ONLINE-ONLY SUPPLEMENTARY MATERIAL

Plasma ceramide measurement

Blood samples for ceramide measurements were obtained in ethylenediamine tetra-acetic acid (EDTA)-containing tubes and stored at −80°C until analysis (13,14). An expert laboratory technician blinded to participants' clinical details performed ceramide measurements of all participants at the Central Laboratory of ‘‘IRCCS Sacro Cuore’’ Hospital of Negrar. Ceramides were purchased from Avanti Polar Lipids Inc. (Alabaster, Alabama, USA) (13,14). Plasma Cer(d18:1/ 16:0), Cer(d18:1/18:0), Cer(d18:1/24:0), and Cer(d18:1/24:1) concentrations were measured by liquid-liquid extraction with 2-propanol:ethyl acetate (4:1 v/v) and gradient reverse phase chromatography on an Agilent Poroshell 120 C18 column (4.6 50 mm, 2.7 mm) (13,14). Cer(d18:1/17:0) was used as an internal standard (13). The apparatus consisted of an Agilent 1290 UHPLC system coupled with an Agilent 6495 Triple Quadrupole LC/MS system (13,14). Mobile phases consisted of LC-MS grade water (A), acetonitrile with 0.1% formic acid (B) and 10 mM ammonium acetate in 2-propanol (C) (13,14). [M + H] + -> 264 MRM transition was selected to quantify each ceramide (13,14). Calibration standards (6 points) were daily prepared in surrogate matrix (5% BSA) at concentration range from 1.0 to 0.031 µmol/L per liter for ceramide (d18:1/16:0), and ceramide (d18:1/18:0), and from 10 to 0.31 µmol/L per liter for ceramide (d18:1/24:0), and ceramide (d18:1/24:1) (13,14). Inter-assay and intra-assay coefficients of variations for precision and accuracy for all measured ceramides were <15%. No matrix interference or carryover was detected (13,14).

Supplementary Table 1. Calculation of the CERT1 risk score at baseline.

CERT1 score components	3rd quartile	4th quartile
Cer(d18:1/16:0)	+1	+2
Cer(d18:1/18:0)	+1	+2
Cer(d18:1/24:1)	+1	+2
Cer(d18:1/16:0) / Cer(d18:1/24:0)	+1	+2
Cer(d18:1/18:0) / Cer(d18:1/24:0)	+1	+2
Cer(d18:1/24:1) / Cer(d18:1/24:0)	+1	+2

Supplementary Table 2. Baseline plasma ceramide concentrations and their ratios to Cer(d18:1/24:0) in participants, stratified by primary composite outcome status at follow-up.

	Without primary composite outcome (n=391)	With primary composite outcome (n=139)	P value
Cer(d18:1/16:0) (umol/L)	0.322±0.004	0.328±0.008	0.552
Cer(d18:1/18:0) (umol/L)	0.127±0.003	0.139±0.006	0.045
Cer(d18:1/24:0) (umol/L)	3.219±0.055	2.682±0.083	<0.001
Cer(d18:1/24:1) (umol/L)	1.048±0.019	1.129±0.037	0.033
Cer(d18:1/16:0) / Cer(d18:1/24:0)	0.108±0.002	0.133±0.004	<0.001
Cer(d18:1/18:0) / Cer(d18:1/24:0)	0.043±0.001	0.560±0.002	<0.001
Cer(d18:1/24:1) / Cer(d18:1/24:0)	0.351±0.008	0.448±0.001	<0.001
CERT1 risk categories (%)			<0.001
Low risk (score 0-2)	40.3	23.7	
Moderate risk (score 3-6)	37.9	36.7	
Increased risk (score 7-9)	12.3	21.6	
High risk (score 10-12)	9.5	18.0	

Sample size, n=530. Data are expressed as means ± standard errors. Differences between the two patient groups were tested using the Mann-Whitney U test.

Supplementary Table 3. Baseline clinical and biochemical characteristics of participants stratified by CERT-1 risk categories at baseline.

	CERT1 Low risk (n=191)	Moderate Risk (n=199)	Increased Risk (n=78)	High Risk (n=62)	P value
Age (years)	67±10	70±10	73±9	73±9	<0.001
Female sex (%)	35.1	31.7	34.6	41.9	0.521
BMI (kg/m²)	27.9±4.2	27.3±4.3	27.4±5.4	27.5±4.4	0.649
Current smokers (%)	11.0	13.1	10.3	12.9	0.881
Obesity (%)	29.8	24.1	28.2	24.2	0.588
Systolic blood pressure (mmHg)	136±17	137±18	142±19	143±27	0.094
Diastolic blood pressure (mmHg)	76±9	75±10	77±12	77±12	0.385
Glucose (mmol/l)	6.8±1.7	6.9±2.3	6.3±1.5	6.6±2.2	0.243
HbA1c (mmol/molHb) (n=271)*	52±8	52±10	49±8	49±9	0.102
Total cholesterol (mmol/l)	4.0±0.9	4.3±1.1	4.4±1.0	4.4±1.3	0.053
HDL cholesterol (mmol/l)	1.3±0.3	1.3±0.4	1.4±0.4	1.3±0.3	0.406
Non-HDL cholesterol (mmol/l)	2.7±0.9	3.0±1.0	3.0±1.0	3.1±1.2	0.035
Triglycerides (mmol/l)	1.2 (0.9-1.7)	1.3 (0.9-1.8)	1.2 (1.0-1.4)	1.4 (1.0-1.7)	0.187
Creatinine (umol/l)	76±17	85±31	82±29	91±28	0.017
$eGFR_{CKD-EPI}$ (ml/min/1.73 m²)	71±17	66±20	65±18	59±20	0.002
CKD (%)	27.2	33.2	35.9	38.7	0.267
Hypertension (%)	78.5	74.9	87.2	88.7	0.032
Type 2 diabetes (%)	24.7	37.2	54.3	61.3	<0.001
Ischemic heart disease (%)	31.4	35.2	34.6	32.3	0.871
Permanent atrial fibrillation (%)	6.3	7.0	5.1	14.5	0.133
Ischemic stroke (%)	2.6	2.0	6.4	6.5	0.142
Anti-platelet drug users (%)	54.9	55.8	57.7	46.8	0.582
Anticoagulant drug users (%)	5.8	9.6	14.1	24.2	<0.005
Beta-blocker drug users (%)	45.0	40.7	35.9	40.3	0.557
ACE-i/ARB drug users (%)	63.4	57.8	62.8	66.1	0.562
Calcium antagonist drug users (%)	19.4	17.6	20.5	9.7	0.317
Diuretic drug users (%)	31.4	30.2	24.4	48.4	0.018
Statin drug users (%)	45.1	53.8	60.8	72.8	<0.005
Insulin therapy (%) (n=271)*	1.6	3.0	2.6	4.8	0.547
Metformin (%) (n=271)*	40.0	77.8	85.9	83.2	0.065
Sulphonylureas (%) (n=271)*	28.4	30.8	22.2	40.0	0.843
Pioglitazone (%) (n=271)*	12.6	7.7	5.6	0	0.533
GLP-1 receptor agonists (%) (n=271)*	22.1	19.2	5.6	0	0.272
SGLT-2 inhibitors (%) (n=271)*	11.6	8.9	5.6	0	0.724

Sample size, n=530. Data are expressed as means ± SD, medians, and interquartile ranges (IQRs) or percentages. Differences among the patient groups were tested by the Chi-squared test for categorical variables, the one-way ANOVA for normally distributed continuous variables, and the Kruskal-Wallis test for non-normally distributed variables. *Data available only for patients with known type 2 diabetes. Obesity was defined as BMI≥ 30 Kg/m². CKD was defined as $eGFR_{CKD-EPI}$ <60 mL/min/1.73 m².

Abbreviations: ACE, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blocker; AST, aspartate aminotransferase; BMI, body mass index; CKD, chronic kidney disease; $eGFR_{CKD-EPI}$, estimated glomerular filtration rate calculated by the CKD-Epidemiology Collaboration study equation.

Supplementary Table 4. Baseline clinical and biochemical characteristics and plasma ceramide levels of participants stratified by study center.

	Patients attending the Cardiology service (n=334)	Patients attending the Diabetes service (n=196)	P value
Age (years)	69±10	70±9	**0.873**
Female sex (%)	24.6	51.5	**0.580**
BMI (kg/m²)	26.7±4.1	28.9±4.7	**<0.001**
Current smokers (%)	9.9	15.3	**0.062**
Obesity (%)	19.2	39.8	**<0.001**
Systolic blood pressure (mmHg)	140±17	136±18	**0.055**
Diastolic blood pressure (mmHg)	75±10	76±10	**0.144**
Glucose (mmol/l)	6.4±2.3	7.2±1.5	**<0.001**
Total cholesterol (mmol/l)	4.4±1.1	4.0±0.9	**<0.001**
HDL cholesterol (mmol/l)	1.3±0.3	1.4±0.4	**0.001**
Non-HDL cholesterol (mmol/l)	3.2±1.0	2.6±0.9	**<0.001**
Triglycerides (mmol/l)	1.4±0.8	1.4±0.6	**0.869**
Creatinine (umol/l)	85±32	77±30	**0.006**
$eGFR_{CKD-EPI}$ (ml/min/1.73 m²)	64±19	71±19	**<0.001**
CKD (%)	32.9	30.6	**0.580**
Abnormal albuminuria (%)	NA	41.0%	**ND**
Hypertension (%)	78.1	82.1	**0.269**
Ischemic heart disease (%)	44.6	14.3	**<0.001**
Permanent atrial fibrillation (%)	9.3	4.1	**0.027**
Ischemic stroke (%)	3.6	3.1	**0.744**
Anti-platelet drug users (%)	58.4	48.5	**0.026**
Anticoagulant drug users (%)	14.7	3.6	**<0.001**
Beta-blocker drug users (%)	46.7	32.7	**0.002**
ACE-i/ARB drug users (%)	60.5	63.3	**0.525**
Calcium antagonist drug users (%)	14.4	23.5	**0.008**
Diuretic drug users (%)	30.8	33.7	**0.499**
Statin drug users (%)	52.7	78.6	**<0.001**
Plasma ceramide concentrations			
Cer(d18:1/16:0) (umol/L)	0.323±0.005	0.319±0.006	**0.454**
Cer(d18:1/18:0) (umol/L)	0.135±0.004	0.123±0.003	**0.186**
Cer(d18:1/24:0) (umol/L)	2.794±0.054	3.561±0.077	**<0.001**
Cer(d18:1/24:1) (umol/L)	1.103±0.02	1.01±0.03	**0.017**
Cer(d18:1/16:0) / Cer(d18:1/24:0)	0.126±0.003	0.095±0.002	**<0.001**
Cer(d18:1/18:0) / Cer(d18:1/24:0)	0.051±0.001	0.037±0.001	**<0.001**
Cer(d18:1/24:1) / Cer(d18:1/24:0)	0.422±0.009	0.299±0.009	**<0.001**

Sample size, n=530. Data are expressed as means ± SD, means ± standard error (for ceramide levels) or percentages. Differences among the patient groups were tested by the Chi-squared test for categorical variables, the Student's t test for normally distributed continuous variables, and the Mann-Whitney U test for non-normally distributed variables.

Obesity was defined as BMI≥ 30 Kg/m². CKD was defined as $eGFR_{CKD-EPI}$ <60 mL/min/1.73 m².

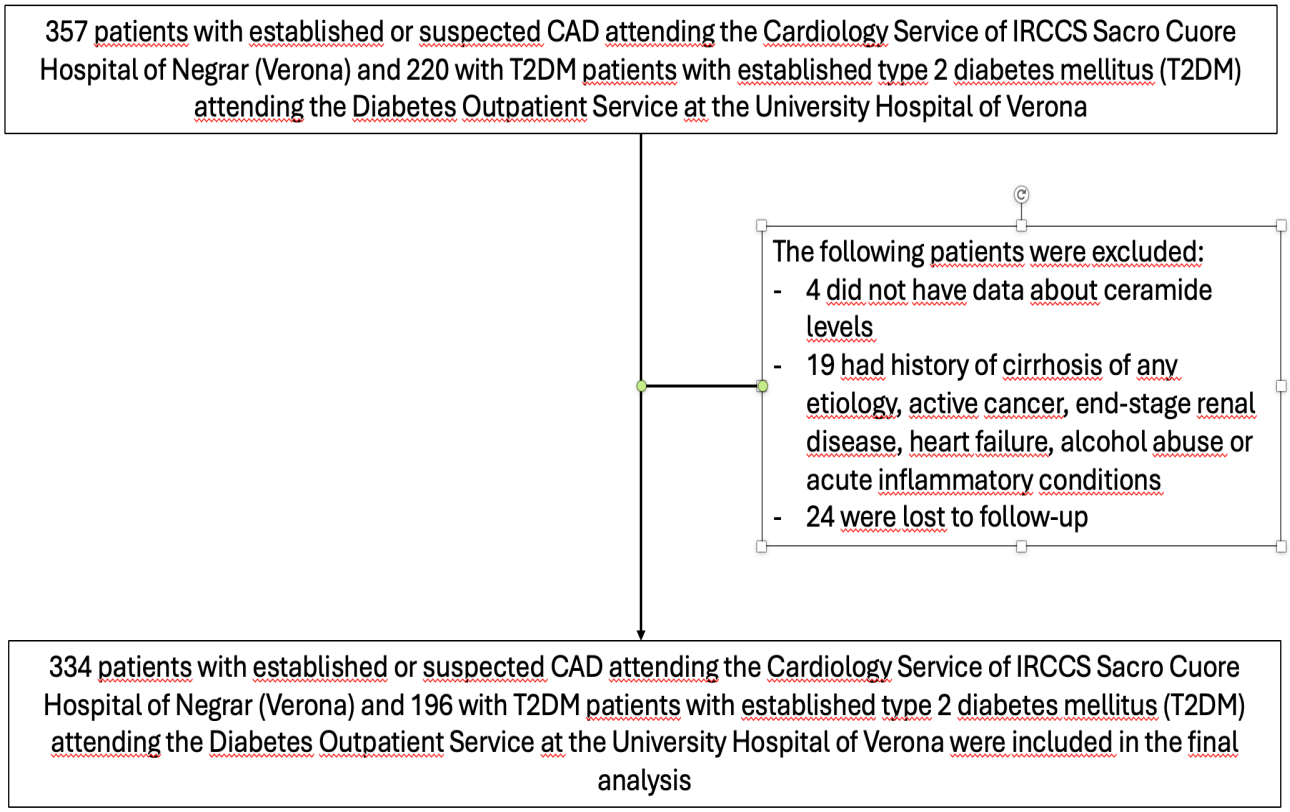
Abbreviations: ACE, angiotensin-converting-enzyme inhibitor; ARB, angiotensin II receptor blocker; AST, aspartate aminotransferase; BMI, body mass index; CKD, chronic kidney disease; $eGFR_{CKD-EPI}$, estimated glomerular filtration rate calculated by the CKD-Epidemiology Collaboration study equation; NA, not available

Supplementary Table 5. Calculation of the Net Reclassification Index (NRI) by the Kaplan Meier estimator with scalar value (t0) of 70 months and a vector to specify the cut-off value of predicted risk for determining *UP* and *DOWN* (cut) of 0.50.

New model / **Old model**	**< 0.50**	**≥ 0.50**
< 0.5 (n)	477	24
≥ 0.5 (n)	23	6

NRI = 0.147; NRI+ = 0.109; NRI- = 0.037; Pr(Up|Case) = 0.169; Pr(Down|Case) = 0.059; Pr(Down|Ctrl) = 0.037; Pr(Up|Ctrl) = 0.000.

Supplementary Figure 1. Flow diagram of the study.



Supplementary Figure 2. Graphical representation of the Net Reclassification Index (NRI) by the Kaplan Meier estimator (t0 = 70 months, cut = 0.50).