

Eligibility criteria:

A. Clinical history and lifestyle data records available at one-time point.

B. At least one previous CCTA examination performed for suspected Coronary heart disease (CHD) and of good quality to allow for: a) non-invasive FFR-CT assessment b) quantitative (automated) 17 segments (American Heart Association (AHA)) analysis and measurement with ≤10% error of minimum lumen diameter (MLA) (mm^2), lumen area stenosis (%), mean plaque burden (mm3), plaque burden at MLA (%), and remodeling index, c) plaque phenotype assessment: Hausfield units (HU) based classification in calcified, non-calcified and mixed, napkin-ring sign, coronary artery calcium (CAC) score.

C. Previous blood and plasma sample available for retrospective analysis.

Inclusion criteria:

1) Male and female subjects.
2) Aged 45–82 years.
3) Caucasian population.
4) Submitted to CCTA for suspected CHD between 2009 and 2012 (in the context of EVINCI and ARTreat FPVII studies) at the hospitals reported in "SMARTool Clinical Center" document and satisfying the eligibility criteria reported above.
5) Submitted to clinical follow-up in the last 6 months with stable clinical conditions and documented CHD or persistent intermediate/high probability of CHD.
6) Signed informed consents (clinical and genetic).

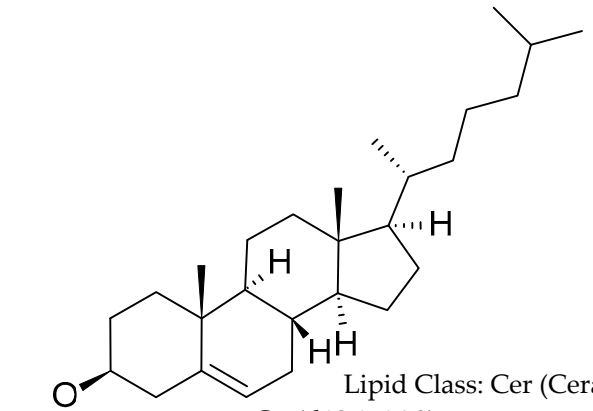
Exclusion criteria:

1) Multi-vessel severe disease (three vessels and/or left main (LM) disease with >90% stenosis).
2) Severe coronary calcification (CAC score > 600).
3) Having undergone surgical procedures related to heart diseases (valve replacement, Cardiac resynchronization therapy (CRT) or CRT-D treatment, any surgery of the heart or arteries).
4) Documented Major adverse cardiovascular events (MACE) at history (myocardial infarction, severe heart failure, recurrent angina) in the last 6 months with/without revascularization.
5) Documented severe peripheral vascular disease (carotid, femoral).
6) Surgery of carotid and/or peripheral arteries or cerebral ischemic attack.
7) History/surgery of abdominal aortic aneurysm (AAA).
8) Severe heart failure (New York Heart Association (NYHA) Class III–IV).
9) LV dysfunction (left ventricle Ejection fraction (EF) < 40%).
10) Atrial fibrillation.
11) Lack of written informed consent (clinical consent and/or genetic consent).
12) Pregnancy (evaluated by urine test) and breastfeeding.
13) Active cancer.
14) Asthma.
15) Cardiomyopathy or congenital heart disease.
16) Significant valvular disease (hemodynamically significant valvular stenosis or insufficiency by echo-Doppler).
17) Renal dysfunction (creatinine > 1.3 mg/dL).

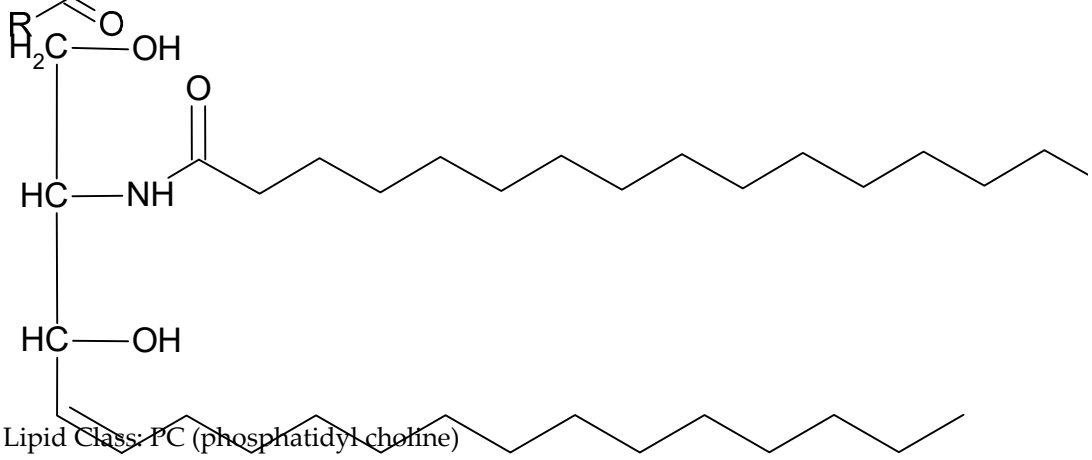
18) Chronic kidney disease (Estimated Glomerular Filtration Rate (eGFR) < 30 mL/min/1.73 m^2).
19) Hepatic failure (at least three of the following: albumin < 3.5 g/dL; prolonged prothrombin time (PT); jaundice; ascites).
20) Waldenström's disease.
21) Multiple myeloma.
22) Autoimmune/acute inflammatory disease.
23) Previous severe adverse reaction to iodine contrast agent.
24) Positivity at blood tests for HIV, Hepatitis B and C.

Exit Criteria:
A) Informed consent retired by the patient (genetic or clinical).
B) Adverse events to contrast medium during C.

Annex 2. Lipids structures
Lipid Class: CE (cholesteryl esters)
CE_R_I
R: total number of carbon atoms in the acyl chains.
I: total number of double bonds in the acyl chains.

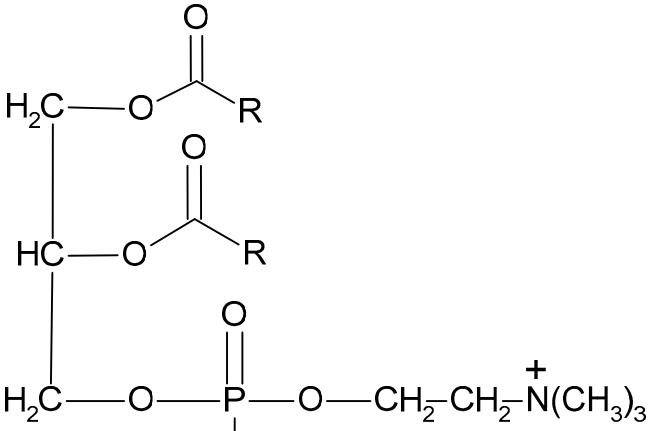


Lipid Class: Cer (Ceramides)
Cer(d18:1, 16:0)



Lipid Class: PC (phosphatidyl choline)
PC_R_I
R: total number of carbon atoms in the acyl chains.

I: total number of double bonds in the acyl chains.

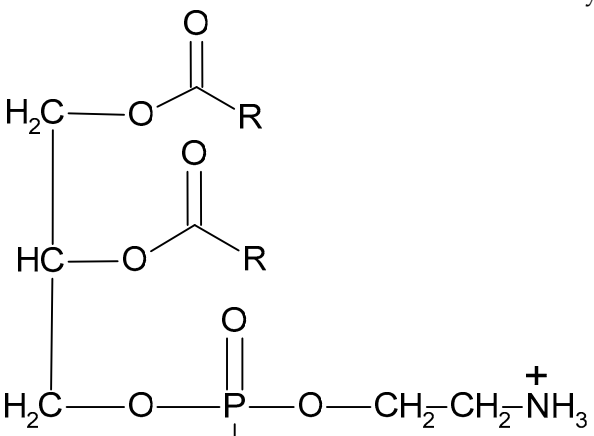


Lipid Class: PE (Phosphatidyl ethanolamine)

PE_R_I

R: total number of carbon atoms in the acyl chains.

I: total number of double bonds in the acyl chains.

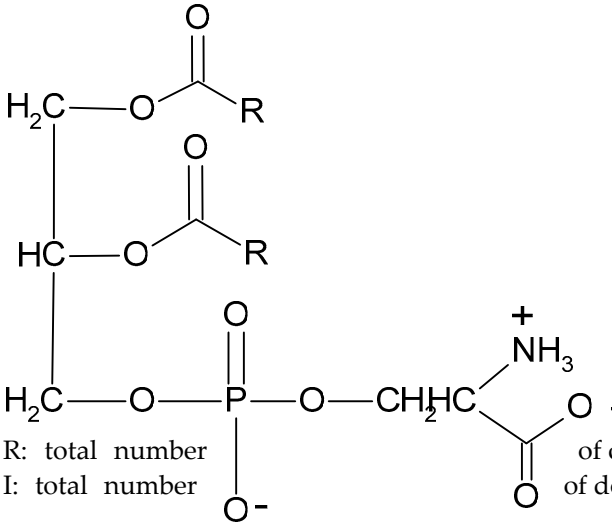


Lipid Class: PS (Phosphatidyl serine)

PS_R_I

R: total number of carbon atoms in the acyl chains.

I: total number of double bonds in the acyl chains.

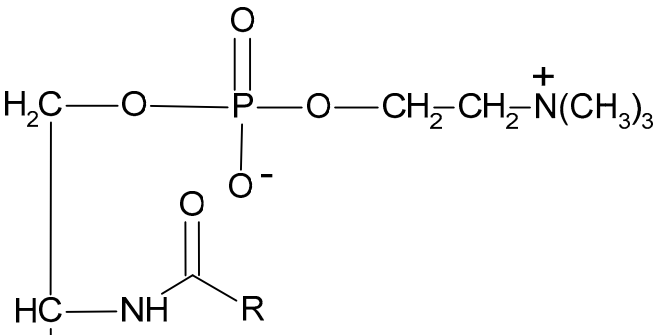


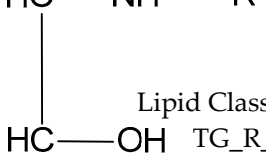
Lipid Class: SM (Sphingomyelin)

SM_R_I

R: total number of carbon atoms in the acyl chains.

I: total number of double bonds in the acyl chains.





Lipid Class: TG (Triacyl glycerol)

TG_R_I

R: total number of carbon atoms in the acyl chains.

I: total number of double bonds in the acyl chains.

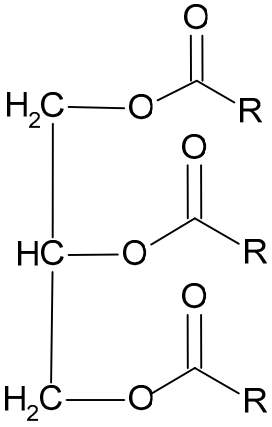


Table S1. Univariate and multivariate linear regression analysis for association of relevant baseline clinical/biohumoral and CCTA variables with single components of plaque progression and their combination.

Dependent Variable	Effect	Univariate Linear Regression Analysis		Multivariate Linear Regression Analysis	
		Estimated Regression Coefficient (95% CI)	*p*-Value	Estimated Regression Coefficient (95% CI)	*p*-Value
Plaque area increase by 20%	Age	0.007 (0.002 to 0.013)	0.0140	0.012 (0.005 to 0.020)	0.0020
	Cer_d18_1_18_0	−0.997 (−2.174 to 0.181)	0.0964	−0.001 (−0.004 to 0.003)	0.7714
	CE_18_3	0.002 (−0.000 to 0.004)	0.0769	0.002 (−0.006 to 0.011)	0.5850
	CE_20_3	0.006 (−0.000 to 0.011)	0.0513	0.001 (−0.002 to 0.003)	0.6043
	SM_42_1	0.003 (0.000 to 0.006)	0.0265	0.003 (−0.001 to 0.006)	0.1017
	Baseline plaque burden	−0.008 (−0.009 to −0.007)	< 0.0001	−0.008 (−0.010 to −0.007)	< 0.0001
	Minimum ESS	0.026 (0.008 to 0.044)	0.0046	0.079 (0.056 to 0.102)	<.0001
	SmartFFR	0.315 (0.053 to 0.576)	0.0187	0.035 (−0.308 to 0.378)	0.8405
Plaque burden increase by 20%	Age	0.007 (0.001 to 0.013)	0.0155	0.012 (0.004 to 0.019)	0.0020
	CE_18_3	0.002 (−0.000 to 0.004)	0.0968	−0.001 (−0.004 to 0.003)	0.6732
	CE_20_3	0.005 (−0.000 to 0.011)	0.0559	0.003 (−0.006 to 0.011)	0.5504
	TG_54_2__TG_18_1_18_	0.002 (−0.000 to 0.004)	0.0950	0.001 (−0.001 to 0.003)	0.4964
	SM_42_1	0.003 (0.000 to 0.006)	0.0280	0.003 (−0.001 to 0.006)	0.0980
	Baseline plaque burden	−0.009 (−0.010 to −0.007)	< 0.0001	−0.008 (−0.009 to −0.006)	< 0.0001
	Minimum ESS	0.016 (−0.002 to 0.034)	0.0750	0.064 (0.041 to 0.086)	< 0.0001
	SmartFFR	0.321 (0.068 to 0.574)	0.0129	0.042 (−0.293 to 0.377)	0.8053

Table S2. Univariate and multivariate binary logistic regression analysis for the plaque progression and lumen area decrease.

Dependent Variable	Effect	Univariate Logistic Regression Analysis		Multivariate Logistic Regression Analysis	
		Odds Ratio	*p*-Value	Odds Ratio	*p*-Value
Plaque area increase by 20%	Age	1.035 (1.007 to 1.064)	0.0141	1.071 (1.035 to 1.108)	<.0001
	Cer_d18_1_16_0	1.159 (1.056 to 1.273)	0.0020	1.005 (0.873 to 1.158)	0.9438
	CE_18_3	1.008 (1.000 to 1.016)	0.0372	1.003 (0.986 to 1.020)	0.7170
	CE_20_3	1.025 (1.002 to 1.048)	0.0320	1.006 (0.969 to 1.045)	0.7452
	PC_36_0	1.038 (0.996 to 1.082)	0.0778	1.008 (0.943 to 1.076)	0.8210
	PC_36_2e__PC_36_1p	1.080 (0.986 to 1.183)	0.0982	1.149 (0.931 to 1.419)	0.1955
	TG_54_2__TG_18_1_18_1_18_0	1.008 (1.000 to 1.015)	0.0395	1.007 (0.998 to 1.017)	0.1417
	SM_38_1	1.018 (1.001 to 1.036)	0.0373	1.012 (0.956 to 1.071)	0.6738
	SM_40_3	1.099 (0.983 to 1.229)	0.0982	1.147 (0.809 to 1.626)	0.4420
	SM_40_2	1.012 (0.998 to 1.026)	0.0926	1.004 (0.947 to 1.064)	0.8928
	SM_41_1	1.013 (1.000 to 1.026)	0.0478	1.014 (0.980 to 1.050)	0.4292
	SM_42_4	1.124 (1.018 to 1.242)	0.0211	1.223 (0.927 to 1.613)	0.1536
	SM_42_1	1.014 (1.003 to 1.025)	0.0161	1.025 (1.001 to 1.051)	0.0451
	Baseline plaque burden	1.049 (1.039 to 1.060)	<0.0001	1.052 (1.037 to 1.066)	<0.0001
	SmartFFR	4.828 (1.712 to 13.617)	0.0029	2.092 (0.445 to 9.839)	0.3503
Plaque burden increase by 20%	Age	1.035 (1.006 to 1.065)	0.0175	1.072 (1.033 to 1.112)	0.0002
	Cer_d18_1_16_0	1.129 (1.033 to 1.234)	0.0076	1.046 (0.923 to 1.186)	0.4787
	CE_18_3	1.008 (1.000 to 1.016)	0.0478	1.005 (0.988 to 1.022)	0.5675
	CE_20_3	1.025 (1.002 to 1.048)	0.0325	1.012 (0.974 to 1.052)	0.5387
	PC_36_0	1.041 (0.999 to 1.085)	0.0563	1.007 (0.958 to 1.058)	0.7755
	TG_54_2__TG_18_1_18_1_18_0	1.008 (1.001 to 1.016)	0.0275	1.006 (0.997 to 1.015)	0.2157
	SM_40_1	1.010 (0.998 to 1.022)	0.0905	1.004 (0.967 to 1.043)	0.8339
	SM_41_1	1.012 (0.999 to 1.026)	0.0679	1.018 (0.981 to 1.056)	0.3544

Dependent Variable	Effect	Univariate Logistic Regression Analysis		Multivariate Logistic Regression Analysis	
		Odds Ratio	*p*-Value	Odds Ratio	*p*-Value
	SM_42_4	1.104 (1.002 to 1.217)	0.0449	1.029 (0.879 to 1.204)	0.7239
	SM_42_1	1.013 (1.002 to 1.025)	0.0227	1.021 (0.991 to 1.053)	0.1752
	Baseline plaque burden	1.051 (1.041 to 1.061)	< 0.0001	1.049 (1.035 to 1.063)	< 0.0001
	SmartFFR	5.456 (1.852 to 16.072)	0.0021	1.736 (0.393 to 7.665)	0.4667