Supporting Information

Patient and control groups

Scripps Venous Thrombosis Registry

Plasma samples from Caucasian subjects, 105 white VTE patients (45 male and 60 female) and age matched controls, were obtained from The Scripps Venous Thrombosis Registry [11, 12]. The protocol was approved by the Institutional Review Board of Scripps Clinic and subjects provided written informed consent. Patients with objectively documented deep venous thrombosis with or without pulmonary embolism were recruited from the Scripps Anticoagulation Service and the community. The diagnosis of VTE was confirmed by objective methods (phlebography, compression duplex ultrasonography, pulmonary computerized tomography, or perfusion-ventilation lung scan). Inclusion criteria for this study were age at thrombosis < 55 years, > 3 months since diagnosis of acute thrombosis, and a life expectancy of at least three years. Eighty-five patients were taking warfarin when blood was donated. The subjects taking lipid lowering medications including statin were excluded to avoid any drug-related effects on dynamic lipid metabolism including glycolipids [11, 13]. Cancer was not known to be present in VTE patients and controls. Age matched (± 2 years) healthy controls were recruited through the Scripps General Clinical Research Center's (GCRC) blood donation program. Blood was collected in the GCRC at least three months after VTE diagnosis and after 12 hours fasting. EDTA-plasma was prepared and stored at -70 ºC. Participants in the blood donation program had normal CBC and were negative for HIV and for hepatitis B and C testing. Some were from the community but most were employees or former employees of Scripps. Clinical data collection included detailed medical history and the presence of risk factors for VTE.

Valencia Venous Thrombosis Registry

Plasma samples from Caucasian subjects, 320 VTE white cases (141 male and 179 female) and 316 white controls (152 male and 164 female), were obtained from the Valencia VTE study [14]. Patients were enrolled with at least one objectively confirmed episode of VTE from the records of patients referred to the University and Polytechnic La Fe Hospital to be examined for thrombophilia over a period of 10 years. Objective diagnoses of VTE and

pulmonary embolism were made by clinical probability, D-dimer levels, compression ultrasonography, ventilation perfusion lung scan and, when necessary, phlebography or pulmonary angiography. The control group was recruited along with the cases and included unrelated volunteers without personal or familial history of thromboembolic disease. Controls were randomly selected to match cases by age, gender and geographic area as the VTE patients. Subjects with known thrombophilic defects, such as antithrombin, protein C, protein S, plasminogen, or heparin cofactor II deficiencies as well as those with factor V Leiden and prothrombin G20210A mutations, or anti-phospholipid antibodies or lupus anticoagulant were excluded as were patients with malignancy, nephrotic syndrome, renal or hepatic dysfunction, inflammatory or infectious disease, heart failure or lupus anticoagulant. Participants with cancer, nephrotic syndrome, renal or hepatic dysfunction, inflammatory or infectious disease, or heart failure, as well as anyone currently taking oral anticoagulants or oral contraceptives were also excluded for the plasma GlcCer and PtdEtn analysis. All subjects gave their informed consent to enter the study which was approved by the Ethics Committee of the University Hospital's Review Board and was performed according to the declaration of Helsinki of 1975, as amended in Edinburgh in 2000. Blood drawing was carried out at least 6 months after the acute event and was performed early in the morning, with all subjects fasting from 6 to 8 hours. Blood was collected into one Vacuette© sodium citrate tube (Greiner Bio-One, Meylan cedex, France) containing 0.129 M trisodium citrate. Plasma was obtained by centrifugation at 1,500 x g for 30 min at 4 ºC and stored in aliquots at -80 ºC.

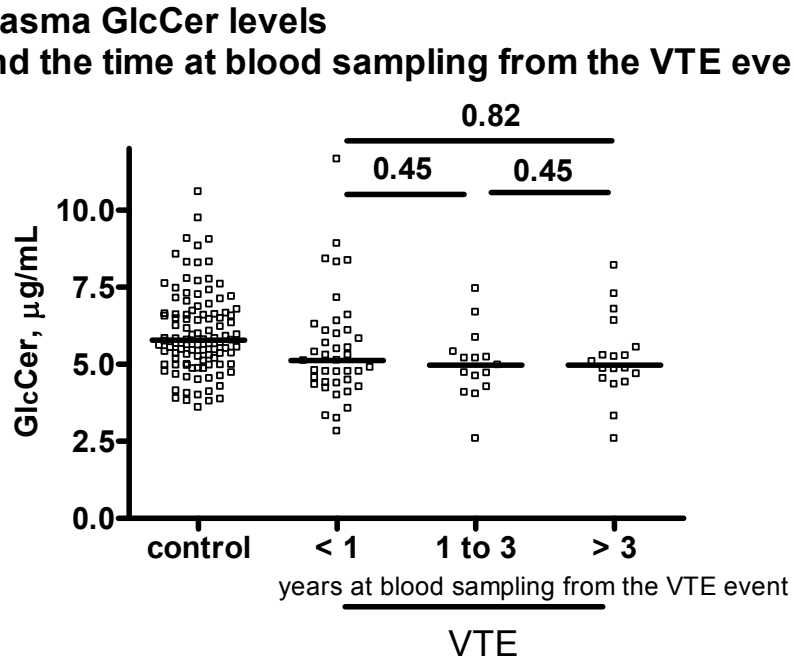
MI study

Plasma samples from 149 Caucasian MI cases (125 male and 24 female) enrolled in the Genetic and Viral Attributes of Myocardial Infarction (GAVAMI) [15] study and 197 Caucasian controls (120 male and 77 female) enrolled in the Genetic Attributes of Thrombosis Epidemiology [16] study were obtained. Cases were adults between ages 18 and 65 hospitalized at Crawford Long Hospital (now Emory University Hospital Midtown, Atlanta, Georgia) for a first or recurrent acute MI. Physician diagnosis of MI was made using standard criteria based on at least one of the following: chest pain lasting more than 30 minutes with abnormal cardiac enzymes, ST segment elevation on EKG in 2 or more leads with abnormal cardiac enzymes, non-ST segment elevation with abnormal cardiac enzymes but with ST depression, T-wave inversion, or non-specific EKG changes. Cases transferred from another

hospital or with known conditions resulting in immunosuppression such as HIV/AIDS, bone marrow or organ transplant, dialysis, or chemotherapy were excluded. Blood specimens from cases were obtained on presentation of MI, 12 hours after presentation, and 1 to 3 months post event. For this project only Caucasian cases with a blood specimen obtained 1-3 months post event were included. Laboratory results for the project are based on the 1-3 moth post event specimen. A standardized questionnaire was administered either during admission or shortly afterward. Controls with no prior history of MI, cardiac surgery, coronary angioplasty, stent, or atherectomy, or conditions resulting in immunosuppression were selected from patient lists of a primary care clinic servicing Crawford Long Hospital. Blood specimens were collected in 0.129 M trisodium citrate from enrolled controls at visits to the Centers for Disease Control and Prevention (Atlanta, GA) where they were also administered a standardized questionnaire. For this project a random selection of 200 Caucasian controls was made, of which 197 had available specimens for testing. Neither cases nor controls were required to fast prior to blood draw, and specimens were not drawn at a standard time of day. The protocols were approved by the Institutional Review Board of Emory University and subjects provided written informed consent.

Figure S1. Plasma GlcCer levels in VTE in Scripps VTE registry by the age at blood sampling from the VTE event.

GlcCer levels were determined in VTE group of Scripps VTE registry excluding recurrence patients, and sub grouped based on the age at blood sampling from the VTE event. The solid thick lines indicate median values. The p-values shown are for the difference of median values between subgroups calculated by Mann-Whitney test using Prism™ 4.03 software (Graph Pad Software Inc., San Diego, CA).



Years at blood sampling from the VTE event	< 1	1-3	> 3
GlcCer level, median (µg/mL) (IQR)	5.1 (4.4–6.1)	5.0 (4.3–5.4)	5.0 (4.5–6.0)

Table S1. Clinical profile of study groups and plasma GlcCer and PtdEtn levels in VTE populations.

	VTE populations		VTE populations			
	Vienna plus Mayo Clinic [5]		Scripps Registry		Valencia Registry	
	control	VTE	control	VTE	control	VTE
Total, N	70	70	105	105	320	316
Race (Caucasian, N)	70	70	105	105	320	316
Age at blood drawing (SD)	42 (14)	50 (14)	45.3 (9.3)	45.0 (9.8)	40.8 (13.7)	43.6 (13.4)
Age at event (SD)		46 (14)		40.2(10.2)		40 (13.4)
Male, N	29	35	45	45	141	152
Female, N	41	35	60	60	179	164
Factor V Leiden	5	17	6	26	0	0
p value	**p = 0.009**		**p = 0.0002**			
Prothrombin G20210A	–	–	4	9	0	0
p value			**p = 0.047**			
Warfarin use	0	20	0	85	0	0
Hormone use	0	2	34	29	0	0
Statin use	–	–	0	0	0	0
BMI [mean, (SD)]	–	–	26.9 (5.0)	29.1 (6.6)	24.8 (3.8)	27.3 (4.9)
p value			**p = 0.01**		**p < 0.0001**	
HDL-C (IQR), mg/dL	–	–	54 (45–66)	52 (41–62)	–	–
p value			**p =** 0.17			
LDL-C (IQR), mg/dL	–	–	113 (91–131)	121 (100–149)	–	–
p value			**p = 0.04**			
HDL particles (IQR), µM	–	–	26.2 (23.2–30.0)	25.3 (21.6–27.8)	–	–
p value			**p = 0.02**			
LDL particles (IQR), µM	–	–	917 (723–1156)	1006 (795-1263)	–	–
p value			**p = 0.03**			
GlcCer (IQR), µg/mL	6.5 (4.4–9.7)	4.9 (3.1–7.1)	5.8 (5.0–6.7)	5.0 (4.4–5.9)	6.0 (5.0–7.0)	5.6 (4.7–6.6)
p value	**0.0007**		**<0.0001**		**0.001**	
PtdEtn (IQR), µg/mL	65.5 (42–102)	70.6 (50–100)	53.6 (42.0–67.3)	54.7 (42.0–67.3)	44.6 (37.3–53.7)	44.9 (36.9–54.7)
p value	0.48		0.82		0.73	

Table S2. Evaluation of correlations of plasma GlcCer levels with lipid/lipoprotein parameters, Age and BMI for the Scripps VTE Registry control subjects (N = 105).
Table shows the relationship of lipid/lipoprotein levels, age and BMI to plasma GlcCer levels. The median values of various parameters by tertile of GlcCer and the correlation of these parameters with plasma levels of GlcCer (N = 105) are shown. The median values of GlcCer were 4.9, 5.8, and 7.2 µg/mL in each tertile, respectively.

	GlcCer tertile			Correlation with GlcCer (N = 105)	
Parameter	**lowest**	**middle**	**highest**	**r**	**p value**
PtdEtn, µg/mL	54.2	46.4	67.7	**0.28**	**0.005**
Total cholesterol, mg/dL	182	189	219	**0.37**	**0.0001**
Triglyceride, mg/dL	111	96	127	0.14	0.16
HDL-C, mg/dL	49.0	58.5	56.5	**0.21**	**0.03**
LDL-C , mg/dL	105	109	133	**0.31**	**0.001**
VLDL Particles, nM	52.7	39.8	47.9	0.03	0.76
Large VLDL/chyromicron, nM	1.9	1.0	1.4	0.04	0.69
Medium VLDL, nM	16.3	16.7	17.1	0.08	0.44
Small VLDL, nM	28.6	20.0	28.5	-0.01	0.95
LDL Particles (total), nM	875	898	1019	0.10	0.31
IDL particles, nM	20.9	8.8	21.3	0.05	0.64
Large LDL particles, nM	296	373	322	0.11	0.28
Small LDL particles, nM	566	412	610	0.01	0.93
HDL Particles (total), µM	25.1	25.7	27.8	**0.30**	**0.002**
Large HDL particles, µM	4.2	7.8	5.2	0.08	0.45
Medium HDL particles, µM	5.0	2.6	4.9	-0.03	0.76
Small HDL particles, µM	15.3	15.1	16.9	**0.23**	**0.02**
VLDL Size, nm	53.8	53.7	52.5	0.07	0.46
LDL Size, nm	20.9	21.4	21.0	0.08	0.40
HDL Size, nm	8.9	9.4	8.9	0.02	0.83
Age	46	47	47.5	0.08	0.40
BMI	25.9	26.1	25.7	-0.06	0.57

Table S3. Evaluation of correlations of plasma PtdEtn levels with lipid/lipoprotein parameters, Age and BMI for the Scripps VTE Registry control subjects.

Table shows the relationship of lipid/lipoprotein levels, age and BMI to plasma PtdEtn levels. The median values of various parameters by tertile of PtdEtn and the correlation of these parameters with plasma levels of PtdEtn (N = 95) are shown. The median values of GlcCer were 38.0, 56.7, and 78.3 μg/mL in each tertile, respectively.

	PtdEtn tertile			Correlation with PtdEtn (N = 95)	
Parameter	**lowest**	**middle**	**highest**	**r**	**p value**
GlcCer, μg/mL	5.5	5.6	7.1	0.28	**0.005**
Total cholesterol, mg/dL	174	192	212	0.34	**0.0007**
Triglyceride, mg/dL	83	112	146	0.37	**0.0002**
HDL-C, mg/dL	52.5	51.5	58.0	0.16	0.11
LDL-C , mg/dL	104	111	127	**0.23**	**0.03**
VLDL Particles, nM	39.2	52.9	52.0	0.14	0.17
Large VLDL/chyromicron, nM	1.1	1.2	1.6	0.18	0.09
Medium VLDL, nM	13.2	18.4	21.4	0.18	0.08
Small VLDL, nM	22.8	28.2	25.8	0.04	0.69
LDL Particles (total), nM	812	989	924	**0.22**	**0.03**
IDL particles, nM	8.2	24.7	18.9	**0.27**	**0.007**
Large LDL particles, nM	314	309	309	-0.04	0.69
Small LDL particles, nM	421	621	600	0.17	0.11
HDL Particles (total), μM	25.5	25.8	27.8	**0.31**	**0.002**
Large HDL particles, μM	5.0	5.6	5.6	0.02	0.83
Medium HDL particles, μM	2.4	3.4	6.9	**0.27**	**0.009**
Small HDL particles, μM	15.2	16.5	16.8	0.14	0.19
VLDL Size, nm	50.7	55.8	52.2	0.18	0.08
LDL Size, nm	21.3	20.9	20.9	-0.12	0.23
HDL Size, nm	9.0	9.0	8.9	-0.12	0.25
Age	46	45.5	46	0.16	0.12
BMI	26.1	25.8	25.6	-0.04	0.67

Table S4. Comparisons of OR for VTE based on low plasma GlcCer level (below 10th percentile of control) with and without adjustments.

	Vienna plus Mayo Clinic [5]	Scripps VTE Registry	Valencia VTE Registry *
10th percentile of GlcCer, µg/mL	4.3	4.5	4.4
Adjustment			
I. none	5.7 (2.3–14)	3.7 (1.8–7.9)	2.1 (1.3–3.3)
II. BMI, Age, Sex	ND	3.3 (1.6–7.2)	2.0 (1.3-3.2)
III. FV Leiden, prothrombin G20210A, BMI, Age, Sex	ND	3.4 (1.6–7.6)	ND
IV. model II plus HDL-C, LDL-C	ND	5.0 (2.1–12)	ND
V. model II plus HDL and LDL particles	ND	3.8 (1.7–8.7)	ND

OR (95% CI) for VTE based on low plasma GlcCer levels (below the 10th percentile) are shown. The subjects above the 10th percentile of GlcCer served as the reference group. 10th percentile cut points were defined in controls. Models II to V were adjusted by variables indicated in the Table. BMI, HDL-C, HDL particles, LDL-C and LDL particles were used as continuous variables. ND indicates not determined.

*The carriers of FV Leiden and/or prothrombin G20210A and the hormone and/or anticoagulant users were excluded from the subjects available for GlcCer analysis for the Valencia VTE Registry study.

Table S5. Odds ratios (OR) (95% CI) for VTE according to tertiles of plasma PtdEtn levels.

	PtdEtn tertiles		
Scripps VTE registry	**lowest**	**middle**	**highest**
PE levels (µg/mL)	< 47.2	≥ 47.2 and < 65.1	≥ 65.1
Adjustment			
I. none	1.0 (0.50–2.1)	1.1 (0.52–2.2)	1
II. FV Leiden, prothrombin G20210A, BMI, Age, Sex	1.1 (0.53–2.5)	1.4 (0.61–3.2)	1
III. model II plus HDL-C, LDL-C	1.4 (0.59–3.3)	1.8 (0.74–4.3)	1
IV. model II plus HDL particles, LDL particles	0.96 (0.37–2.5)	1.5 (0.62–3.6)	1
Valencia VTE registry *	**lowest**	**middle**	**highest**
PtdEtn levels (µg/mL)	< 40.1	≥ 40.1 and < 50.4	≥ 50.4
Adjustment			
I. none	1.1 (0.71–1.61)	1.0 (0.67–1.53)	1
II. BMI, Age, Sex	1.2 (0.72-1.97)	1.2 (0.73-1.89)	

The tertile-based odds ratios (OR) (95% CI) for VTE based on the plasma PtdEtn are shown. Tertile cut points were defined in controls. The subjects with the highest tertile of PtdEtn served as reference. Models II to IV were adjusted by variables indicated in the Table. BMI, HDL-C, HDL particles, LDL-C and LDL particles were used as continuous variables.

*The carriers of FV Leiden and/or prothrombin G20210A and the hormone and/or anticoagulant users were excluded from the subjects available for PtdEtn analysis for the Valencia VTE Registry study.

Table S6. Clinical profile of study groups and plasma GlcCer and PtdEtn levels in the MI population.

	MI population	
	control	MI
Total, N	197	149
Race (Caucasian, N)	197	149
Age at blood drawing (SD)	50.2 (12.5)	52.0 (7.4)
Age at event (SD)		52.0 (7.4)
Male, N	120	125
Female, N	77	24
BMI (SD)	26.6 (5.5)	29.9 (6.0)
Warfarin Use, N	1	62
Hormone use, N	33	4
Statin use, N	25	129
Aspirin use, N	46	131
Hypertension, N	57	69
p value	**p = 0.0010**	
Diabetes, N	7	19
p value	**p = 0.003**	
Smoking at time of blood draw , N	24	68
p value	**p < 0.001**	
Alcohol consumption, N		
0 /week	30	53
< 1 /week	36	17
1-7 /week	95	34
≥ 7/week	36	45
	p = 0.065	
Obesity (BMI > 30), N	37	63
	p< 0.0001	
Family history	13	39
	p < 0.0001	
HDL-C	ND	ND
LDL-C	ND	ND
HDL particles	ND	ND
LDL particles	ND	ND
GlcCer, µg/mL(IQR)	5.6 (4.8–6.8)	4.3 (3.6–5.2)
p value	**<0.0001**	
PtdEtn, µg/mL(IQR)	54.5 (44.2–66.2)	46.1 (38.7–57.4)
p value	**<0.0001**	

ND indicates not determined.