Emerging risk factors for venous thromboembolism: The role of commonly prescribed drugs for cardiovascular disease and inflammatory disorders

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Chapter 4

Association of Apolipoproteins C-I, C-II, C-III and E with Coagulation Markers and Venous Thromboembolism Risk.

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Summary

Background: Apolipoproteins C-I, C-II, C-III and E have been associated with risk of arterial thrombotic diseases. **Objectives?** We investigated whether these apolipoproteins have prothrombotic properties and are associated with risk of venous thromboembolism (VTE). **Patients and methods:** A total of 127 VTE patients and 299 controls were randomly selected from the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis study (1999–2004), in the Netherlands. The apolipoproteins were quantified using mass spectrometry (LC/MS/MS) and their levels were analysed as continuous variable (per SD increase). **Results:** In controls, increases in levels of apolipoproteins were associated with increases in levels of vitamin K dependent factors, factor XI, antithrombin and clot lysis time. Additionally, increasing apolipoproteins C-III and E levels were associated with higher factor VIII and von Willebrand factor levels. Levels of C-reactive protein were not associated with any apolipoprotein. The age and sex adjusted odds ratios of apolipoproteins E, C-III, CII and CI to the risk of venous thrombosis were 1.21 (95% CI, 0.98-1.49), 1.19 (0.99-1.44), 1.24 (0.95-1.61) and 1.06 (95% CI, 0.87-1.30) per SD increase, respectively. These odds ratios did not attenuate after adjustments for statin use, estrogen use, BMI, alcohol use, and self-reported diabetes. **Conclusions:** Levels of apolipoproteins C-I, C-II, C-III and E are associated with those of several coagulation factors. However, whether these apolipoproteins are also associated with an increased risk of VTE remains to be established.

Keywords: Thrombosis; Proteomics; Lipids and Cholesterol; Coagulation; Risk Factors

INTRODUCTION

Venous thromboembolism (VTE) and arterial thrombosis are distinct diseases linked by several clinical aspects. Patients with VTE have a 1.6 to 3 fold- increase in the risk of subsequent arterial cardiovascular events [1, 2], and drugs such as aspirin and statins prevent the occurrence of both arterial and venous thrombosis [3-5]. Furthermore, the two diseases share risk factors, such as age, sex, lifestyle and body mass index (BMI) [6-8].

Disorders of lipid metabolism are risk factors for arterial thrombosis that can potentially play a role in the risk of VTE, as these disorders affect hemostasis and lead to a hypercoagulable state [9, 10]. However, an association between lipid disorders and VTE has not been confirmed so far. Lipid levels were not associated with a risk of VTE in several clinical studies [6, 11, 12], and a relationship between apolipoproteins (apo) and VTE risk remains controversial [11, 13-16]. Although higher levels of apoB and lower levels of apoA-I were reported to increase the risk for VTE in selected populations [14, 16], these observations were not confirmed in larger cohorts [11, 13]. A recent report from a large, unselected population, has demonstrated that decreasing levels of apoA-I and B were associated with increased risk of VTE [12].

Recently, apoC-II, C-III and E have emerged as potential risk factors for cardiovascular disease [17-20] and as target for new lipid lowering agents [21]. Whether these newly described risk factors for cardiovascular disease also play a role in the risk of VTE has not been evaluated thus far. In this study, we apply recent advances in liquid chromatography tandem-mass spectrometry (LC/MS/MS) [22] to perform a comprehensive analysis of the association between serum apoC-I, C-II, C-III and E and levels of hemostatic factors (proteins C and S, antithrombin, TFPI activity, fibrinogen, factors II, VII, VIII, IX, X, XI, von Willebrand and clot lysis time) and inflammatory markers (C-reactive protein). We also investigate the association of these serum apos with the risk of VTE.

MATERIAL AND METHODS

Study population

For this study patients and random digit-dialing (RDD) controls were randomly selected from a population-based case-control study, i.e., the Multiple Environmental and Genetic

Assessment of Risk Factors for Venous Thrombosis (MEGA) study and from a cohort study, the MEGA follow-up.

Details of the MEGA study were described previously [23]. Briefly, between March 1999 and August 2004, 4956 consecutive patients aged 18-70 years with a first acute VTE (deep vein thrombosis or pulmonary embolism) were included. A questionnaire on putative risk factors for VTE was filled in and blood was sampled on the day of enrolment for the study. Participants were asked to provide blood samples up to June 2002, and 2377 patients and 1459 RDD controls provided blood samples. Blood samples were collected from at least 3 months after discontinuation of anticoagulation, or during anticoagulant therapy if this was continued for more than 1 year. None of the included patients with VTE had a recurrence before blood sampling.

RDD controls were frequency matched for sex and age to the cases. RDD controls were aged 18-70 y and had no history of VTE. Patients were followed until 2007-2009 when the vital status of all MEGA follow-up participants was accessed, as described previously (MEGA follow-up study) [24]. The MEGA study was approved by the Ethics Committee of the Leiden University Medical Center, and written informed consent was obtained from all participants at the date of the inclusion in the study. This study was conducted in accordance with the Declaration of Helsinki.

A total of 127 patients with a first VTE event were selected from the MEGA study and MEGA follow-up study, of whom 63 (50%) had had one VTE event and 64 (50%) recurrent VTE. Patients with recurrent VTE were oversampled *a-priori* as we considered them to be more likely to have aberrant apo levels leading to first and recurrent VTE. Otherwise the sampling was completely random. The control group comprised of 300 controls randomly chosen from the RDD control population of the MEGA study. In all groups apoC-I, C-II, C-III and E were measured. Figure 1 illustrates the flow chart of the participants' selection for the study.

Clinical outcomes

The information about the diagnosis of VTE was obtained from hospital discharge reports and general practitioners. The diagnosis of deep vein thrombosis was confirmed with Doppler ultrasonography and the diagnosis of pulmonary embolism was confirmed with a ventilation perfusion lung scan, computed tomography of the chest or angiogram.

Between June 2008 and July 2009, participants were invited to answer questions

regarding recurrent VTE by mail or by telephone interview. Information about recurrences was also retrieved from the anticoagulation clinics where patients were initially included for their first event and at the clinic nearest to their address in case they moved house. Discharge letters were requested from the clinician who had diagnosed the recurrence. A decision rule regarding certainty of the diagnosis was made according to the information collected per patient, as described previously [25]. Reported recurrences were classified as certain when 1) a discharge letter stated a diagnosis of a recurrent event based on clinical and radiological data, or 2) both the anticoagulation clinic and the patient reported a recurrent event that was at a clearly different location than the first event or occurred more than one year since the first event, or 3) a registered death from a recurrent event at least six months after the first event was found. For this analysis, we considered certain recurrences as outcome event only.

Laboratory procedures

ApoC-I, C-II, C-III and E were measured on stored (-80 °C) and once previously thawed fasting serum samples. To determine the apo profile, a mass spectrometric method was developed for multiplexed quantification of apolipoproteins [26]. This multiplex mass spectrometry-based analytical method was validated according to Clinical and Laboratory Standards Institute (CLSI) protocols and its **total coefficient of variation (CV) ranges from 2.5% to 5.9% for the apolipoproteins [27]**.

In contrast to classical high density lipoprotein –cholesterol (HDLc) and low density lipoprotein –cholesterol (LDLc) tests and lipoprotein particle counting methods, the quantitative proteomics test allows quantitation of unequivocally characterized apos with an analytical performance that meets test requirements derived from biological variation. The peptides in the serum digest are separated by liquid chromatography (LC) and detected by tandem mass spectrometry (MS/MS). In MS/MS, ions of the selected peptides are pre-filtered by their molecular mass-to-charge ratio before fragmentation by a collision gas and detection of the generated, highly specific, product ions, relative to stable isotope labelled internal standard peptides. The trace of a precursor-to-product ion transition is used for protein identification and/or quantification.

Serum lipids and lipoprotein fractions were measured using reagents and calibrators from Roche Diagnostics on Modular P analyzers. Traceability of serum lipid and lipoprotein test results to the CDC Reference Measurement System is guaranteed and monitored through stringent vigilance by the Dutch SKML EQA-programme [28].

Procoagulant factors (fibrinogen, factors II, VII, VIII, IX, X and XI, and von Willebrand factor), natural anticoagulants (antithrombin, protein C, and total protein S), clot lysis time, and high sensitive C-reactive protein (hsCRP) levels were determined according to previously described methods [29, 30]. Briefly, measurements of antithrombin and protein C levels were performed with a chromogenic assay and factors II, VII, VIII, X and XI activities were measured with a mechanical clot detection method on a STA-R coagulation analyzer following the instructions of the manufacturer (Diagnostica Stago, Asnieres, France). Total protein S levels and levels of factor IX antigen were determined by ELISA (Diagnostica Stago). Fibrinogen activity was measured on the STA-R analyzer. Von Willebrand factor antigen was measured by immunoturbidimetry (STA Liatest®, Diagnostica Stago), following the instructions of the manufacturer. CRP was measured by automated particle-enhanced immunoturbidimetric assay (Tina-quant® CRP detection method; Roche Diagnostics, West Sussex, UK).

Total tissue factor pathway inhibitor (TFPI) activity in citrated plasma was determined using the Actichrome TFPI activity assay (Sekisui Diagnostics, Stamford, CT, USA). Briefly, TFPI activity is assessed by measuring TFPI inhibition of the catalytic tissue factor (TF)-factor VIIa (FVIIa) complex; one unit of TFPI activity corresponds to 55 ng/mL plasma TFPI. All laboratory analyses were performed without knowledge of whether the sample was from a patient or a control subject.

Statistical analysis

Baseline characteristics are presented as counts and percentages if they are categorical variables or mean +/- standard deviation (SD) if they are continuous variables.

To assess the association of apoC-I, C-II, C-III and E with hemostatic factors and inflammatory markers, the apo-levels were considered as per standard deviation (SD) increase and linear regression models were performed. Data that were not normally distributed were log-transformed. The models were adjusted for age (continuous), sex (dichotomous), statin use (dichotomous), estrogen use (dichotomous), alcohol intake (dichotomous), body mass index (continuous), and self-reported diabetes (dichotomous).

The association between apoC-I, C-II, C-III and E and VTE was assessed by logistic regression analysis. Three adjusted logistic regression models were used: age and sex-adjusted model; age, sex and statin use –adjusted model and age, sex, statin-use, estrogen use, alcohol intake, body mass index, and self-reported diabetes adjusted model.

In each logistic regression model, odds ratios (OR) and their 95% CI were used to estimate the association with all VTE (n=127), or one VTE event only (n= 63), using the levels of apolipoproteins as continuous variables.

As 64 patients also had a recurrent VTE, differences in the outcome between patients with only one VTE event (n=63) and recurrent VTE (n=64) were studied in a case-control analysis within the MEGA follow-up study. For this purpose, an age and sex-adjusted logistic regression model was applied to estimate the odds ratio of recurrent VTE, as compared with first VTE only, for apo levels as continuous values.

All statistical analyses were performed with SPSS version 23.0 for Windows (SPSS Inc, IBM, Armonk, NY, USA). Graphs were plotted using GraphPad Prism version 6.0 for MAC (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Clinical characteristics

Clinical characteristics of the participants are shown in Table 1. The main site of thrombosis was deep vein thrombosis (65%). There were no substantial clinical differences between patients and RDD controls selected for the current analysis and the total patients and RDD control participants of MEGA study (data not shown).

Association of apoC-I, C-II, C-III and E with hemostatic and inflammatory markers

Graphic material on the correlation between apos C-I,-II, -III, E and vitamin K dependent coagulation factors and FVIII and VWF is provided as Supplementary Figures 1-8.

In controls, the mean levels (and SD) of apoC-I, C-II, C-III and E were 21.38 mg/L (5.33), 39.89 mg/L (27.64), 103.59 mg/L (37.11), 30.71 mg/L (13.03), respectively. The associations of apoC-I, C-II, C-III and E with hemostatic factors and inflammatory markers in control subjects are shown in detail in Table 2.

Increases in the levels of all measured apos (apoC-I, C-II, C-III and E) were associated with higher levels of FII, FVII, FIX, FX, FXI, natural anticoagulants (protein C, protein S, antithrombin) and clot lysis time. Additionally, an increase in the levels of FVIII and VWF was observed with increases in the levels of apoC-III and apoE (Table 2). There was no association of apoC-I, C-II, C-III or E with levels of CRP.

Association of apoC-I, C-II, C-III and E with venous thromboembolism

In VTE patients, the mean levels (and SD) of apoC-I, C-II, C-III and E were 21.59 mg/L (6.29), 43.08 mg/L (32.22), 110.53 mg/L (46.04), 32.92 mg/L (12.69), respectively. Figure 2 illustrates the association between apo levels and first VTE risk. There was no association between first VTE and levels of apoC-I. The sex and age adjusted OR and 95% CI of first VTE was 1.24 (95% CI: 0.95, 1.61) with apoC-II, 1.19 (OR 95% CI: 0.99, 1.44) with apoC-III and 1.21 (95% CI: 0.98, 1.49) with apoE, as compared with RDD controls. The observed associations remained similar after further adjustments for statin use, estrogen use, BMI, alcohol use, and self-reported diabetes. Results were also similar when considering patients with only one VTE event. Age and sex adjusted odds ratios of venous thromboembolism for apolipoproteins C-I, C-II, C-III, and E levels when comparing patients with only one VTE event with RDD controls were 1.03 (95% CI 0.78 – 1.37), 1.26 (95% CI 0.88 – 1.81), 1.07 (95% CI 0.81 – 1.41) and 1.21 (95% CI 0.93 – 1.57) respectively.

The sex- and age-adjusted ORs and 95% CIs of recurrent VTE with apoC-I, C-II, C-III and E levels (per SD increase) were 1.03 (95% CI: 0.77, 1.39), 1.14 (95% CI: 0.74, 1.78), 1.22 (95% CI: 0.90, 1.66) and 0.99 (0.69-1.42), respectively, as compared with first VTE only.

DISCUSSION

In individuals without VTE, levels of apoC-I, C-II, C-III and E were associated with levels of several hemostatic factors, in particular with vitamin K dependent pro- coagulant factors and natural anticoagulants. The association of these apos with coagulation factors remained significant after adjustment for confounding factors. These observations are consistent with previous results from the MEGA study, in which a positive association of apoA-I and B with vitamin K dependent factors and coagulation inhibitors was reported [12].

Additionally, higher levels of both apoC-III and E were associated with increases in levels of FVIII and VWF, which are well known risk factors for VTE [31-33]. It is also worth noticing that apoC-III and apoE have consistently been associated with cardiovascular diseases [34]. Previous studies demonstrated that apoE (per SD increase)

is associated with 15 to 30% increased risk of ischemic heart disease [20, 35] and 2-fold increased risk of cardiovascular mortality.[36] Therefore, our findings provide additional evidence for an association between the proteomic signature and a worse or impaired lipid/coagulation pattern due to cross-talks between these systems.

Multiple mechanisms are potentially at the basis of this association. As the major source of serum apoC-I, C-II, C-III and E are the hepatocytes, it is possible that the synthesis of apos and hepatocyte-derived coagulation factors and natural anticoagulants is regulated by common mechanisms [33, 34]. Additionally, biochemical studies have shown that triglycerides-rich lipoproteins, which contain apoC-I, C-II, C-III and E, stimulate coagulation *in vitro* by binding vitamin K-dependent coagulation factors, promoting enzymatic activity of prothrombinase complex and enhancing thrombin activation [37, 38]. Although these hypotheses may explain the association between apoC-I, C-II, C-III and E and hepatocyte-derived coagulation factors, it does not explain the association of apoC-III and E with coagulation factors secreted by the endothelium and not dependent of vitamin K, such as VWF and FVIII [35, 36]. Biological mechanisms linking apoE and FVIII have been described [39-42]. In animal models of atherogenesis, the absence of FVIII had a protective effect against the development of atherosclerosis in *APOE* knock-out mice [39, 40]. Moreover, low-density lipoprotein receptor-related protein-1 (LRP-1) is a common receptor for both apoE and FVIII [41]. The receptor is also capable of mediating the degradation of FVIII [42] and contributes to variations in FVIII plasma levels [43]. Therefore, the observed association between apoC-I, C-II, C-III and E and coagulation factors may be explained by various mechanisms, such as common pathways of synthesis in hepatocytes [33, 34] coagulation activation [37, 38] and competition binding between apoE and FVIII for a common receptor [43].

Since VTE patients are characterized by a high prevalence of classic cardiovascular risk factors [6], we further evaluated whether apoC-I, C-II, C-III and E were associated with risk of VTE. Although our analyses pointed towards an association between apoC-II, C-III and E with VTE risk, numbers were small and confidence intervals included unity. Therefore, our results need larger studies to definitely conclude on the relation between these apos and VTE risk.

Some aspects in this study need to be considered in order to interpret our results. First, the sample was randomly selected from a large population-based study, the MEGA study, but was not large enough for strong conclusions on the association with VTE risk.

Second, we cannot rule out that some associations between apos and hemostatic factors occurred by chance or due to residual confounding such as hepatic function or diet, variables on which we have no information in MEGA. However, similar associations were described previously both in basic research and in population-based studies [9, 20, 37-44] and can be explained biologically. Third, despite patients with recurrent VTE being overrepresented, the study sampling was otherwise completely random. As a result, clinical characteristics of patients and RDD controls selected for this study were similar to that reported for the total MEGA study population [12]. Furthermore, the availability of the blood samples was based on logistical reasons only, where blood sampling was performed for all participants included up to June 2002 [45]. It is possible, though, that a participant's clinical condition contributed to his/her decision to partake on blood sampling. Forth, it is possible to argue that since apos were tested after the VTE event, reverse causality was present. Indeed, after VTE, patients may have modified certain lifestyle factors, which could have affected their apo profile. However, results from randomized trials have shown that changes in diet hardly affect lipid levels without concomitant statin use or aerobic-exercise programs.[46, 47] Finally, since the investigation of apoC-I, C-II, C-III and E for associations with coagulation factors, inflammatory markers and VTE is unprecedented, our findings require replication.

Conclusion

In conclusion, we have shown that serum apoC-I, C-II, C-III and E were associated with multiple plasma coagulation factors and natural anticoagulants. Particularly, higher levels of apoC-III and E were associated with an increase in levels of FVIII and VWF. Whether these apolipoproteins are also associated with an increased risk of VTE remains to be established.

Authors' contributions

Fernanda A. Orsi performed the statistical analyses and drafted the manuscript. Willem M. Lijfering designed and performed the statistical analyses and revised the manuscript. Arnoud Van der Laarse and L. Renee Ruhaak were responsible for the laboratory analyses and revised the manuscript. Frits R. Rosendaal was responsible for the MEGA study concept, designed the analyses and revised the manuscript, Suzanne C. Cannegieter designed the analyses and revised the manuscript and Christa Cobbaert was

responsible for the development of the laboratory methods, designed the analyses and revised the manuscript.

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Disclosure

The authors declare no competing financial interests.

List of Abbreviations

Apo Apolipoprotein
CI confidence interval
CLT clot lysis time
F Factor
HDLc high density lipoprotein –cholesterol
Hs-CRP high sensitivity C-reactive protein
LC/MS/MS liquid chromatography detected tandem mass spectrometry
LDLc low density lipoprotein –cholesterol
PC Protein C
PS Protein S
RDD random digit-dialing
TFPI Tissue factor pathway inhibitor
VTE Venous Thromboembolism
VWF von Willebrand factor

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Table 1 Clinical Characteristics of Patients and RDD Controls Randomly Selected from the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis Case-Control Study, the Netherlands, 1999–2004

Characteristic[a]	Patients			RDD controls
	All (n=127)	First VTE (n=63)	Recurrent VTE (n=64)	(n=299)
Age at enrollment[b], mean (SD)	46 (13)	45 (12)	47 (13)	47 (13)
Men, n (%)	59 (46)	20 (32)	39 (61)	138 (46)
Statin use, n (%)	3 (2.4)	3 (4.8)	0 (0)	22 (7)
BMI, kg/m^2, mean (SD)	27.4 (4.8)	27.6 (5.2)	27 (4.4)	25.3 (4.2)
Estrogen use, n (% in women)	43 (64.2)	26 (41.3)	17 (26.6)	40 (25.3)
Diabetes (self-reported), n (%)	3 (2.4)	1 (1.6)	2 3.1	5 (1.7)
Alcohol use, n (%)				
No	26 (21)	15 (25)	11 (17)	35 (12)
Yes	96 (79)	45 (75)	52 (83)	263 (88)

Abbreviations: BMI, body mass index; RDD, random digit-dialing.

[a] There were some missing data on BMI, estrogen use, statin use, alcohol use and diabetes.

[b] Age is expressed in years.

Table 2 Association of Apolipoproteins With Levels of Hemostatic Factors and C-Reactive Protein in RDD Controls Randomly Selected From the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis Case-Control Study, the Netherlands, 1999–2004

Parameters	**ApoC-I** (n=292)			**ApoC-II**[f] (n=286)			**ApoC-III** (n=292)*			**ApoE** (n=292)*		
	per SD increase (5.33 mg/L)	(95%CI)		per SD increase (0.97 mg/L)	(95%CI)		per SD increase (37.11 mg/L)	(95%CI)		per SD increase (13.03 mg/L)	(95%CI)	
Anticoagulant factors												
Protein C (IU/dL)[e]	8.21	(6.02;	10.40)	6.25	(3.77;	8.72)	8.00	(5.73;	10.26)	7.34	(5.00;	9.68)
Protein S antigen (IU/dL)[e]	5.51	(3.52;	7.50)	5.26	(3.10;	7.42)	5.74	(3.71;	7.78)	3.86	(1.72;	5.99)
Antithrombin (IU/dL)	2.78	(1.56;	4.00)	2.12	(0.77;	3.46)	2.41	(1.19;	3.64)	1.74	(0.45;	3.02)
TFPI activity (IU/mL)	0.07	(0.01;	0.12)	0.04	(-0.02;	0.10)	0.00	(-0.05;	0.06)	0.04	(-0.01;	0.10)
Procoagulant factors												
Fibrinogen (g/L)	-0.07	(-0.14;	0.00)	0.02	(-0.06;	0.10)	0.01	(-0.07;	0.08)	0.06	(-0.01;	0.14)
Factor II (IU/dL)[e]	4.47	(3.07;	5.86)	3.10	(1.55;	4.65)	4.83	(3.41;	6.24)	3.42	(1.92;	4.92)
Factor VII (IU/dL)[e]	8.10	(5.56;	10.63)	4.90	(2.07;	7.73)	9.87	(7.35;	12.39)	8.80	(6.17;	11.43)
Factor VIII (IU/dL)	1.07	(-3.72;	5.85)	2.83	(-2.37;	8.02)	6.60	(1.91;	11.30)	5.12	(0.23;	10.02)
VWF (IU/dL)	2.07	(-2.72;	6.85)	3.01	(-2.20;	8.21)	5.74	(1.02;	10.45)	6.08	(1.19;	10.96)
Factor IX antigen (IU/dL)[e]	3.62	(1.62;	5.61)	2.80	(0.63;	4.96)	5.45	(3.46;	7.43)	2.74	(0.64;	4.84)
Factor X (IU/dL)[e]	7.06	(5.22;	8.90)	4.81	(2.73;	6.90)	7.96	(6.11;	9.80)	4.70	(2.67;	6.73)
Factor XI (IU/dL)	3.67	(1.52;	5.82)	2.26	(-0.07;	4.58)	2.14	(-0.02;	4.31)	3.68	(1.46;	5.89)
Fibrinolytic factor												
Clot lysis time (min)[e]	5.91	(3.71;	8.11)	3.40	(0.95;	5.85)	5.95	(3.69;	8.20)	6.36	(4.07;	8.64)
Inflammatory marker												
hsCRP (mg/L)[f]	-0.10	(-0.21;	0.02)	0.03	(-0.10;	0.16)	0.02	(-0.10;	0.14)	0.09	(-0.03;	0.21)

Abbreviations: ApoC-I, apolipoproteinC-I; ApoC-II, apolipoproteinC-II; ApoC-III, apolipoproteinC-III; ApoE, apolipoproteinE; CI, confidence interval; TFPI, tissue factor pathway inhibitor; VWF, von Willebrand factor. [a] Adjusted for age, sex, statin use, estrogen use, BMI, alcohol use, and self-reported diabetes. [e] Individuals using VKA are excluded when analysing vitamin K dependent coagulation factors (3 RDD were using anticoagulants) [f] values were log-transformed.

1

Legends to figures

Figure 1. Flow chart of participants selection. Patients and RDD controls were randomly selected from the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis case-control study, the Netherlands, 1999–2004. Abbreviations: RDD, random digit dialing; VTE, venous thromboembolism; apo, apolipoprotein.

Figure 2. Odds ratio of venous thromboembolism for apolipoproteins C-I, C-II, C-III, and E levels when comparing patients and RDD controls randomly selected from the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis case-control study, the Netherlands, 1999–2004. The models were adjusted for: A) age and sex; B), age, sex and statin use; C) and age, sex, statin use, estrogen use, alcohol intake, body mass index, and self-reported diabetes adjusted model. Data shows odds ratio (OR) and 95% confidence interval (CI). Abbreviations: RDD, random digit dialing; VTE, venous thromboembolism; apo, apolipoprotein.

Supplementary Figure 1. The scatter plot matrix graphic illustrates the correlation between apoC-I and vitamin K dependent coagulation factors (protein C, protein S, FII, FVII, FIX, FX)

Footnotes: Abbreviations: ApoC-I, apolipoproteinC-I. Individuals using VKA are excluded when analysing vitamin K dependent coagulation factors (3 RDD were using anticoagulants).

Supplementary Figure 2. The scatter plot matrix graphic illustrates the correlation between apoC-II and vitamin K dependent coagulation factors (protein C, protein S, FII, FVII, FIX, FX).

Footnotes: Abbreviations: ApoC-II, apolipoproteinC-II. Individuals using VKA are excluded when analysing vitamin K dependent coagulation factors (3 RDD were using anticoagulants). ApoC-II values were log-transformed.

Supplementary Figure 3. The scatter plot matrix graphic illustrates the correlation between apoC-III and vitamin K dependent coagulation factors (protein C, protein S, FII, FVII, FIX, FX).

Footnotes: Abbreviations: ApoC-III, apolipoproteinC-III. Individuals using VKA are excluded when analysing vitamin K dependent coagulation factors (3 RDD were using anticoagulants).

Supplementary Figure 4. The scatter plot matrix graphic illustrates the correlation between apoE and vitamin K dependent coagulation factors (protein C, protein S, FII, FVII, FIX, FX).

Footnotes: Abbreviations: ApoE, apolipoproteinE. Individuals using VKA are excluded when analysing vitamin K dependent coagulation factors (3 RDD were using anticoagulants).

Supplementary Figure 5. The scatter plot matrix graphic illustrates the correlation between apoC-I and factor VIII and VWF.

Footnotes: Abbreviations: ApoC-I, apolipoproteinC-I.

Supplementary Figure 6. The scatter plot matrix graphic illustrates the correlation between apoC-II and factor VIII and VWF.

Footnotes: Abbreviations: ApoC-II, apolipoproteinC-II. ApoC-II values were log-transformed.

Supplementary Figure 7. The scatter plot matrix graphic illustrates the correlation between apoC-III and factor VIII and VWF.

Footnotes: Abbreviations: ApoC-III, apolipoproteinC-III.

Supplementary Figure 8. The scatter plot matrix graphic illustrates the correlation between apoE and factor VIII and VWF.

Footnotes: Abbreviations: ApoE, apolipoproteinE.

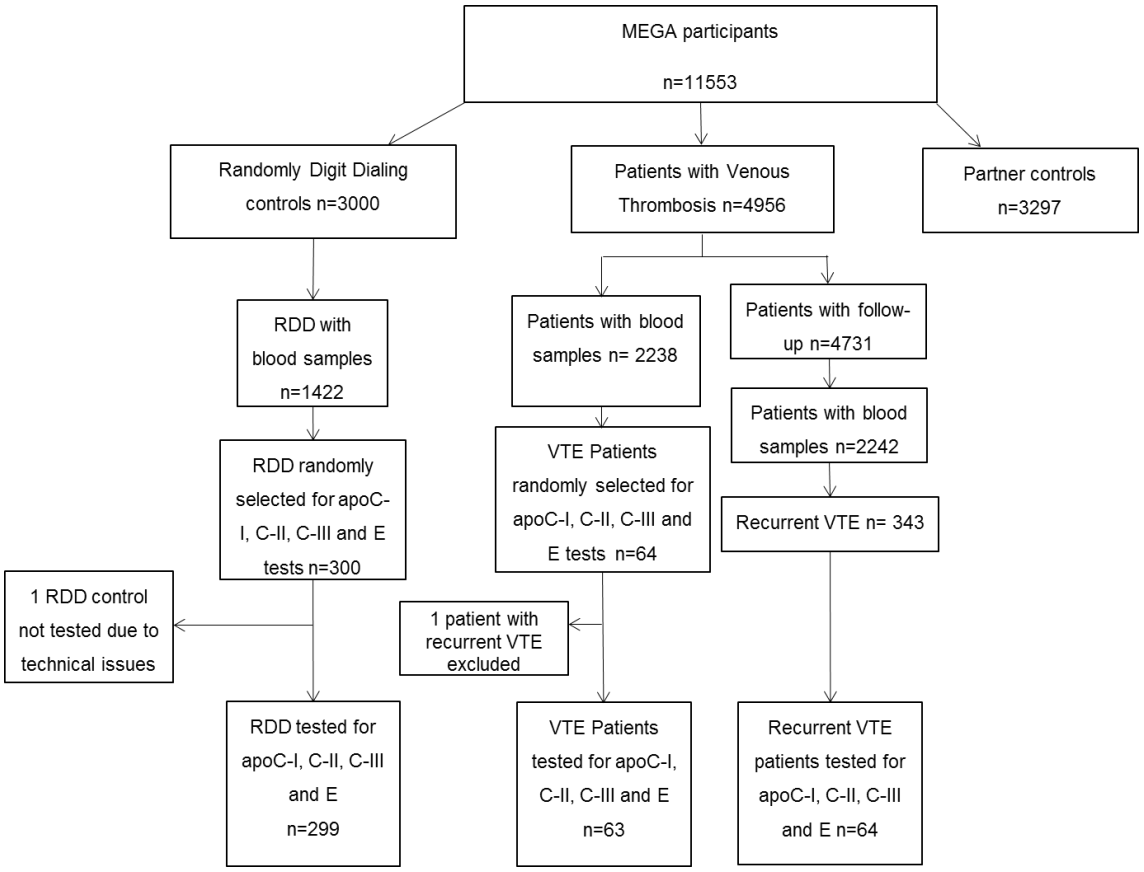


Figure 1. Flow chart of participants selection. Patients and RDD controls were randomly selected from the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis case-control study, the Netherlands, 1999–2004. A total of 127 patients with a first VTE event were selected from the MEGA and MEGA follow-up studies, 63 had only one VTE event and 64 had recurrent VTE. Abbreviations: RDD, random digit dialing; VTE, venous thromboembolism; apo, apolipoprotein.

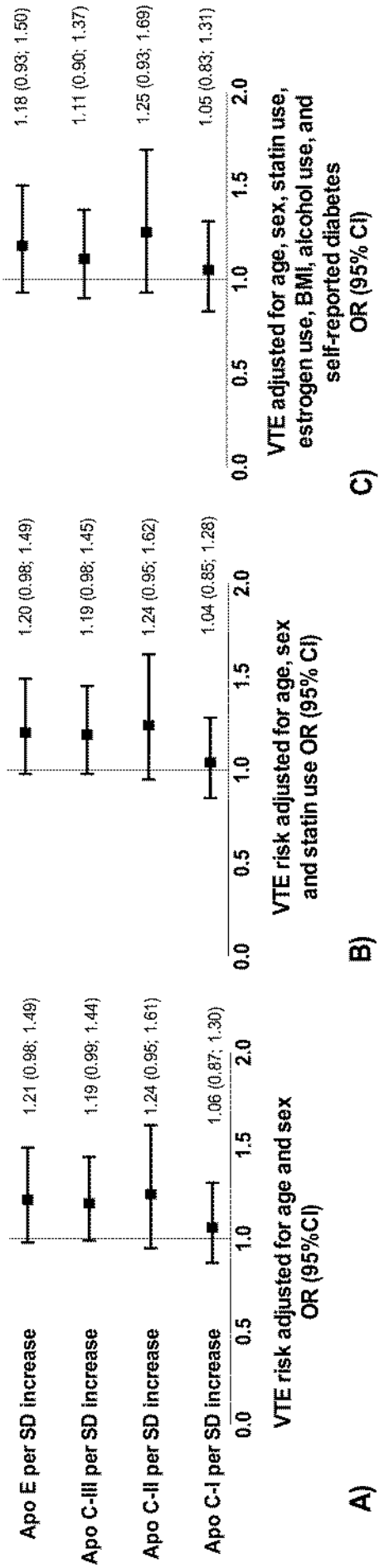
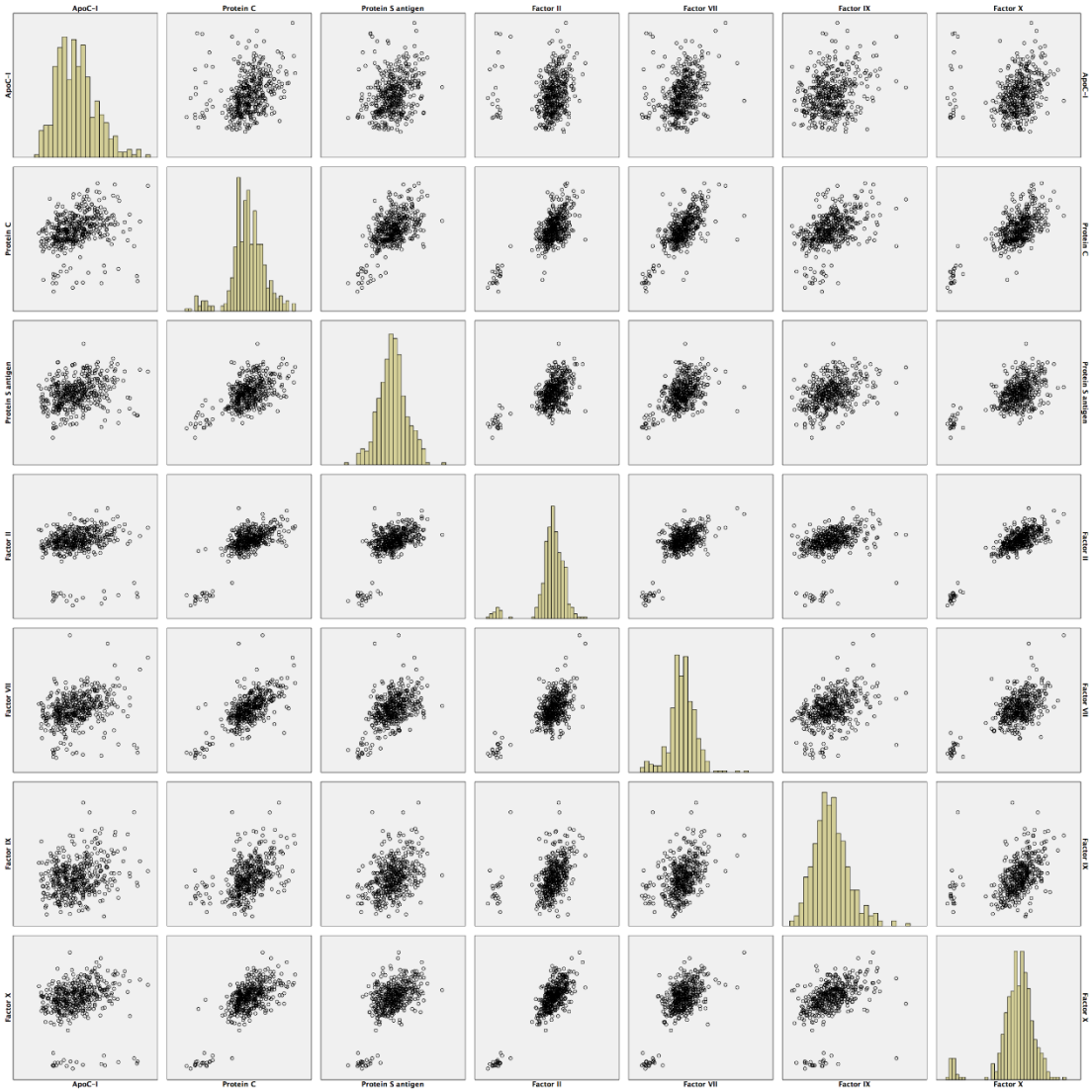
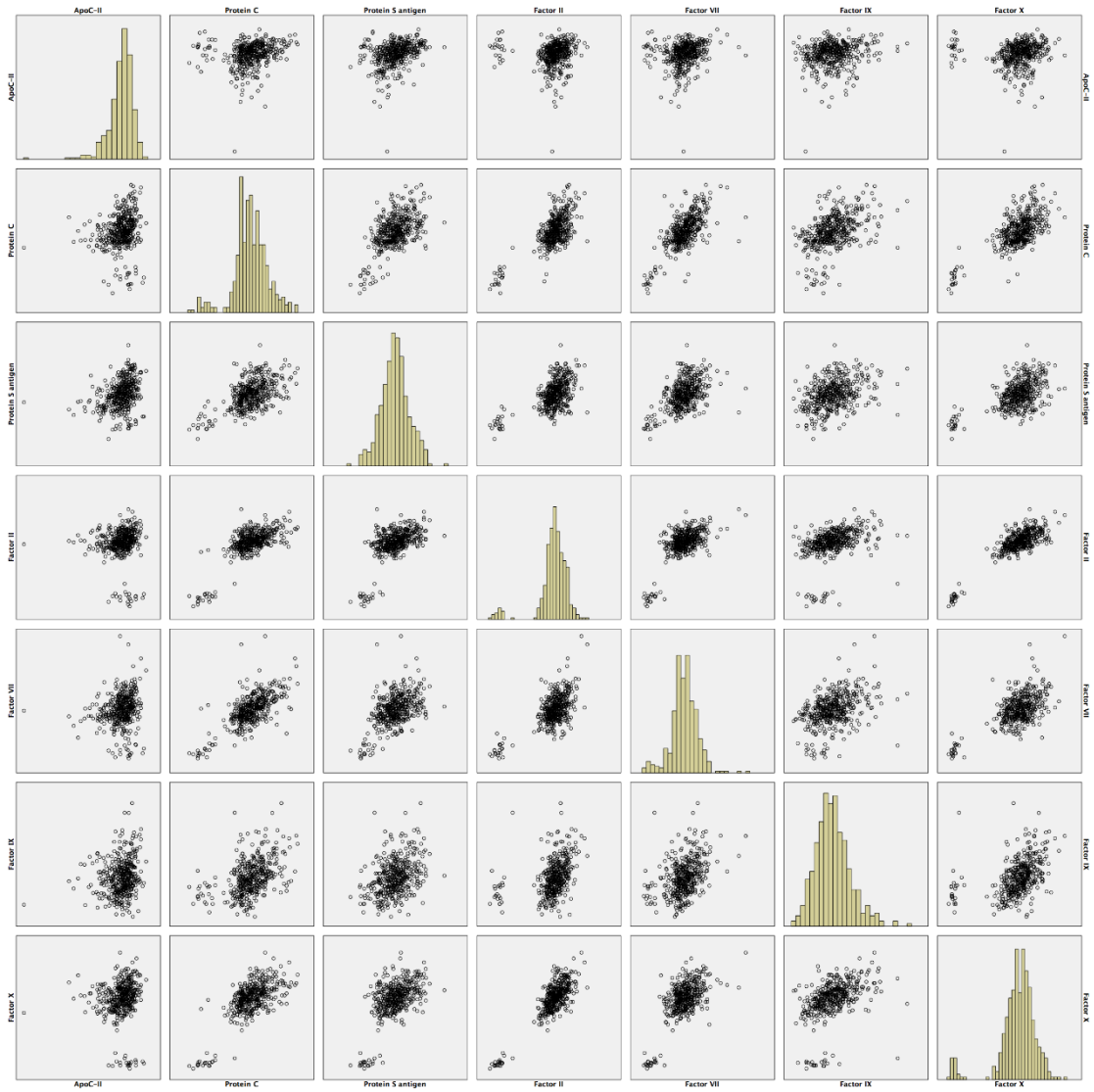


Figure 2. Odds ratio of venous thromboembolism for apolipoproteins C-I, C-II, C-III, and E levels when comparing patients with first VTE (n=127) and RDD (n=299) controls randomly selected from the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis case-control study, the Netherlands, 1999–2004. The models were adjusted for: A) age and sex; B), age, sex and statin use; C) and age, sex, statin use, estrogen use, alcohol intake, body mass index, and self-reported diabetes adjusted model. Data shows odds ratio (OR) and 95% confidence interval (CI). Abbreviations: RDD, random digit dialing; VTE, venous thromboembolism; apo, apolipoprotein.



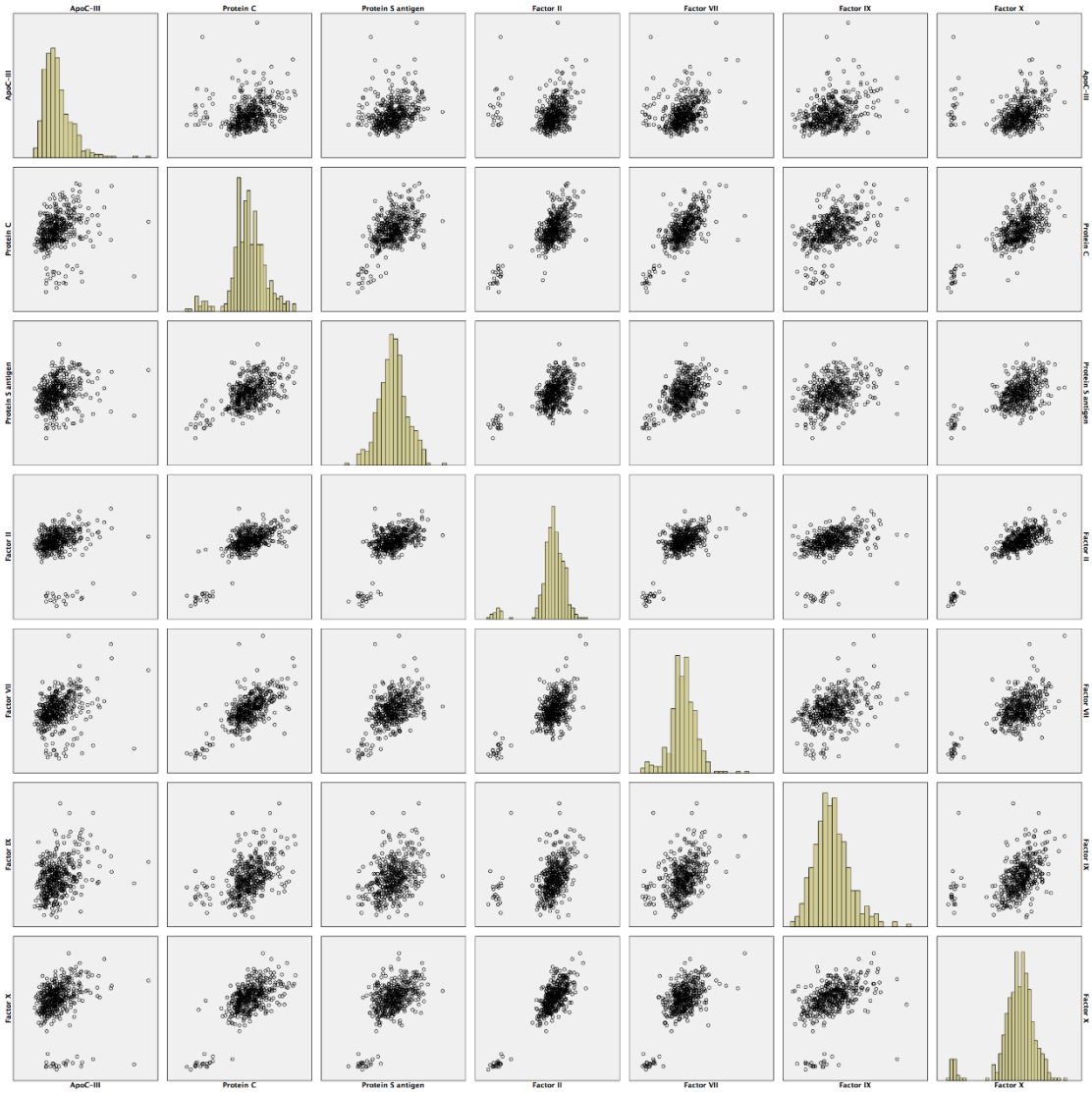
Supplementary Figure 1. The scatter plot matrix graphic illustrates the correlation between apoC-I and vitamin K dependent coagulation factors (protein C, protein S, FII, FVII, FIX, FX)

Footnotes: Abbreviations: ApoC-I, apolipoproteinC-I. Individuals using VKA are excluded when analysing vitamin K dependent coagulation factors (3 RDD were using anticoagulants).



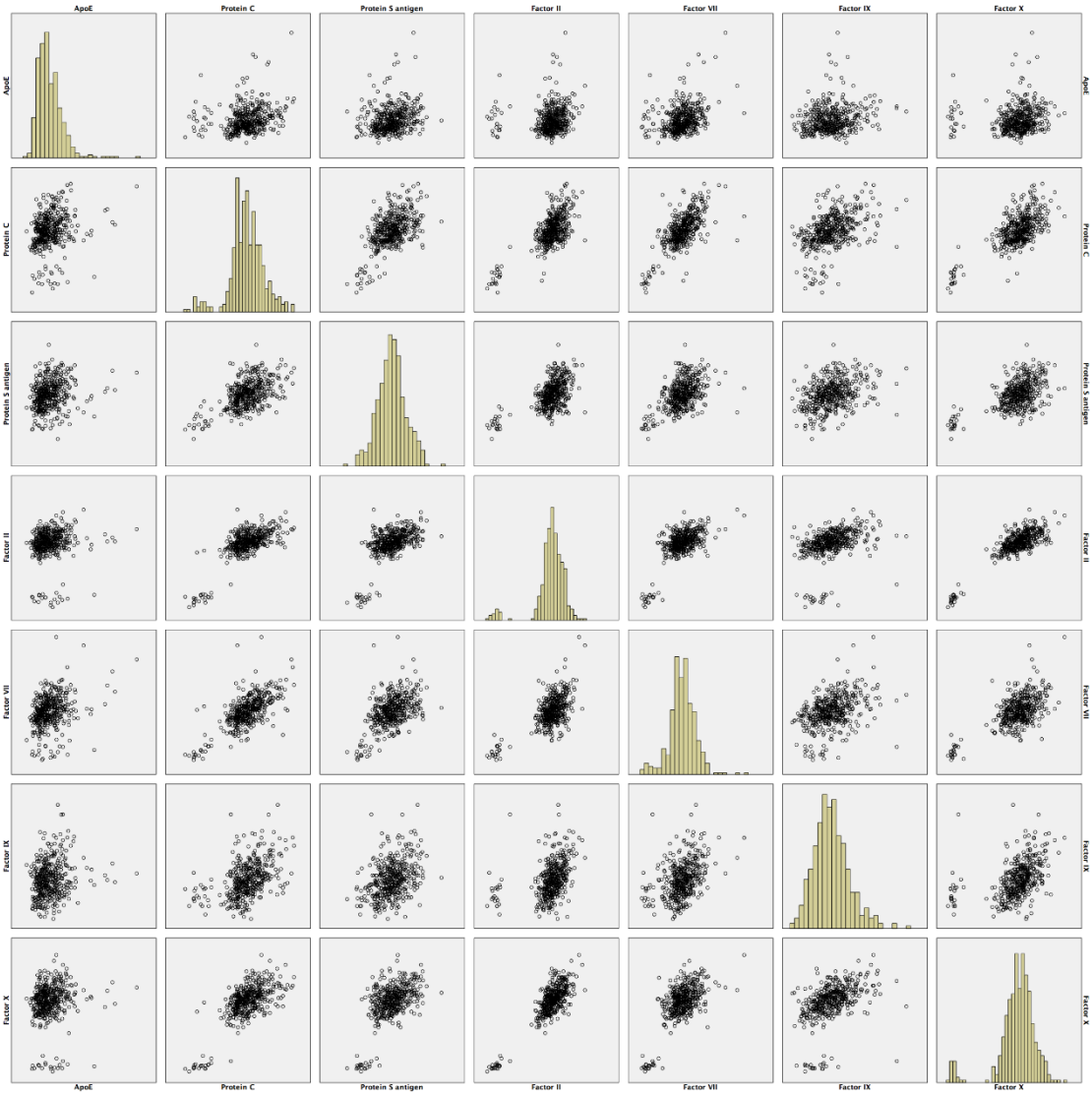
Supplementary Figure 2. The scatter plot matrix graphic illustrates the correlation between apoC-II and vitamin K dependent coagulation factors (protein C, protein S, FII, FVII, FIX, FX).

Footnotes: Abbreviations: ApoC-II, apolipoproteinC-II. Individuals using VKA are excluded when analysing vitamin K dependent coagulation factors (3 RDD were using anticoagulants). ApoC-II values were log-transformed.



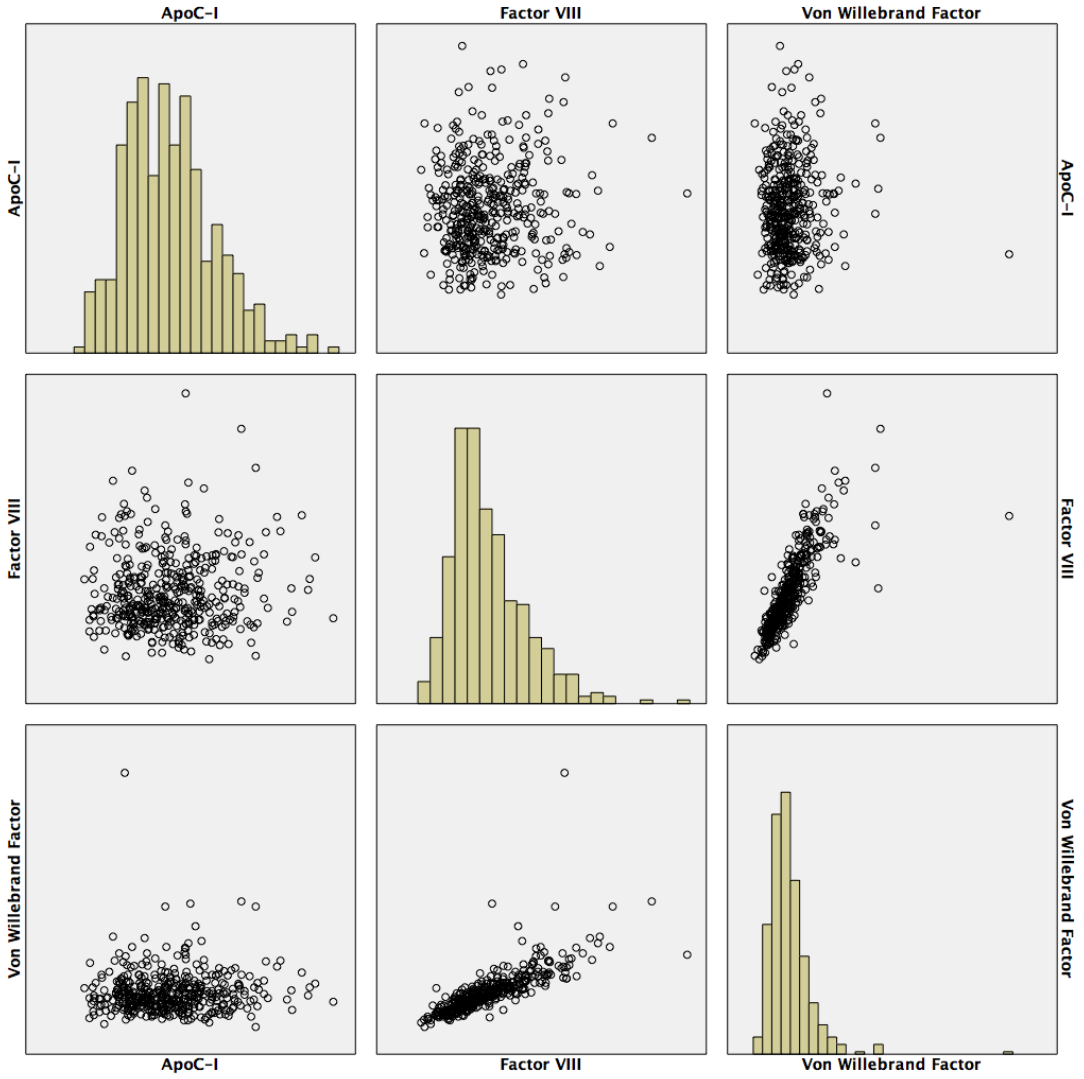
Supplementary Figure 3. The scatter plot matrix graphic illustrates the correlation between apoC-III and vitamin K dependent coagulation factors (protein C, protein S, FII, FVII, FIX, FX).

Footnotes: Abbreviations: ApoC-III, apolipoproteinC-III. Individuals using VKA are excluded when analysing vitamin K dependent coagulation factors (3 RDD were using anticoagulants).



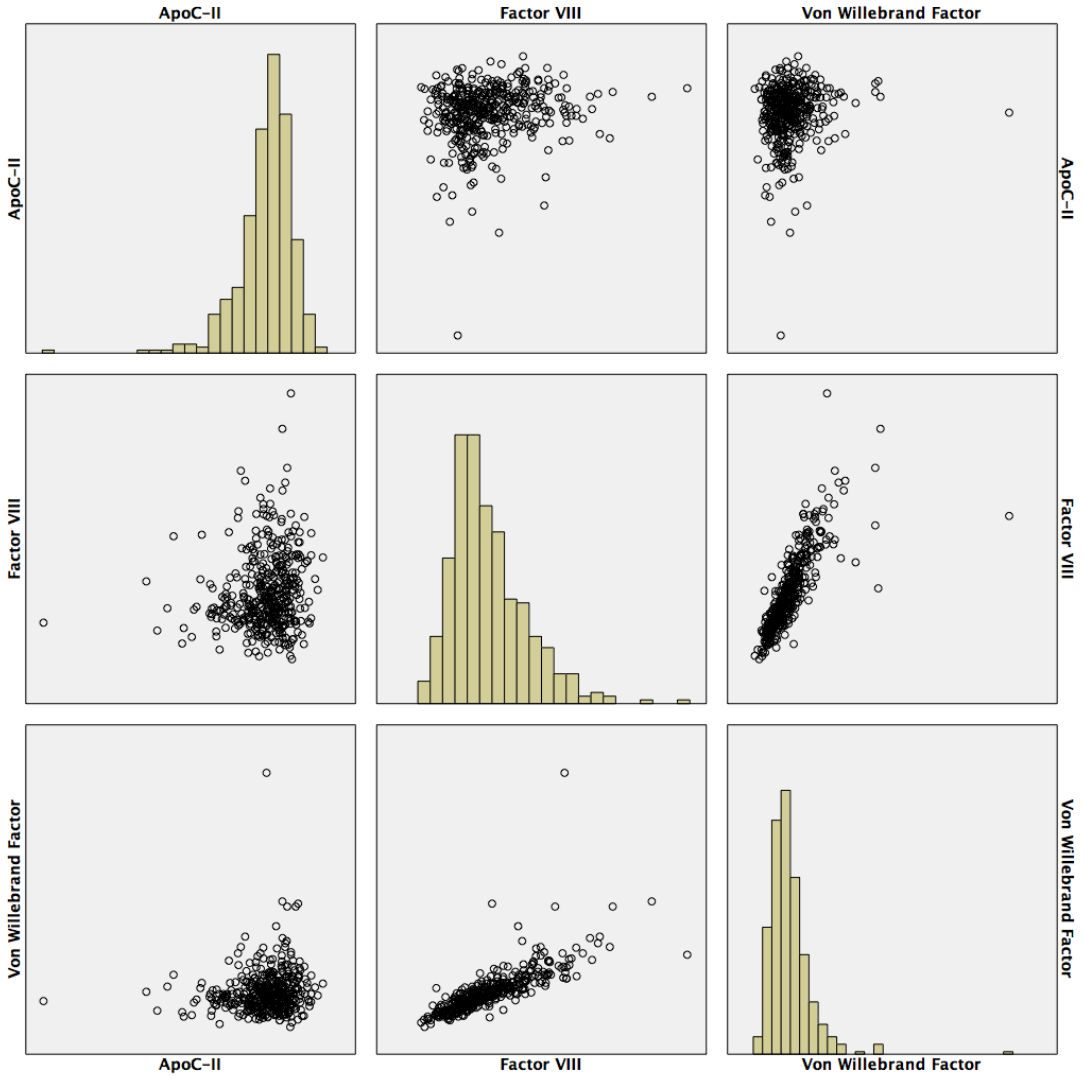
Supplementary Figure 4. The scatter plot matrix graphic illustrates the correlation between apoE and vitamin K dependent coagulation factors (protein C, protein S, FII, FVII, FIX, FX).

Footnotes: Abbreviations: ApoE, apolipoproteinE. Individuals using VKA are excluded when analysing vitamin K dependent coagulation factors (3 RDD were using anticoagulants).



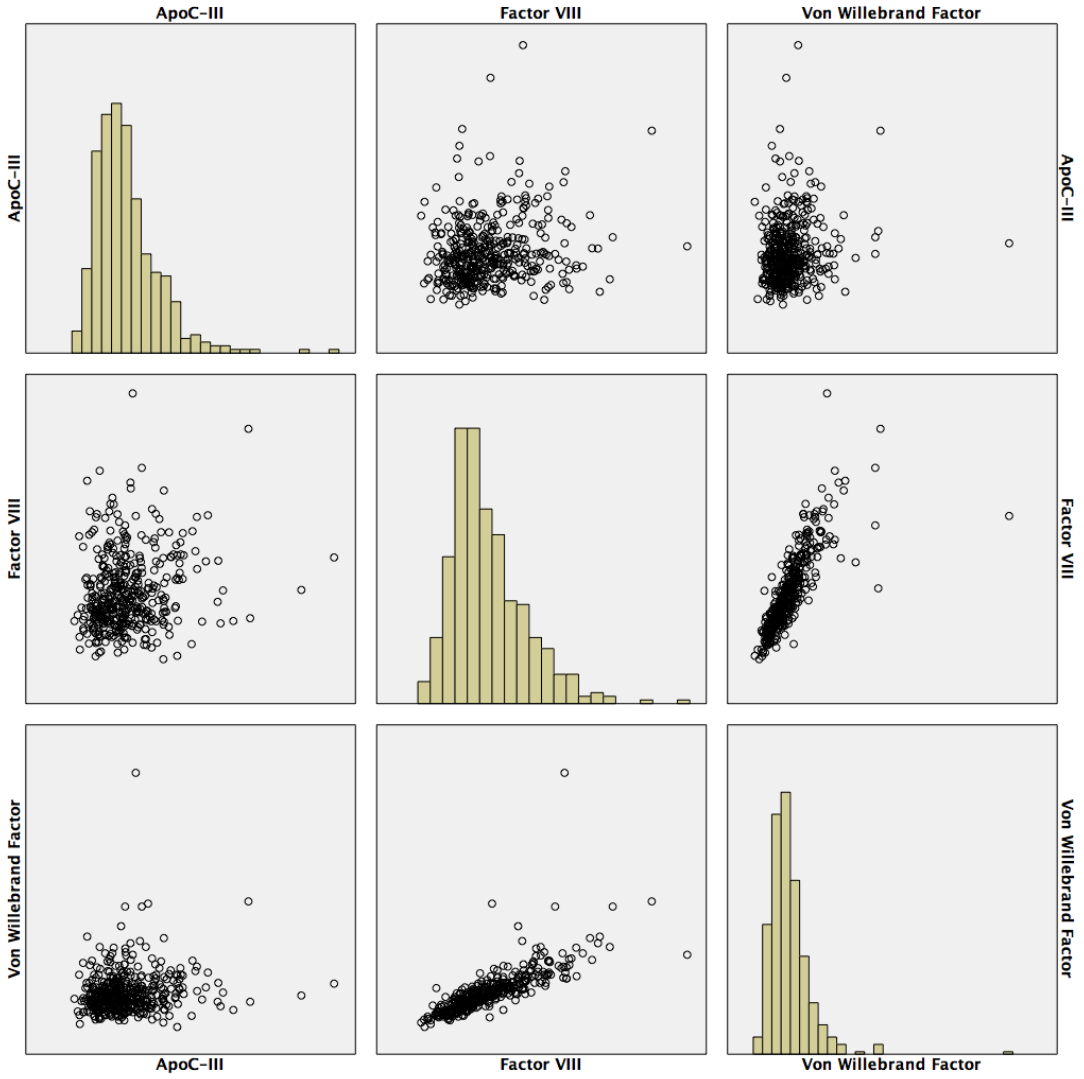
Supplementary Figure 5. The scatter plot matrix graphic illustrates the correlation between apoC-I and factor VIII and VWF.

Footnotes: Abbreviations: ApoC-I, apolipoproteinC-I.



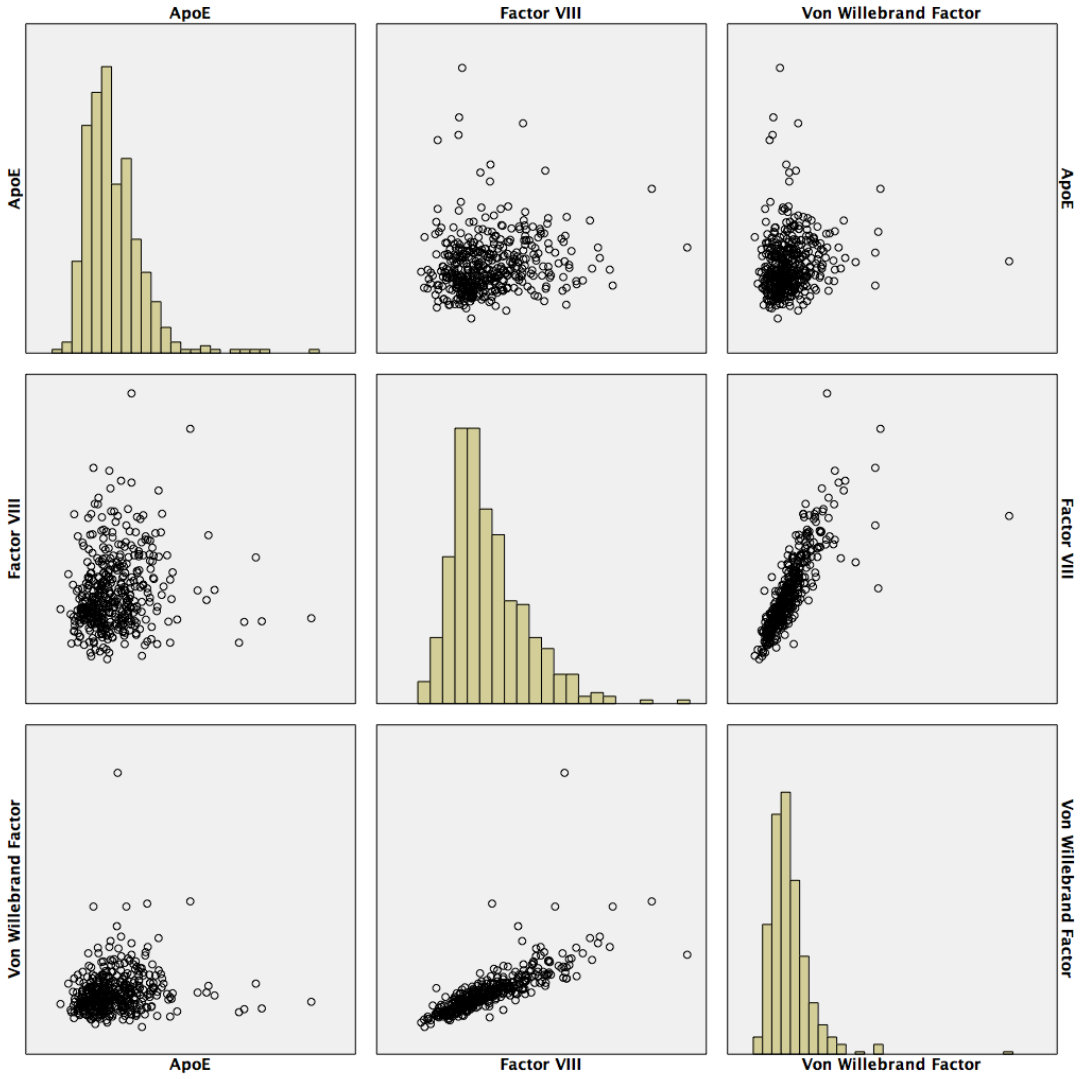
Supplementary Figure 6. The scatter plot matrix graphic illustrates the correlation between apoC-II and factor VIII and VWF.

Footnotes: Abbreviations: ApoC-II, apolipoproteinC-II. ApoC-II values were log-transformed.



Supplementary Figure 7. The scatter plot matrix graphic illustrates the correlation between apoC-III and factor VIII and VWF.

Footnotes: Abbreviations: ApoC-III, apolipoproteinC-III.



Supplementary Figure 8. The scatter plot matrix graphic illustrates the correlation between apoE and factor VIII and VWF.

Footnotes: Abbreviations: ApoE, apolipoproteinE.