

Detailed Methods - Lipidomic analysis reveals metabolism alteration associated with subclinical carotid atherosclerosis in type 2 diabetes

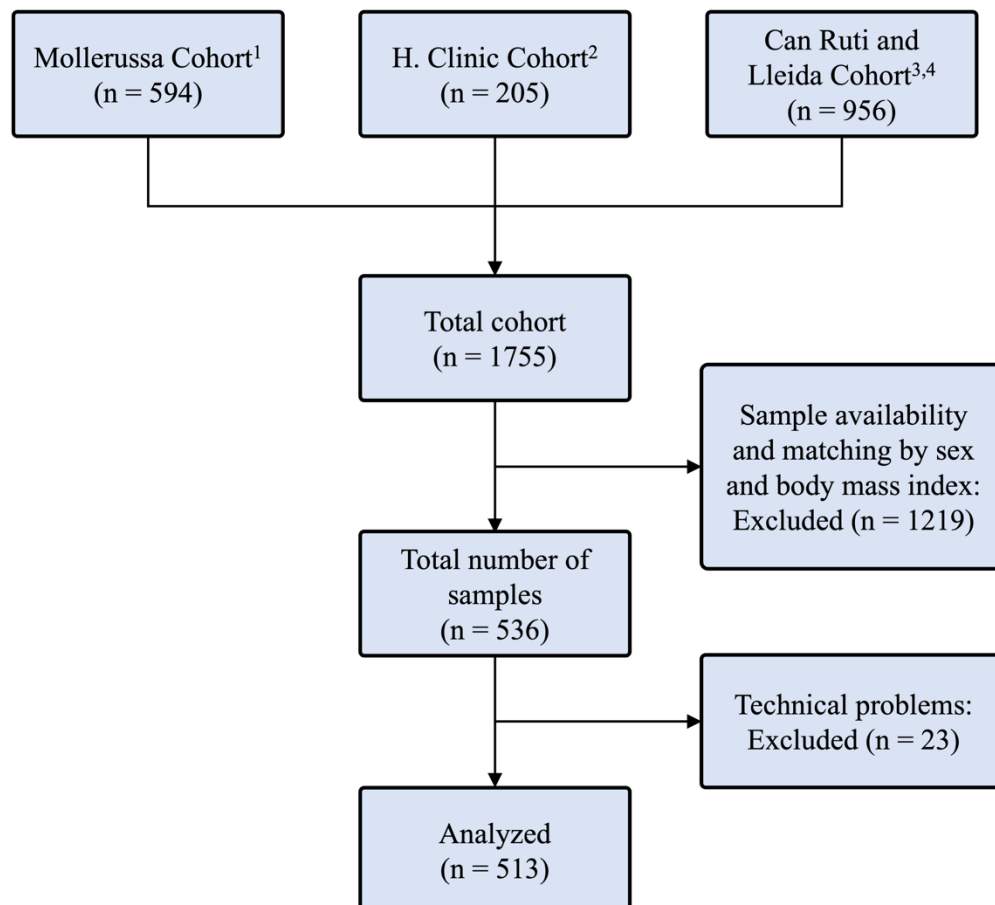


Figure S1. Flow chart of the participants recruitment.

- (1) Vilanova MB, Falguera M, Marsal JR, Rubinat E, Alcubierre N, Castelblanco E, Granado-Casas M, Miró N, Molló À, Mata-Cases M, Franch-Nadal J, Mauricio Di. Prevalence, clinical features and risk assessment of pre-diabetes in Spain: The prospective Mollerussa cohort study. *BMJ Open* 2017;7.
- (2) Catalan M, Herreras Z, Pinyol M, Sala-Vila A, Amor AJ, Groot E de, Gilbert R, Ros E, Ortega E. Prevalence by sex of preclinical carotid atherosclerosis in newly diagnosed type 2 diabetes. *Nutrition, Metabolism and Cardiovascular Diseases* 2015;25:742–748.
- (3) Carbonell M, Castelblanco E, Valldeperas X, Betriu À, Traveset A, Granado-Casas M, Hernández M, Vázquez F, Martín M, Rubinat E, Lecube A, Franch-Nadal J, Fernández E, Puig-Domingo M, Avogaro A, Alonso N, Mauricio D. Diabetic retinopathy is associated with the presence and burden of subclinical carotid atherosclerosis in type 1 diabetes. *Cardiovasc Diabetol* 2018;17:1–10.
- (4) Alonso N, Traveset A, Rubinat E, Ortega E, Alcubierre N, Sanahuja J, Hernández M, Betriu A, Jurjo C, Fernández E, Mauricio D. Type 2 diabetes-associated carotid plaque burden is increased in patients with retinopathy compared to those without retinopathy. *Cardiovasc Diabetol* 2015;14:1–9.

Supplementary Table S1. Description of the analyses implemented.

Analysis	Subjects	Formula
SCA presence	T1D	$f_i \sim \text{Sex} + \text{age} + \text{HT} + \text{DLP} + \text{BMI} + \text{waist} + \text{SCA presence} + \text{HbA1c} + \text{glucose} + \text{sample origin} + \text{smoking} + \text{diabetes duration} + \text{Antiplatelet} + \text{ALT}$
SCA presence	T2D	$f_i \sim \text{Sex} + \text{age} + \text{HT} + \text{DLP} + \text{BMI} + \text{waist} + \text{SCA presence} + \text{HbA1c} + \text{glucose} + \text{sample origin} + \text{smoking} + \text{diabetes duration}$
SCA presence	CT	$f_i \sim \text{Sex} + \text{age} + \text{HT} + \text{DLP} + \text{BMI} + \text{waist} + \text{SCA presence} + \text{HbA1c} + \text{glucose} + \text{sample origin} + \text{smoking}$
SCA burden	T1D	$f_i \sim \text{Sex} + \text{age} + \text{HT} + \text{DLP} + \text{BMI} + \text{waist} + \text{SCA burden} + \text{HbA1c} + \text{glucose} + \text{sample origin} + \text{smoking} + \text{diabetes duration} + \text{Antiplatelet} + \text{ALT}$
SCA burden	T2D	$f_i \sim \text{Sex} + \text{age} + \text{HT} + \text{DLP} + \text{BMI} + \text{waist} + \text{SCA burden} + \text{HbA1c} + \text{glucose} + \text{sample origin} + \text{smoking} + \text{diabetes duration}$
SCA burden	CT	$f_i \sim \text{Sex} + \text{age} + \text{HT} + \text{DLP} + \text{BMI} + \text{waist} + \text{SCA burden} + \text{HbA1c} + \text{glucose} + \text{sample origin} + \text{smoking}$
SCA presence sub-group	T1D and T2D	$f_i \sim \text{Sex} + \text{age} + \text{HT} + \text{DLP} + \text{BMI} + \text{waist} + \text{SCA presence} + \text{HbA1c} + \text{glucose} + \text{sample origin} + \text{smoking} + \text{DM} + \text{diabetes duration} + \text{Antiplatelet} + \text{ALT} + \text{SCA presence: DM} + \text{SCA presence: subgroup}$
SCA presence sub-group	CT	$f_i \sim \text{Sex} + \text{age} + \text{HT} + \text{DLP} + \text{BMI} + \text{waist} + \text{SCA presence} + \text{HbA1c} + \text{glucose} + \text{sample origin} + \text{smoking} + \text{SCA presence: subgroup}$
SCA burden sub-group	T1D and T2D	$f_i \sim \text{Sex} + \text{age} + \text{HT} + \text{DLP} + \text{BMI} + \text{waist} + \text{SCA burden} + \text{HbA1c} + \text{glucose} + \text{sample origin} + \text{smoking} + \text{DM} + \text{diabetes duration} + \text{Antiplatelet} + \text{ALT} + \text{SCA burden: DM} + \text{SCA burden: subgroup}$
SCA burden sub-group	CT	$f_i \sim \text{Sex} + \text{age} + \text{HT} + \text{DLP} + \text{BMI} + \text{waist} + \text{SCA burden} + \text{HbA1c} + \text{glucose} + \text{sample origin} + \text{smoking} + \text{SCA burden: subgroup}$

HT, hypertension; DLP, dyslipidemia; BMI, body mass index; SCA, subclinical carotid atherosclerosis; HbA1c, glycated hemoglobin, DM, diabetes mellitus; ALT, Alanine aminotransferase levels. Subgroup represents each risk variable used in the contrast analysis, i.e., smoking habit, hypertension, dyslipidaemia, antiplatelet use and sex.

Contrast analysis: For smoking-specific differences, multiple linear regression models were applied to each metabolite feature adding an interaction term between SCA presence or burden and smoking habit (No smoker = 0 and Former/current smoker = 1). Using this configuration, the p-value and regressor associated to presence/burden of SCA were assigned to non-smokers ($\beta_{\text{non-smoker}}$). The effect of SCA presence/burden in smokers (β_{smoker}) was computed by summing the regressor of the variable SCA presence/burden and the interaction between SCA presence/burden and smoking habit. Then, the t-value was computed dividing the effect (β_{smoker}) by its standard error and the p-value associated to SCA presence/burden in smokers was computed. Again, False Discovery Rate (FDR) was controlled using the R package qvalue. The same procedure was repeated to determine the rest of sub-group-specific differences associated to SCA presence and burden. In Supplementary Table S1, a detailed description of the analyses performed is provided.

Supplemental Tables and supporting information - Lipidomic

analysis reveals metabolism alteration associated with subclinical

carotid atherosclerosis in type 2 diabetes

Supplementary Table S2. Summary of lipid species identified using LipidSearch in positive acquisition mode. Values represent the mean (standard deviation) of their relative abundance per lipid class for all the population, subjects without diabetes, subjects with type 1 diabetes and type 2 diabetes.

Lipid class	Lipids	Total	Control	T1D	T2D
AcCa	23	15.6504 (1.1630)	15.6200 (1.1439)	15.6368 (1.1788)	15.7043 (1.1715)
Cer	31	15.0622 (1.3304)	15.0561 (1.3366)	14.9234 (1.3758)	15.2033 (1.2621)
CerG3GNAc 1	1	14.7634 (0.2902)	14.7544 (0.2853)	14.9134 (0.2467)	14.6294 (0.2677)
ChE	13	17.2875 (1.8992)	17.2867 (1.9005)	17.2865 (1.9389)	17.2893 (1.8603)
Co	1	15.0964 (0.3995)	15.2322 (0.3665)	15.0790 (0.3633)	14.9170 (0.4081)
DG	15	15.6459 (1.5055)	15.6382 (1.4852)	15.4054 (1.5153)	15.8709 (1.4898)
GM3	4	14.7840 (0.5519)	14.7739 (0.5308)	14.7811 (0.6052)	14.8003 (0.5248)
Hex1Cer	8	14.9589 (1.0657)	14.9742 (1.0615)	15.0562 (1.0508)	14.8430 (1.0759)
Hex2Cer	3	16.0605 (0.5960)	16.0809 (0.5942)	16.1608 (0.5640)	15.9355 (0.6081)
Hex3Cer	1	15.1726 (0.3157)	15.1409 (0.3023)	15.3604 (0.2707)	15.0319 (0.2861)
LPC	55	16.4042 (2.0078)	16.4230 (1.9994)	16.5488 (2.0144)	16.2382 (2.0006)
LPE	8	14.6192 (1.0677)	14.5823 (1.0726)	14.6978 (1.0648)	14.5920 (1.0608)
MePC	43	17.1592 (2.3907)	17.1958 (2.3694)	17.1280 (2.3820)	17.1404 (2.4271)
PC	120	17.0603 (2.0103)	17.0730 (1.9921)	17.0643 (2.0207)	17.0394 (2.0244)
PE	28	16.1572 (1.3546)	16.1098 (1.3531)	16.2143 (1.3800)	16.1646 (1.3296)
PI	17	15.4816 (1.2519)	15.5027 (1.2364)	15.4195 (1.2697)	15.5137 (1.2535)
SM	70	17.1457 (2.0819)	17.1662 (2.0742)	17.1215 (2.0879)	17.1416 (2.0861)
ST	1	14.2100 (0.3108)	14.1445 (0.2882)	14.3595 (0.2769)	14.1494 (0.3222)
SiE	1	14.1977 (0.7080)	14.1503 (0.5719)	14.6668 (0.6394)	13.8034 (0.6733)
TG	66	17.0302 (2.0877)	17.0272 (2.0905)	16.9762 (2.0937)	17.0865 (2.0768)
ZyE	1	14.3213 (0.2939)	14.3737 (0.2997)	14.1423 (0.2126)	14.4179 (0.2795)

Supplementary Table S3. Summary of lipid species identified using LipidSearch in negative acquisition mode. Values represent the mean (standard deviation) of their relative abundance per lipid class for all the population, subjects without diabetes, subjects with type 1 diabetes and type 2 diabetes.

Lipid class	Lipids	Total	Control	T1D	T2D
Cer	14	14.9484 (1.1287)	14.9742 (1.1271)	14.8611 (1.1450)	15.0034 (1.1088)
CerP	11	14.4098 (1.1189)	14.4142 (1.1084)	14.4728 (1.1210)	14.3354 (1.1279)
GM3	3	13.9847 (0.4036)	13.9595 (0.3893)	14.0683 (0.3819)	13.9318 (0.4322)
Hex1Cer	11	14.2450 (0.8336)	14.2664 (0.8331)	14.2788 (0.8640)	14.1785 (0.7974)
Hex2Cer	1	15.2328 (0.2901)	15.2497 (0.2596)	15.3612 (0.2477)	15.0719 (0.2992)
Hex3Cer	1	13.7767 (0.2342)	13.7523 (0.2119)	13.9042 (0.2000)	13.6761 (0.2394)
LPA	1	14.8373 (1.1603)	15.0011 (0.9908)	15.4027 (0.5886)	13.9967 (1.3659)
LPC	11	16.3196 (1.4546)	16.3332 (1.4438)	16.4163 (1.5133)	16.1970 (1.3975)
LPE	6	15.1843 (0.4471)	15.1290 (0.4131)	15.3292 (0.5052)	15.1089 (0.3883)
LdMePE	2	13.3037 (0.6468)	13.3069 (0.6496)	13.4972 (0.5201)	13.0931 (0.6993)
PA	1	13.7444 (0.1882)	13.7382 (0.2003)	13.7715 (0.1682)	13.7245 (0.1886)
PC	50	15.6312 (1.5933)	15.6408 (1.5841)	15.5972 (1.5899)	15.6537 (1.6096)
PE	26	15.2201 (0.9825)	15.1634 (0.9784)	15.2282 (0.9993)	15.2924 (0.9652)
PI	14	15.3496 (1.1831)	15.3602 (1.1730)	15.2985 (1.1911)	15.3896 (1.1875)
SM	1	14.2222 (0.3059)	14.2496 (0.2791)	14.1523 (0.3127)	14.2573 (0.3244)

Supplementary Table S4. Number of significant LC-MS features in each analysis and ionization mode and for each group of subjects. Counts of significant features are shown in (positive acquisition mode, negative acquisition mode).

Risk-group	SCA presence			SCA burden		
	T1D	T2D	Controls	T1D	T2D	Controls
General	(0, 0)	(26, 0)	(0, 0)	(33, 35)	(0, 0)	(2, 0)
Non-smokers	(0, 0)	(0, 0)	(0, 0)	(0, 1)	(0, 0)	(0, 0)
Smokers	(1, 0)	(66, 1)	(0, 0)	(6, 14)	(40, 109)	(3, 0)
HT No	(0, 0)	(0, 0)	(0, 0)	(2, 6)	(0, 0)	(0, 0)
HT Yes	(0, 0)	(0, 0)	(0, 0)	(0, 1)	(0, 0)	(1, 3)
DLP No	(0, 0)	(4, 0)	(0, 0)	(1, 15)	(53, 4)	(0, 0)
DLP Yes	(0, 0)	(0, 0)	(0, 0)	(0, 1)	(0, 0)	(0, 0)
Antiplatelet No	(0, 0)	(0, 0)	-	(5, 28)	(0, 0)	-
Antiplatelet Yes	(0, 0)	(0, 0)	-	(0, 1)	(0, 0)	-
Men	(0, 0)	(6, 0)	(0, 0)	(0, 1)	(0, 0)	(0, 0)
Women	(0, 0)	(0, 0)	(0, 0)	(9, 25)	(0, 4)	(1, 0)