Cardiometabolic risk factors and venous thrombosis

Morelli, V.M.

Chapter 5

Interrelation between levels of hemostatic factors, lipids and C-reactive protein in population controls

V.M. Morelli, R. Li-Gao, W.M. Lijfering, F.R. Rosendaal,
S.C. Cannegieter, A. van Hylckama Vlieg

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ABSTRACT

Background

Previous studies have shown that hemostatic factor levels are interrelated and clustered together. However, results were not consistent, probably due to differences in sample sizes, study population, and variables studied. Furthermore, since arterial and venous thrombosis share some traditional risk factors, we questioned if and how hemostatic factors cluster with lipids and C-reactive protein (CRP).

Objective

To assess the clustering of hemostatic factor levels, and how these clusters relate to lipid and CRP levels.

Methods

We included 2874 individuals (47% men) who had participated as population controls in a previous study. Clusters of interrelated factors were identified by principal component analysis. A factor loading >0.40 was used as the marginal value to include factors in a cluster.

Results

We identified 3 clusters among the hemostatic factors: a vitamin K-dependent factor (VKDF) cluster (factors [F]II, VII, IX, X, protein C and protein S [PS]) that also included FXI and antithrombin (AT); another comprising fibrinogen, FVIII, von Willebrand factor (VWF) and D-dimer, and a third one including FV, tissue factor pathway inhibitor, PS and AT. The addition of lipid fractions and CRP led to two extra lipid clusters, with triglycerides also clustering with VKDFs. VWF and FVIII now formed a separate cluster, and CRP clustered with fibrinogen, D-dimer and FIX. When individuals with malignancies or self-reported chronic diseases were excluded (n=570), the clustering pattern remained virtually the same.

Conclusions

In this comprehensive study, we confirmed and extended clustering patterns of previous reports between levels of hemostatic factors, lipids and CRP.

INTRODUCTION

Previous studies have shown that hemostatic factor levels are interrelated and clustered together [1-3], suggesting that there are common mechanisms located outside the hemostatic factor genes that could regulate levels of several proteins in the hemostatic system [2]. In these studies, levels of procoagulant vitamin K-dependent factors (VKDFs), i.e. factors(F) II, VII, IX and X, consistently clustered together [1-3]. However, the clustering pattern of other hemostatic factors substantially differed among reports, probably due to distinct samples sizes, study population, and hemostatic factors studied [1-3].

It is noteworthy that some hemostatic factors have been shown to cluster with cardiometabolic risk factors. For instance, procoagulant VKDF levels clustered together with lipids, in particular, triglyceride levels [1,4], whereas levels of fibrinogen, FVIII, FIX, and D-dimer clustered with levels of C-reactive protein (CRP) [1], an inflammatory risk marker of arterial cardiovascular disease (CVD) [5]. However, the interrelation between hemostatic factor, lipid and CRP levels is not known in detail, as studies on this topic are few, involve small sample sizes, and study a limited number of lipids and hemostatic factors [1,4]. Clarification of this issue is relevant since several studies in the past decade have shown that venous thrombosis is associated with an increased risk of subsequent arterial CVD [6-10], and that the two diseases may share common risk factors [8,11]. Indeed, cardiometabolic risk factors, such as dyslipidemia, inflammation and obesity, are well known to increase the risk of arterial CVD [12-14] but are also associated with venous thrombosis [15-18]. Knowledge on the interrelation between levels of hemostatic factors, lipids and inflammation could provide insights on mechanisms underlying the associations of cardiometabolic risk factors with venous thrombosis. In addition, these insights may further reveal why lipid-lowering drugs (statins) are not only able to decrease the risk of arterial CVD, but also of venous thrombosis [19].

The aim of this study was to investigate the clustering of hemostatic factor levels, and how these clusters relate to lipid and CRP levels in population controls. For this purpose, we used data from the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study.

METHODS

Study population

The study population was comprised of the control group from the MEGA study, details of which have been previously described [20]. Briefly, between March 1999 and September 2004, 4956 consecutive patients aged 18-70 years with a first objectively confirmed deep vein thrombosis of the leg or pulmonary embolism were enrolled

from six anticoagulation clinics in the Netherlands. During the same time period, 6297 control subjects without a history of venous thrombosis were included. Control subjects were either partners of the patients (n = 3297) or individuals approached by random digit dialing [RDD] (n = 3000). In the MEGA study, blood sampling was determined by calendar time, i.e., for logistic reasons participants were asked to provide blood samples up to June 2002 only. Of the 6297 control subjects, 2943 provided blood samples. We excluded 41 individuals because the quality of their plasma was not adequate for blood coagulation assays, leaving 2902 control subjects. For the current analysis, to eliminate any influence of anticoagulant treatment on the interpretation of the results of the hemostatic factors, control subjects on anticoagulation at blood sampling were excluded (n = 28). Therefore, the present study included 2874 population controls, of whom 570 had a history of malignancy or chronic disease, defined as self-reported liver disease, kidney disease, rheumatoid arthritis, multiple sclerosis, diabetes mellitus, hypothyroidism, hyperthyroidism, chronic bronchitis, emphysema, thrombophlebitis, heart failure, hemorrhagic stroke, and arterial CVD (angina, myocardial infarction, peripheral vascular disease, ischemic stroke, and transient ischemic attack). This study was approved by the Ethics Committee of the Leiden University Medical Center, and written informed consent was obtained from all participants.

Laboratory measurements

Blood collection and laboratory measurements have been previously described in the MEGA study [21-24]. Briefly, fibrinogen activity was measured according to the method of Clauss on a STA-R analyzer [25]. FII activity, FVII activity, FVIII activity, FX activity, and FXI activity were measured with a mechanical clot detection method on a STA-R analyzer (Diagnostica Stago) [21]. FV and FIX antigen levels were determined by enzyme-linked immunosorbent assay (ELISA), and von Willebrand factor (VWF) antigen was measured by an immunoturbidimetric method using STA Liatest kit (Diagnostica Stago) [21]. Measurements of antithrombin and protein C levels were performed with a chromogenic assay, whereas total protein S levels were determined by ELISA (Diagnostica Stago) [21]. Total tissue factor pathway inhibitor (TFPI) activity levels were assessed in citrated plasma by measuring TFPI inhibition of the catalytic tissue factor (TF)-factor VIIa (FVIIa) complex using the Actichrome TFPI activity assay (Sekisui Diagnostics); one unit of TFPI activity corresponds to 55 ng/ml plasma TFPI. D-dimer was measured with the D-dimer HemosIL assay on an ACL TOP 700 analyzer (Instrumentation Laboratory) [22]. CRP levels and lipid levels, i.e. triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), apolipoprotein A1 (apo A1), and apolipoprotein (apo B) were measured as previously described [23,24].

Statistical analysis

Principal component analysis with orthogonal varimax rotation was used to assess the interrelations between levels of hemostatic factors, lipids and CRP, as previously described [2]. The central idea of principal component analysis is to reduce the dimensionality of a data set consisting of a large number of interrelated variables, while retaining as much as possible of the variation present in the data set [26]. This is achieved by transforming to a new set of variables, the principal components, which are uncorrelated, and which are ordered so that the first few retain most of the variation present in all of the original variables [26]. Orthogonal varimax rotation is used to achieve a strong correlation between each of the original variables and only one of the new principal components (simple structure). After rotation, the new principal components remain relatively independent and this rotation has no effect on the variation in the data that is explained by the new components.

For each principal component, the analysis generates an eigenvalue, which is the sum of squared correlations between the original variables and the principal component. The eigenvalue indicates the variance attributable to a principal component. Standardized variables have a variance equal to 1. An eigenvalue greater than 1 indicates that the corresponding principal component accounts for more total variance than the original standardized variables. Only principal components with an eigenvalue greater than 1 were selected in this analysis.

The inclusion of variables in a cluster depends on their factor loadings, which can be interpreted as the correlation coefficient between the original variable and the newly formed (principal) components. We used a factor loading of 0.40 as a marginal value, i.e. only factor loadings >0.40 are considered in the identification of a clustering pattern. This marginal value is arbitrarily chosen in such a manner that there will be little overlap between any of the newly formed components, thereby leading to an understandable interpretation of the results.

Since D-dimer, CRP and TG levels were not normally distributed they were natural log-transformed. Principal component analysis was performed first on hemostatic factors (procoagulant, anticoagulant and fibrinolytic factors), and second with the addition of CRP and lipid levels. All analyses were repeated excluding controls with malignancy or self -reported chronic diseases. Statistical analyses were performed with SPSS for Windows, release 23.0 (SPSS Inc, Chicago, IL).

RESULTS

Table 1 shows the characteristics of the study population. In the group of 2874 control subjects, the median age was 49.7 years (interquartile range 39.2-58.1 years], and 1353 (47.1%) were men. Levels of procoagulant, anticoagulant and fibrinolytic hemostatic factors, lipids and CRP are described in Table 1.

Table 1. Characteristics of the study population

Characteristics	Total study population	n = 2874
Demographic factors		
Male, n (%)	1353	(47.1)
Age (years)	49.7	(39.2-58.1)
Procoagulant factors		
Fibrinogen (g/L)	3.3	(0.7)
Factor II (IU/dL)	110.7	(15.3)
Factor V (U/mL)	0.93	(0.17)
Factor VII (IU/dL)	111.3	(24.7)
Factor VIII (IU/dL)	112.2	(38.2)
VWF (IU/dL)	111.4	(46.0)
Factor IX (IU/dL)	104.6	(18.4)
Factor X (IU/dL)	117.1	(18.6)
Factor XI (IU/dL)	100.2	(19.1)
Anticoagulant factors		
Antithrombin (IU/dL)	105.3	(10.7)
Protein C (IU/dL)	117.7	(21.1)
Protein S (IU/dL)	102.5	(19.7)
TFPI (U/dL)	174.5	(46.0)
Fibrinolytic factor		
D-dimer (ng/mL) †	236.6	(167.6-357.0)
Lipid profile		
Triglycerides (mmol/L) †	1.32	(1.00-1.87)
TC (mmol/L)	5.62	(1.11)
LDL-C (mmol/L)	3.56	(0.95)
HDL-C (mmol/L)	1.35	(0.39)
Apo A1 (g/L)	1.43	(0.29)
Apo B (g/L)	0.99	(0.26)
Inflammatory marker		
CRP (mg/L) †	1.43	(0.68-3.13)

Continuous variables are shown as mean (± standard deviation), or median (25th percentile - 75th percentile) when non-normally distributed (as marked with '†'). Categorical variables are shown as number (%).

Data were missing for some participants in some subgroups.

apo A1, apolipoprotein A1; apo B, apolipoprotein B; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TFPI, tissue factor pathway inhibitor; VWF, von Willebrand factor.

As depicted in Table 2, there were 3 clusters among the hemostatic factors with an eigenvalue greater than 1, which together accounted for more than 50% of the total variance. We identified a VKDF cluster (component 1 in Table 2), composed of all VKDFs (FII, FVII, FIX, FX, protein C, and protein S), that also included FXI and antithrombin. Another cluster (component 2 in Table 2) included FVIII, VWF,

Table 2. Principal component analysis with hemostatic factors in 2874 population controls. Factor loadings between the original variables and the newly formed components are shown

	Components		
Original variable	**1**	**2**	**3**
Procoagulant factors			
Fibrinogen	0.371	**0.556***	0.154
Factor II	**0.741***	-0.011	0.051
Factor V	0.147	0.175	**0.641***
Factor VII	**0.657***	0.125	0.164
Factor VIII	0.064	**0.818***	0.117
VWF	-0.003	**0.833***	0.096
Factor IX	**0.690***	0.298	-0.012
Factor X	**0.818***	0.021	-0.036
Factor XI	**0.517***	0.085	0.227
Anticoagulant factors			
Antithrombin	**0.415***	-0.301	**0.455***
Protein C	**0.748***	-0.042	0.226
Protein S	**0.475***	0.134	**0.456***
TFPI activity	-0.003	0.055	**0.785***
Fibrinolytic factor			
D-dimer†	0.034	**0.611***	-0.031
Variance			
% total variance	29.56	14.92	8.65
% cumulative variance	29.56	44.48	53.13

Data were missing for some participants in some subgroups.

The principal components were ranked according to their eigenvalues from 1 to 3.

TFPI, tissue factor pathway inhibitor; VWF, von Willebrand factor.

*Factor Loading > 0.40.

†Log-transformed variable.

fibrinogen and D-dimer, and a third one (component 3 in Table 2) was comprised of TFPI, FV, protein S, and antithrombin. Exclusion of control subjects with malignancy or self-reported chronic diseases yielded virtually the same clustering pattern, with the exception of antithrombin that was no longer included in the VKDF cluster (Table S1).

The inclusion of lipid fractions and CRP in the analysis led to three additional clusters with an eigenvalue greater than 1, now resulting in the formation of 6 principal components, which together accounted for almost 70% of the total variance. As shown in Table 3 and Fig. 1, there were two extra lipid clusters: one composed of TC, LDL-C, and apo B that also included TFPI (component 2 in Table 3), and another comprising HDL-C, apo A1 and TG that did not include any hemostatic factor (component 3 in Table 3). The VKDF cluster (component 1 in Table 3) now includes

Table 3. Principal component analysis with hemostatic factors, lipids and C-reactive protein in 2874 population controls. Factor loadings between the original variables and the newly formed components are shown

	Components					
Original variable	**1**	**2**	**3**	**4**	**5**	**6**
Procoagulant factors						
Fibrinogen	0.259	0.039	-0.083	**0.725***	0.159	0.268
Factor II	**0.675***	0.139	0.047	0.121	-0.080	0.131
Factor V	0.113	0.130	0.016	0.118	0.128	**0.625***
Factor VII	**0.656***	0.192	0.102	0.056	0.145	0.093
Factor VIII	0.087	0.003	0.044	0.171	**0.907***	0.098
VWF	0.017	0.061	-0.007	0.190	**0.909***	0.047
Factor IX	**0.649***	0.059	-0.110	**0.418***	0.096	0.031
Factor X	**0.794***	0.083	0.035	0.131	-0.032	0.007
Factor XI	**0.468***	-0.030	0.116	0.108	0.031	**0.409***
Anticoagulant factors						
Antithrombin	**0.452***	-0.057	-0.032	-0.375	-0.073	**0.542***
Protein C	**0.746***	0.212	0.103	-0.084	0.052	0.183
Protein S	**0.416***	0.204	-0.161	0.120	0.073	**0.451***
TFPI	-0.067	**0.403***	-0.066	0.075	0.002	**0.612***
Fibrinolytic factor						
D-dimer†	-0.087	0.021	0.093	**0.649***	0.218	0.091
Lipid profile						
Triglycerides†	**0.547***	0.365	**-0.460***	0.003	0.107	-0.155
TC	0.273	**0.911***	0.204	-0.005	0.048	0.136
LDL-C	0.133	**0.928***	-0.012	0.019	0.012	0.203
HDL-C	-0.003	-0.026	**0.970***	-0.046	0.019	0.007
Apo A1	0.209	0.046	**0.913***	-0.004	0.045	-0.101
Apo B	0.252	**0.893***	-0.197	0.075	0.026	0.116
Inflammatory marker						
CRP†	0.336	0.019	-0.097	**0.794***	0.036	-0.098
Variance						
% total variance	26.62	11.45	10.66	9.07	6.12	5.14
% cumulative variance	26.62	38.07	48.73	57.80	63.92	69.06

Data were missing for some participants in some subgroups.

The principal components were ranked according to their eigenvalues from 1 to 6.

apo A1, apolipoprotein A1; apo B, apolipoprotein B; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TFPI, tissue factor pathway inhibitor; VWF, von Willebrand factor.

*Factor Loading > 0.40.

†Log-transformed variables.

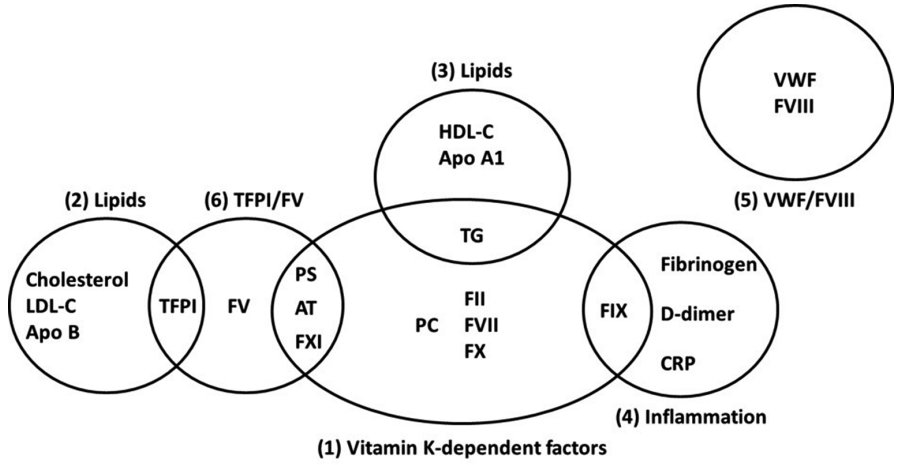

Figure 1. Factor loading pattern of hemostatic factors, lipids and C-reactive protein in 2874 population controls from the the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA) study. The principal components are represented by ellipses, and were ranked according to their eigenvalues from 1 to 6 (numbers between parentheses). AT, antithrombin; apo A1, apolipoprotein A1; apo B, apolipoprotein B; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PC, protein C; PS, protein S; TFPI, tissue factor pathway inhibitor; TG, triglycerides; VWF, von Willebrand factor.

TG. FVIII and WVF formed a new separate cluster (component 5 in Table 3), whereas CRP levels clustered with fibrinogen, D-dimer, and factor IX levels (component 4 in Table 3). The cluster composed of TFPI, FV, protein S, and antithrombin (component 6 in Table 3) remained practically unchanged, with the addition of FXI only. The exclusion of individuals with malignancy or self-reported chronic diseases resulted in minor changes in the clustering pattern, in which the 6 principal components also accounted for almost 70% of the total variance (Tables S2).

DISCUSSION

In this study, we investigated the interrelation between levels of hemostatic factors, lipids and CRP in 2874 population controls. We included several procoagulant and anticoagulant hemostatic factors and lipids, which enabled us to perform a comprehensive analysis on their clustering pattern. Among our main findings is that VKDFs, including procoagulant (FII, FVII, FIX, and FX) and anticoagulant (protein C and protein S) factors, clustered together. Upon addition of lipids to the analysis, all VKDFs consistently clustered with TG levels. Furthermore, we found that CRP levels clustered together with fibrinogen, D-dimer, and FIX levels. The clustering patterns

observed in this study suggest that there might be common mechanisms regulating hemostatic factor and lipid levels, and the inflammatory response. Whether and to what extent the interrelation between levels of hemostatic factors, lipids and CRP contributes to the risk of venous thrombosis and arterial CVD remains unsettled. This is a relevant issue from both a mechanistic and a clinical viewpoint, as therapeutic strategies targeting possible common regulatory mechanisms of hemostatic factors and cardiometabolic risk factors, like statins [19,27], have the potential to decrease the risk of both venous thrombosis and arterial CVD. Indeed, experimental data have demonstrated that statins may have antithrombotic effects that are unrelated to their lipid-lowering activity [27].

Since the liver is the main site of production of coagulation factors [28], it is biologically plausible that the interrelation between hemostatic factors could be explained, at least in part, by common mechanisms regulating their biosynthesis. For instance, hepatocyte nuclear factor 4 (HNF-4) is a transcription factor highly expressed in the liver, that has been shown to regulate the expression of several genes, such as those encoding FII, FVII, FIX, FX, and FXI, and anticoagulant factors protein S and antithrombin [29-35]. Therefore, one may consider that common determinants in the regulation of the transcription of hemostatic factors, such as HNF-4, could contribute to the clustering of VKDF, FXI and antithrombin levels, as depicted in Fig. 1. The clustering of VKDFs could be further explained by a fundamental post-translational step during the biosynthesis of these factors. This step is a vitamin K-dependent enzymatic reaction mediated by γ-glutamyl carboxylase, which leads to the conversion of glutamic acid to γ- carboxyglutamic acid [36]. The modification of glutamic acid residues enables these factors to bind to phospholipid membranes at physiological calcium concentration [37], which is key for their activity [38]. Although previous studies have also shown that VKDFs clustered together, including the Leiden Thrombophilia Study (LETS) [2], not all factors were measured in these studies [1], or showed a consistent clustering pattern between all procoagulant and anticoagulant VKDFs [1-3], as observed in the present analysis.

Here we found that TG, but not the other lipids studied, clustered with VKDFs. This result is in line with a previous finding from Sakkinen *et al*. [1], in which TG levels clustered together with FVII, FIX and FX in the Cardiovascular Health Study that included 322 elderly participants. Vanschoonbeek *et al*. [4] also described clustering between VKDF (FII, FVII, and FX) and TG levels in a study comprising 57 overweight men and 42 overweight individuals with type 2 diabetes. Interestingly, results from an animal model study also pointed to a relationship between VKDF and TG levels. In this study, which involved a murine model of type III hyperlipidemia, mice kept on fish oil diet (n-3 polyunsaturated fatty acids [n-3 PUFAs]) for 21 days had a reduction in plasma triglyceride levels, thrombin generation, and activity of VKDFs, but not in

VKDF mRNA levels, which remained unchanged [39]. Analysis of mouse livers showed that n-3 PUFA was associated with upregulation of genes related to lipid degradation, and downregulation of genes related to lipid synthesis and of γ-glutamyl carboxylase. The latter gene encodes the enzyme responsible for the γ-carboxylation of VKDFs, which, as previously pointed out, is a fundamental post-translational step for the activity of these factors in blood coagulation [38]. On the basis of our and other results [1,4], and the murine model study [39], one may speculate that common mechanisms lying outside the genes coding for VKDFs and TG could explain, at least in part, the clustering between VKDFs and TG.

In the present study, TC, LDL-C and apo B levels clustered together, whereas HDL-C levels formed a separate cluster with apo A1 and TG levels. These clustering patterns seem logical, as apo B is the protein component of the very low-density/low-density lipoprotein spectrum, and apo A1 is the major protein component of HDL-C [40]. Of note, total TFPI activity levels clustered with TC, LDL-C, and apo B, which is consistent with the fact that the majority of the TFPI in plasma is bound to apo B-containing lipoproteins [41]. Importantly, TFPI and FV composed a cluster that also included total protein S and antithrombin. This clustering pattern is in line with previous findings showing a close relationship between TFPI, FV, and protein S levels [42-44]. In the study by Duckers *et al*. [42], TFPI levels were markedly reduced in individuals with severe FV deficiency as compared with healthy control subjects. In the same study, FV and TFPI levels were also found to be strongly correlated in both healthy and FV-deficient individuals [42]. Thereafter, Dahm *et al*. [43] showed by means of linear regression that protein S and FV levels were the strongest determinants of TFPI levels in healthy individuals from the LETS. In experimental studies, TFPI has been suggested to bind to FV in plasma [42], and protein S has been shown to be a cofactor of TFPI that facilitates optimal FXa-inhibition and efficient down-regulation of thrombin generation in plasma [44]. Finally, we have no explanation for the clustering of antithrombin with the aforementioned factors other than that they are inhibitors of the coagulation system (with the exception of FV).

FVIII, VWF, fibrinogen and D-dimer levels are known to increase during inflammatory disorders [45-48], and pro-inflammatory cytokines can up-regulate the expression of fibrinogen [46] and FVIII [45]. Therefore, our result on the clustering of these factors is biologically reasonable, as inflammation could be a common mechanism affecting their levels. However, upon addition of CRP to the analysis, FVIII and VWF levels formed a separate cluster, whereas CRP levels clustered together with fibrinogen, D-dimer, and FIX levels. In the study by Sakkinen *et al*. [1], CRP levels also clustered with fibrinogen, D-dimer, and factor IX levels, like we found, but in addition with FVIII and plasmin-α_2-antiplasmin levels. However, in their study, VWF was not assessed. In line with our results, Vossen *et al*. [3] also demonstrated that FVIII and

VWF composed a separate cluster. FVIII and VWF plasma levels are strongly related, as VWF regulates FVIII levels by acting as a carrier protein [49], and this could explain the formation of a separate cluster when an extra variable (i.e. CRP) was added to the analysis.

The strengths of this study include that to our knowledge this is the largest study that has been performed on this issue so far, in which levels of several hemostatic factors and lipids were measured. Moreover, the detailed knowledge of self-reported disease status enable us to repeat all analyses in a healthy population. The main limitation is related to our study design, i.e. we could investigate the interrelation between hemostatic factor, lipid and CRP levels but not the mechanisms, related to either genetic or environmental factors, underlying their interrelation.

In conclusion, in this comprehensive study, we confirmed and extended clustering patterns of previous reports between levels of hemostatic factors, lipids and CRP.

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SUPPLEMENTAL MATERIAL

Supplementary Table 1. Principal component analysis with hemostatic factors in 2304 population controls without malignancy or self-reported chronic diseases. Factor loadings between the original variables and the newly formed components are shown

	Components		
Original variable	**1**	**2**	**3**
Procoagulant factors			
Fibrinogen	0.384	**0.542***	0.132
Factor II	**0.797***	0.008	0.077
Factor V	0.130	0.184	**0.642***
Factor VII	**0.641***	0.085	0.189
Factor VIII	0.033	**0.833***	0.152
VWF	-0.004	**0.845***	0.135
Factor IX	**0.696***	0.273	-0.021
Factor X	**0.825***	0.025	-0.054
Factor XI	**0.515***	0.072	0.209
Anticoagulant factors			
Antithrombin	0.375	-0.326	**0.469***
Protein C	**0.734***	-0.041	0.240
Protein S	**0.442***	0.091	**0.482***
TFPI activity	-0.004	0.062	**0.768***
Fibrinolytic factor			
D-dimer†	0.063	**0.596***	-0.070
Variance			
% total variance	29.23	15.19	9.02
% cumulative variance	29.23	44.42	53.44

Data were missing for some participants in some subgroups.

The principal components were ranked according to their eigenvalues from 1 to 3.

TFPI, tissue factor pathway inhibitor; VWF, von Willebrand factor.

*Factor Loading > 0.40.

†Log-transformed variable.

Supplementary Table 2. Principal component analysis with hemostatic factors, lipids and C-reactive protein in 2304 population controls without malignancy or self-reported chronic diseases. Factor loadings between the original variables and the newly formed components are shown

	Components					
Original variable	**1**	**2**	**3**	**4**	**5**	**6**
Procoagulant factors						
Fibrinogen	0.249	0.062	-0.081	**0.724***	0.159	0.257
Factor II	**0.734***	0.132	0.040	0.145	-0.057	0.166
Factor V	0.079	0.165	0.028	0.108	0.154	**0.626***
Factor VII	**0.630***	0.253	0.150	0.020	0.129	0.090
Factor VIII	0.064	0.003	0.044	0.169	**0.921***	0.097
VWF	0.024	0.062	-0.009	0.192	**0.919***	0.052
Factor IX	**0.636***	0.091	-0.093	**0.429***	0.071	0.026
Factor X	**0.802***	0.085	0.018	0.148	-0.024	-0.013
Factor XI	**0.455***	-0.030	0.107	0.125	0.004	**0.425***
Anticoagulant factors						
Antithrombin	**0.441***	-0.070	-0.060	**-0.404***	-0.076	**0.536***
Protein C	**0.731***	0.227	0.106	-0.082	0.060	0.181
Protein S	0.366	0.234	-0.177	0.112	0.036	**0.467***
TFPI	-0.087	**0.422***	-0.078	0.072	0.014	**0.588***
Fibrinolytic factor						
D-dimer†	-0.087	0.008	0.125	**0.626***	0.214	0.096
Lipid profile						
Triglycerides†	**0.538***	**0.405***	**-0.449***	0.005	0.071	-0.153
TC	0.283	**0.907***	0.192	0.005	0.037	0.148
LDL-C	0.150	**0.919***	-0.032	0.021	0.013	0.217
HDL-C	-0.002	-0.048	**0.969***	-0.029	0.021	0.002
Apo A1	0.212	0.034	**0.912***	0.015	0.034	-0.106
Apo B	0.262	**0.886***	-0.196	0.074	0.032	0.129
Inflammatory marker						
CRP†	0.345	0.014	-0.097	**0.791***	0.042	-0.095
Variance						
% total variance	27.03	11.60	10.59	9.01	6.16	5.09
% cumulative variance	27.03	38.63	49.22	58.23	64.39	69.48

Data were missing for some participants in some subgroups.

The principal components were ranked according to their eigenvalues from 1 to 6.

apo A1, apolipoprotein A1; apo B, apolipoprotein B; CRP, C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TFPI, tissue factor pathway inhibitor; VWF, von Willebrand factor.

*Factor Loading > 0.40.

†Log-transformed variables.