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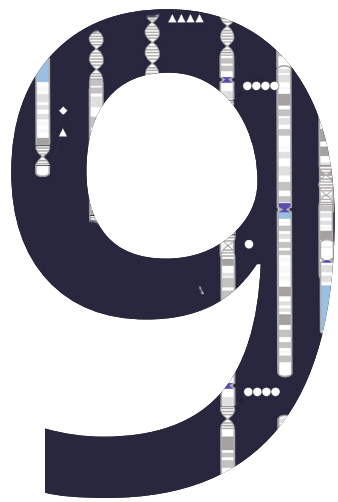
The association of genetic variants in *cholesteryl ester transfer protein* with haemostatic factors and a first venous thrombosis



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SUMMARY

Background: Cholesteryl ester transfer protein (CETP) plays an important role in lipoprotein metabolism. Previous studies suggested that the *CETP TaqI* B1/B2 allele is associated with the risk of venous thrombosis (VT).

Aim: To investigate the associations between genetically determined CETP concentration and 22 haemostatic factors in healthy individuals as well as the risk of a first event of VT in a large VT case-control study.

Methods: Analyses were performed in the Multiple Environmental and Genetic Assessment of Risk Factors for Venous Thrombosis (MEGA) case-control study. CETP unweighted/weighted genetic risk scores (GRSs) were derived from three SNPs that were identified from a recent genome-wide association study (GWAS) on serum CETP concentrations. The associations between CETP GRSs and 22 haemostatic factors (pro-/anti-coagulant and fibrinolytic factors) were assessed by linear regression from an additive model in controls (n=2,813). The associations between CETP GRSs and a first VT risk were assessed by logistic regression analyses in 3950 VT cases and 4765 controls.

Results: In the controls (median age, 49 years; 53% women), both unweighted and weighted GRSs showed that factor VII activity was negatively associated with genetically determined CETP concentration (weighted GRS β -3.08 IU/dL per μg/mL genetically determined CETP, 95% CI: [-5.73, -0.42]). No association was observed with the risk of a first VT.

Conclusions: Genetically determined CETP concentration only showed a weak negative association to factor VII activity. However, this did not lead to an association to the risk of a first VT.

Essentials

- Cholesteryl ester transfer protein (CETP) *TaqI* B1/B2 allele has been associated with the risk of venous thrombosis (VT) in males.
- Whether CETP is a risk factor for venous thrombosis (VT) is still unclear.
- We assessed the associations between CETP genotype and 22 haemostatic factors and the risk of a first VT.
- Genetically determined CETP concentration only showed a weak negative association to factor VII activity.
- No association was found with a higher risk of a first VT.

INTRODUCTION

Cholesteryl ester transfer protein (CETP) plays an important role in the lipoprotein metabolism, facilitating the net flux of cholesteryl esters from high-density lipoproteins (HDL) towards low- and very-low density lipoproteins (LDL and VLDL) [1]. Since elevated CETP concentrations contribute to an adverse lipoprotein profile, and high HDL-C levels have been associated with reduced cardiovascular disease (CVD) risk [2], CETP inhibitors have been developed with the aim to reduce the risk of cardiovascular disease [3, 4]. However, despite the fact that all CETP inhibitors increased HDL-C, only one of the CETP inhibitors that have been tested extensively in humans showed a significant yet small benefit on CVD risk [5]. Thus, the biological role and function of CETP remains to be further uncovered.

The first association between CETP and the risk of venous thrombosis (VT) was described by Deguchi and colleagues, who showed that the CETP *Taq*I B2 allele frequency, which was associated with decreased plasma levels of CETP antigen and activity, was lower in VT cases than controls [6]. However, the study was performed only in men and had a small size of individuals with VT. In another recent study on the association between plasma CETP activity and coagulability, a negative correlation was observed between plasma CETP activity and activated partial thromboplastin time (aPTT), suggesting an association between CETP activity and plasma coagulability [7]. The mechanism of CETP procoagulant activity was postulated to relate to the direct binding of CETP to FXa with enhanced prothrombinase activity [7, 8].

A genome-wide association study (GWAS) was recently performed to identify genetic variants that are associated with serum CETP concentrations [9]. From this GWAS, three independent single nucleotide polymorphisms (SNPs), i.e., hg19 chr16:g.56989590C>T, chr16:g.57000885A>G and chr16:g.57010948G>T, were identified and strongly associated with serum CETP concentrations, with effect sizes ranging from 0.32 to 0.12 μm/mL changes on CETP concentrations by adding one risk allele [9]. The newly identified SNPs shared moderate linkage disequilibrium (LD) to the previously used *CETP* variants in the literature (e.g. *TaqI B1/B2*, Arg451Gln, Ala373Pro). Altogether, the three SNPs explained some 16% of variation in the CETP concentration. Thus, it opens the possibility of performing Mendelian randomization (MR) studies to investigate the causal relationships between CETP concentration, the levels of haemostatic factors in plasma and the disease risk [10].

In the current study, we aimed to use the three newly identified CETP SNPs as genetic instrumental variables to explore the association between genetically determined CETP concentration and (1) haemostatic factors including procoagulant, anticoagulant as well as fibrinolytic factors and (2) the risk of a first VT in a large, population-based

case-control study, the Multiple Environmental and Genetic Assessment (MEGA) of Risk Factors for VT study.

MATERIALS AND METHODS

Study population

The MEGA study is a population-based case-control study with the aim of studying the aetiology of VT. The study design was approved by the Ethics Committee of the Leiden University Medical Center, the Netherlands, and written informed consent was obtained from all participants. From March 1999 to September 2004, 4956 consecutive patients aged between 18 and 70 years (>90% of European-ancestry) with an objectively confirmed first event of VT or pulmonary embolism (PE) were recruited from six anticoagulation clinics in the Netherlands [11]. The control subjects were recruited from two sources, i.e., partners of VT patients when between 18 and 70 years of age and without a history of VT (n=3297); and from the general population, by random-digit dialling (RDD), further frequency matched for age and sex with the VT cases (n=3000). For logistic reasons, a blood sample was provided only by patients and controls recruited before June 2002. Of the participants who were not available for a blood draw, buccal swabs were collected for DNA analysis. In total, around 60% DNA materials were extracted from blood samples, and 40% from buccal swabs (Fig.1).

For the analyses on the association between genetically determined CETP concentration and haemostatic factors, the following participants were selected (1) controls with blood sample available, (2) without history of malignancy within five years before the index date, and (3) without using vitamin K antagonists (Fig. 1b). For the analyses on the association between genetically determined CETP concentration and the risk of a first VT, participants were selected (1) with DNA samples available and good quality, (2) without history of malignancy within five years before the index date, and (3) without Klinefelter syndrome (Fig. 1a).

Laboratory measurements

Detailed information about laboratory measurements of haemostatic factors in the MEGA study has been described elsewhere [12]. In brief, factor II activity (FII), factor VII activity (FVII), factor VIII activity (FVIII), factor X activity (FX), and factor XI activity (FXI) were measured with a mechanical clot detection method on a STA-R coagulation analyzer (Diagnostica Stago, Asnières, France), to quantify the potential of producing active coagulation factors. The Factor V (FV) levels were determined employing an in-house developed sandwich enzyme-linked immunosorbent assay (ELISA) using two monoclonal antibodies recognizing the light chain (V-6) or the heavy chain (V-39) of

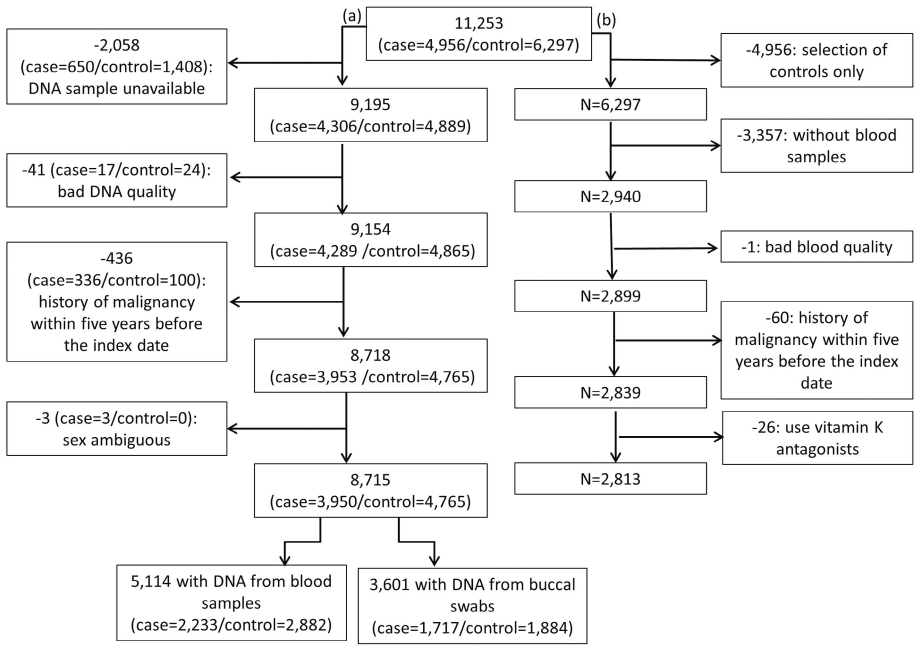

FIG.1 Flowchart of sample selection from the MEGA study. (a). The selection for the analysis of CETP GRS and the risk of VT. (b). The selection for the analysis of CETP GRS and haemostatic factors.

FV, which was specific for single chain FV, including FV-short [13]. The factor IX (FIX) antigen was measured with an in-house ELISA with rabbit anti-FIX antibodies and anti-FIX IgG conjugated to HRP (Dako A/S, Glostrup, Denmark) [14]. Antithrombin activity and protein C levels (PC) was measured with a chromogenic assay on a STA-R coagulation analyzer following the instructions of the manufacturer (Diagnostica Stago, Asnières, France) [15]. Total protein S levels (PS) were measured by ELISA (Diagnostica Stago, Asnières, France). Plasminogen activator inhibitor-1 (PAI-1) antigen levels were measured with a Technozym PAI-1 enzyme-linked immunosorbent assay (ELISA) reagent kit (Kordia Life Sciences, Leiden, The Netherlands; Biopool, Umea, Sweden) and D-dimer was determined by the HemosIL D-dimer assay on an ACL TOP 700 analyzer [16]. The clot lysis time (CLT) was derived from a clot-lysis turbidity profile, which has been described previously with details [12]. Several fibrinolytic factors were only measured in a subsample of the MEGA study, including PAI-1, tissue type plasminogen activator (tPA), plasminogen concentration, antiplasmin (α2-antiplasmin) concentration, and thrombin activatable fibrinolysis inhibitor (TAFI) activity. All the laboratory measures were performed without knowing VT case/control status. The measurements of PAI-1, D-dimer, and clot lysis time were logarithm transformed to obtain normal distributions.

SNP genotyping and genetic risk score

Three SNPs (rs247616:C>T, rs12720922:A>G, rs196890:G>T) identified from the previous CEPT GWAS [9] were genotyped in the MEGA study. DNA samples were obtained from blood samples or buccal swabs, with a concentration of 3 ng/µl. Genotyping of individual DNA samples was performed with kPCR assays using 0.3 ng of DNA or multiplexed oligo ligation assays. Genotyping accuracy of both systems has been assessed in previous studies, and the concordance of the genotype calls from these methods was >99% [17-19].

An unweighted and a weighted genetic risk score (GRS) was calculated. The unweighted genetic risk score was defined as the counts of the total number of CETP concentration increasing (risk) alleles. To take the SNP effect size into account, the weighted genetic risk score was derived by the sum of numbers of CETP concentration increasing alleles multiplied by the SNP effect sizes reported from the original GWAS. Weighted genetic risk scores were considered as the least biased estimates for genetically determined CETP concentration.

Statistical analyses

Linear regression models were used to estimate the effect sizes (beta) with 95% confidence intervals (95% CI) for the associations of individual SNPs as well as the derived CETP GRS (both weighted and unweighted) with 22 haemostatic factors. The beta can be interpreted as difference in haemostatic factor measures (units used in the measures) per unit (µg/mL) in genetically determined CETP concentration. For the haemostatic factors that were logarithmically transformed (namely D-dimer, clot lysis time and PAI-1), the beta represents the percentage change in the factor measures per unit (µg/mL) in genetically determined CETP concentration. For this analysis, extreme measures of each of the 22 haemostatic factors were excluded, i.e., when the individual value was beyond five standard deviations (SD) from the mean. To correct for the multiple testing, the significance level was set to p-value<0.0083, where the standard significance level (p-value = 0.05) was divided by the number (N=6) of principal components that explain over 95% of the variation of 22 haemostatic factors. To identify the associations between genetically determined CETP concentration and the risk of a first VT, logistic regression models were applied to estimate the odds ratios (ORs) with 95% CI for both the single SNPs and the weighted/unweighted CETP GRSs.

In addition to the analysis on the total population, a stratified analysis was performed for men and women. The risk of VT associated with genetically determined CETP concentration was estimated for provoked and unprovoked VT events separately. Unprovoked thrombosis was defined as the absence of any of these provoking factors: surgery, trauma, hospitalization, immobilization, plaster cast, hormone use (oral

contraceptives and hormone therapy), pregnancy within three months before the first event, within four weeks postpartum, and long-haul flight (>4 hours) in the two months before the first thrombosis [20]. Similarly, a separate analyses on the risk of deep vein thrombosis (DVT) and pulmonary embolism (PE) was performed for both the single SNPs and CETP GRSs.

A power calculation was performed based on the current study population (with 45% VT cases) by an online power calculator for Mendelian randomization [21], and 5,839 samples (N=8,715 in the current study) were found to be needed to achieve 80% power by the settings of type I error rate (α) = 0.05, proportion of CETP concentration variance explained by the three SNPs (R^2_{xz}) = 16%, and OR=1.2. All statistical analyses were performed with SPSS for Windows, release 23 (SPSS Inc, Chicago, IL).

RESULTS

Table 1 summarizes the baseline characteristics of all participants in both analyses. Among 2813 controls used for the haemostatic factor analyses, the minor allele frequencies of the three selected SNPs were similar to the previously published GWAS [9]. The median BMI was higher in the VT patients (26.3 kg/m^2) than in controls (25.1 kg/m^2) for the association analysis to the risk of first VT. Of all VT events, over 68% was provoked. VT patients and controls showed similar clinical lipid profiles including total cholesterol, LDL-C, HDL-C and triglycerides. All three SNPs passed the Hardy-Weinberg equilibrium (HWE) tests in controls (P-value>0.05). The call rates for all the three SNPs did not differ between VT cases and controls, and the SNP calling missing-ness was mainly due to the weaker DNA integrity in buccal swabs than blood-derived DNA (over 90% of missing SNP genotypes were from DNA samples extracted by buccal swabs).

The association between individual genetic variants and the levels of haemostatic factors is shown in Supplemental Table 1. After Bonferroni correction (P-value<0.0083), an association was observed between rs247616:C>T and FVII activity (i.e., FVII activity decreased with 1.93 IU/dL per μg/mL increase in genetically determined CETP: β=-1.93, 95%CI: [-3.24, -0.61]) (Supplemental Table 1). Similarly, another two positive associations were found with the uncorrected significance level (P-value<0.05) between rs247616:C>T and FVIII activity (β=2.34 IU/dL per μg/mL genetically determined CETP, 95%CI: [0.37, 4.32]) as well as rs12720922:A>G and clot lysis time (β=1.71% per μg/mL genetically determined CETP, 95%CI: [0.20%, 3.36%]) (Supplemental Table 1).

With the GRS based on the three CETP SNPs, only weak associations were present (Table 2): FVII activity exhibited associations with both weighted (β=-3.08 IU/dL per μg/mL genetically determined CETP, 95%CI: [-5.73, -0.42]) and unweighted CETP GRS (β=-0.92 IU/dL per μg/mL genetically determined CETP, 95%CI: [-1.71, -0.13]). FVIII

activity also showed an association to the weighted CETP GRS (β=4.26 IU/dL per μg/mL genetically determined CETP, 95%CI: [0.26, 8.26]).

TABLE 1. Baseline characteristics

Characteristics	Association to haemostatic factors in controls with blood samples (n=2,813)	A first event of VT	
		Cases (n=3,950)	Controls (n=4,765)
Age, years	49 [39, 58]	49 [38, 58]	49 [39, 58]
Women, n (%)	1,490 (53%)	2161 (54.7%)	2536 (53.2%)
BMI (kg/m^2)	25.1 [22.7, 27.7]	26.3 [23.7, 29.3]	25.1 [22.8, 27.8]
DVT, n(%)	NA	2316 (58.6%)	NA
PE, n(%)	NA	1263 (32.0%)	NA
DVT & PE, n(%)	NA	371 (9.4%)	NA
A first unprovoked VT, n (%)	NA	1149 (29.1%)*	NA
A first provoked VT, n (%)	NA	2715 (68.7%)*	NA
Total cholesterol (mmol/L)	5.5 [4.8, 6.3]	5.5 [4.8, 6.3]	5.5 [4.8, 6.3]
Triglyceride (mmol/L)	1.3 [1.0, 1.9]	1.4 [1.0, 1.9]	1.3 [1.0, 1.9]
HDL-C (mmol/L)	1.3 [1.1, 1.6]	1.3 [1.0, 1.5]	1.3 [1.1, 1.6]
LDL-C (mmol/L)	3.5 [2.9, 4.2]	3.5 [2.9, 4.2]	3.5 [2.9, 4.2]
rs247616_C			
Call rate (%)	98.4%	79.5%	83.4%
Minor allele frequency	32.3%	27.8%	27.1%
HWE p-value	0.78	NA	0.67
rs12720922_A			
Call rate (%)	99.3%	92.6%	95.3%
Minor allele frequency	16.7%	15.8%	15.3%
HWE p-value	0.49	NA	0.074
rs1968905_G			
Call rate (%)	99.5%	92.5%	95.6%
Minor allele frequency	19.9%	17.5%	17.5%
HWE p-value	0.14	NA	0.33

Results are presented as median (inter quartile range [IQR]) or number (percentage).
BMI, body mass index; DVT, deep vein thrombosis; PE, pulmonary embolism; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; HWE, Hardy-Weinberg equilibrium.
*86 VT patients with unknown provoking factors.

TABLE 2. The association of CETP concentration GRS to coagulation factors by additive models

	Unweighted GRS			Weighted GRS		
	N	**Beta**	**95%CI**	**N**	**Beta**	**95%CI**
Anticoagulant factors						
Protein C activity (IU/dL)	2753	-0.30	[-0.99, 0.39]	2753	-1.31	[-3.63, 1.00]
Total Protein S antigen (IU/dL)	2753	0.17	[-0.46, 0.80]	2753	0.69	[-1.42, 2.80]
Free Protein S (%)	2725	0.63	[-0.054, 1.32]	2725	1.66	[-0.66, 3.97]
TFPI activity (U/mL)[§]	2752	-0.0060	[-0.020, 0.0090]	2752	-0.019	[-0.069, 0.031]
Antithrombin activity (IU/dL)	2750	-0.12	[-0.47, 0.24]	2750	-0.55	[-1.75, 0.65]
Procoagulant factors						
Fibrinogen activity (g/L)	2748	0.0050	[-0.015, 0.026]	2748	0.020	[-0.049, 0.089]
Factor II activity (IU/dL)	2751	-0.019	[-0.48, 0.44]	2751	-0.17	[-1.71, 1.37]
Factor V antigen (U/mL)	2749	0.0020	[-0.0040, 0.0070]	2749	0.0020	[-0.016, 0.020]
Factor VII activity (IU/dL)	2753	-0.92	[-1.71, -0.13]	2753	-3.08	[-5.73, -0.42]
Factor VIII activity (IU/dL)	2750	1.00	[-0.19, 2.19]	2750	4.26	[0.26, 8.26]
Von Willebrand factor antigen (IU/dL)	2747	0.72	[-0.62, 2.07]	2747	2.99	[-1.53, 7.51]
Factor IX antigen (IU/dL)	2752	-0.053	[-0.65, 0.55]	2752	-0.29	[-2.31, 1.74]
Factor X activity (IU/dL)	2752	0.12	[-0.50, 0.74]	2752	0.13	[-1.96, 2.23]
Factor XI activity (IU/dL)	2752	0.061	[-0.56, 0.68]	2752	0.24	[-1.85, 2.33]
Global assay measurements						
Clot lysis time (minute)[a]	2747	0.40	[-0.30, 1.21]	2747	1.21	[-1.29, 3.87]
ELPLT (nM.min)	2741	-0.30	[-4.50, 3.90]	2741	-1.43	[-15.57, 12.70]
Fibrinolytic factors						
PAI-1 (ng/mL)[a§]	704	0.90	[-3.82, 5.76]	704	5.97	[-9.79, 24.61]
tPA (ng/mL)[§]	704	0.057	[-0.086, 0.20]	704	0.38	[-0.10, 0.86]
D-dimer (ng/mL)[a]	2749	0.40	[-1.69, 2.43]	2749	1.41	[-5.26, 8.55]
Plasminogen concentration (%)[§]	702	-0.18	[-1.22, 0.86]	702	-0.90	[-4.38, 2.58]
α2-antiplasmin concentration (%)[§]	702	0.056	[-0.65, 0.76]	702	-0.11	[-2.46, 2.24]
TAFI activity (%)[§]	701	0.48	[-0.65, 1.60]	701	1.12	[-2.65, 4.89]

[a.] Natural logarithm transformed, with the beta estimated as the percentage change in the factor measures per unit (μg/mL) in genetically determined CETP concentration.
[§]TFPI: Tissue factor pathway inhibitor; ETPLP: Endogenous thrombin potential (area under the curve) obtained at low tissue factor concentration; t-PA: Tissue plasminogen activator; PAI-1: Plasminogen activator inhibitor; TAFI: Thrombin activatable fibrinolysis inhibitor.

For each participant, the number of CETP concentration increasing (risk) alleles were counted. The number of risk alleles ranged from zero to six (Fig.2), and similar proportions of VT cases and controls were falling into each allele count category. By

logistic regression models, neither single SNPs (Supplemental Table 2) nor weighted and unweighted CETP GRS (Table 3) were associated with an increased risk of a first VT, with an OR of 0.98 (95%CI: [0.85, 1.13]) for the weighted CETP GRS in all the study population (Table 3). No associations were observed in separate analyses on provoked and unprovoked VT, i.e., ORs were 1.01 (95% CI: [0.86-1.19]) for provoked VT and 0.87 (95% CI: [0.69-1.10]) for unprovoked VT by the weighted CETP GRS, as well as for the separate analyses on PE and/or DVT. Stratified analysis on sex yielded the same null results.

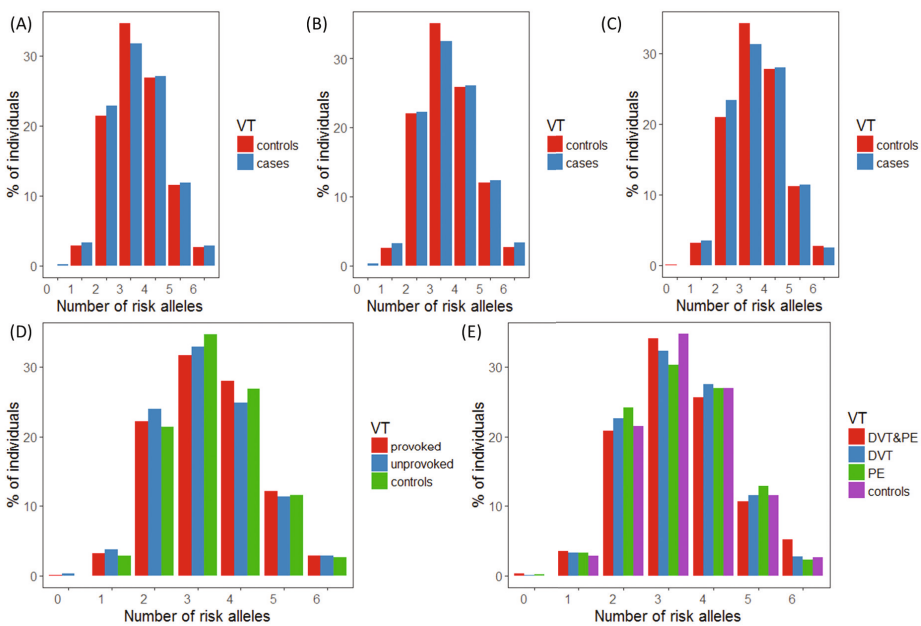

FIG.2 The three-SNP risk allele distributions between VT cases and controls. (A). The entire cohort; (B). Men only; (C). Women only; (D). VT cases separated for provoked and unprovoked VT; (E). VT cases separated for PE and DVT.

DISCUSSION

In the current large-scale population-based VT case-control study, of 22 haemostatic factors studied, the genetically determined CETP concentration was found to be weakly associated with FVII activity levels, which was predominantly attributable to a single SNP (rs247616:C>T). However, no association was observed between genetically determined CETP concentration and the risk of a first VT, which is in line with the absence of an association between FVII levels and the risk of VT in the literature [22].

Deguchi and colleagues first described the association of the *CETP TaqI B1/B2* polymorphism (rs708272) with VT in a small case-control study composed of 98

TABLE 3. The association of CETP concentration GRS to the first event of VT by additive models

	Unweighted GRS				Weighted GRS			
	N_{case}	$N_{control}$	OR	95%CI	N_{case}	$N_{control}$	OR	95%CI
	All participants							
All participants	3101	3906	0.99	[0.95, 1.03]	3101	3906	0.98	[0.85, 1.13]
	Stratified by sex							
Men only	1406	1853	1.02	[0.96, 1.08]	1406	1853	1.09	[0.89, 1.35]
Women only	1695	2053	0.98	[0.92, 1.03]	1695	2053	0.91	[0.74, 1.11]
	Provoked/unprovoked VT							
Provoked VT only	2130	3906	1.01	[0.96, 1.06]	2130	3906	1.01	[0.86, 1.19]
Unprovoked VT only	902	3906	0.95	[0.89, 1.02]	902	3906	0.87	[0.69, 1.10]
	PE/DVT							
PE&DVT	309	3906	1.03	[0.93, 1.14]	309	3906	1.04	[0.73, 1.49]
DVT only	1804	3906	0.99	[0.94, 1.04]	1804	3906	0.97	[0.82, 1.15]
PE only	988	3906	0.98	[0.92, 1.05]	988	3906	0.96	[0.78, 1.19]

male participants [6], which was not observed in the current study. In this study, the frequency of the *TaqI B2* allele was lower in the VT cases than controls (0.33 versus 0.47). Pecheniuk et al. further investigated the allele frequencies of two other non-synonymous *CETP* SNPs (Ala373Pro and Arg451Gln), in the same case-control study population. Both variants were found to have more often CETP increasing allele in the male VT cases than controls [23]. Based on this evidence, the authors suggested that *CETP* genotypes were associated with VT in men. However, in contrast to the previous findings, in the current VT case-control study with a much larger sample size, we found no association with the risk of VT in either men or women. This null association was found despite the observation that the GRS explained over 16% of variation in CETP concentration [9]. The three SNPs used in the current analyses are in moderate LDs with the previously used SNPs (pairwise LDs between 0.51 and 0.55), which is unlikely to explain the discrepant results to the previous studies. However, it is noteworthy that in the previous case-control study with 98 male participants, the controls were recruited from a blood donation program, which is likely to recruit controls screened for good health, and in particular with beneficial lipid profiles [6]. As a result, selection bias might have been introduced to their study, which hypothesis is in line with a higher allele frequency of *TaqI B2* in the controls than the Caucasian reference population from 1000 Genome project (0.47 versus 0.42) [24].

A previous study showed inverse correlations of endogenous plasma CETP antigen levels with prothrombin time induced by tissue factor (reflecting the extrinsic coagulation pathways) as well as factor XIa induced clotting time (reflecting the intrinsic pathways), which implied a potential association between CETP and coagulability through a common pathway, namely prothrombin activation [25]. Another recent study further demonstrated enhanced CETP prothrombin activation was through a direct binding of CETP to activated factor X (Xa) [7]. In addition, thrombin generation measured by prothrombin activation assays was reported as five-fold increased with Gln451 (rs1800777 A/G, with CETP concentration increasing allele A) CETP mutation than the wild type CETP [7]. Based on this evidence, our null associations between genetically determined CETP concentration and prothrombin (FII) as well as FX activity were unexpected. Nevertheless, our result is in line with our previous findings where we observed no association of lipid levels and the risk of a first VT [26]. This is relevant, since CETP concentrations are highly inversely associated with HDL-C levels [27, 28]. Previous findings have reported the associations between FVII and lipid profiles [29], however, FVII levels is not a risk factor of VT in the literature [22]. Taken together, this may explain the association between genetically determined CETP GRS and FVII levels, while absence of association to the risk of VT in the current study. In addition, thrombin generation was also measured in the current study by endogenous thrombin potential (area under the curve) obtained at low tissue factor (ETPLT) concentration, and null result was observed between ETPLT and CETP GRS, which questioned the previously observed associations between CETP genetic mutations and thrombin generation.

There are several strengths in the current study. Firstly, to our knowledge, this is the largest study with extensive haemostatic factors measured in the study population, to investigate the associations between CETP concentrations and haemostatic factors as well as the risk of a first VT. Secondly, we used strong genetic instruments (explaining some 16% of phenotypic variation) to reflect CETP concentrations, which increased the statistical power for the analyses. Thirdly, by using the genetic risk scores, we performed two-sample Mendelian randomization analyses, which are less vulnerable to revere causation and residual confounding issues in the observational studies. For etiological studies, the associations observed in these results are more likely to be causal.

Several limitations should also be acknowledged in the current study. Firstly, although it is a well-powered study for the main analysis, the sample size might still be insufficient for the sex stratified analysis as well as the separate analyses on provoked/unprovoked VT and DVT/PE. Secondly, CETP genetic risk scores were derived based on Caucasian populations, and the findings of current study might not apply to the other ethnicities. Thirdly, DNA materials extracted from buccal swabs had higher genotyping failure rates than DNA extracted from blood samples due to decreased DNA integrity.

However, the call rates for each SNP were similar between cases and controls, and the missing-ness is unlikely to be related to the disease status.

In conclusion, genetically determined CETP concentration showed weak associations with FVII activity. However, no association was found between genetically determined CETP concentrations and the risk of a first VT.

ADDENDUM

R. Li-Gao analysed and drafted the manuscript; R. Li-Gao, A. van Hylckama Vlieg interpreted the data; F. R. Rosendaal, A. van Hylckama Vlieg designed the study. All authors reviewed the manuscript.

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CONFLICT OF INTEREST

Dennis O. Mook-Kanamori is a part-time clinical research consultant for Metabolon, Inc. All other authors: Ruifang Li-Gao, Suzanne C. Cannegieter, Ko Willems van Dijk, Frits R. Rosendaal and Astrid van Hylckama Vlieg have nothing to disclose.

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SUPPLEMENTAL TABLE 1. The associations of CETP concentration determined SNPs to haemostatic factors by additive models

	rs247616 (allele-C)			rs12720922 (allele-A)			rs1968905 (allele-G)		
	N	**Beta**	**95%CI**	**N**	**Beta**	**95%CI**	**N**	**Beta**	**95%CI**
				Anticoagulant factors					
Protein C activity (IU/dL)	2768	-0.91	[-2.05, 0.24]	2794	-0.43	[-1.85, 1.00]	2798	0.42	[-0.94, 1.78]
Total Protein S antigen (IU/dL)	2768	0.12	[-0.92, 1.16]	2794	0.63	[-0.67, 1.93]	2798	0.070	[-1.17, 1.31]
Free Protein S (%)	2740	0.86	[-0.28, 2.00]	2766	0.011	[-1.42, 1.44]	2770	1.20	[-0.16, 2.56]
TFPI activity (U/mL)[§]	2767	-0.0010	[-0.025, 0.024]	2793	-0.016	[-0.046, 0.015]	2797	-0.003	[-0.032, 0.026]
Antithrombin activity (IU/dL)	2765	-0.24	[-0.84, 0.35]	2791	-0.43	[-1.17, 0.30]	2795	0.21	[-0.49, 0.92]
				Procoagulant factors					
Fibrinogen activity (g/L)	2763	0.025	[-0.009, 0.060]	2788	-0.012	[-0.055, 0.030]	2792	-0.0060	[-0.047, 0.034]
Factor II activity (IU/dL)	2766	-0.16	[-0.93, 0.60]	2792	-0.064	[-1.02, 0.89]	2796	0.19	[-0.72, 1.10]
Factor V antigen (U/mL)	2764	-0.0010	[-0.010, 0.008]	2790	-0.0010	[-0.012, 0.010]	2794	0.0080	[-0.0030, 0.019]
Factor VII activity (IU/dL)	2768	-1.93	[-3.24, -0.61]	2794	-0.41	[-2.05, 1.23]	2798	-0.36	[-1.92, 1.21]
Factor VIII activity (IU/dL)	2765	2.34	[0.37, 4.32]	2791	1.70	[-0.77, 4.16]	2795	-0.90	[-3.24, 1.44]
Von Willebrand factor antigen (IU/dL)	2762	1.53	[-0.70, 3.76]	2788	1.38	[-1.40, 4.15]	2792	-0.57	[-3.21, 2.08]
Factor IX antigen (IU/dL)	2767	-0.033	[-1.04, 0.97]	2793	-0.29	[-1.54, 0.96]	2797	0.34	[-0.85, 1.53]
Factor X activity (IU/dL)	2767	-0.40	[-1.44, 0.63]	2792	0.40	[-0.89, 1.69]	2796	0.60	[-0.62, 1.83]
Factor XI activity (IU/dL)	2767	0.33	[-0.70, 1.37]	2793	-0.33	[-1.62, 0.95]	2797	0.015	[-1.21, 1.24]
				Global assay measurements					
Clot lysis time (minute)[a]	2762	-0.40	[-1.59, 0.90]	2788	1.71	[0.20, 3.36]	2792	0.80	[-0.70, 2.22]
ELPLT (nM.min)	2756	0.74	[-6.26, 7.74]	2780	-1.04	[-9.74, 7.67]	2784	-0.072	[-8.37, 8.23]

SUPPLEMENTAL TABLE 1. Continued.

	rs247616 (allele-C)			rs12720922 (allele-A)			rs1968905 (allele-G)		
	N	**Beta**	**95%CI**	**N**	**Beta**	**95%CI**	**N**	**Beta**	**95%CI**
	Fibrinolytic factors								
PAI-1 (ng/mL)[a§]	706	-0.010	[-7.78, 8.11]	713	8.00	[-1.88, 18.89]	712	-3.15	[-12.19, 6.82]
tPA (ng/mL) [§]	706	0.24	[-0.0030, 0.47]	713	0.16	[-0.13, 0.44]	712	-0.29	[-0.58, 0.0020]
D-dimer (ng/mL)[a]	2764	0.40	[-2.96, 3.87]	2790	1.11	[-3.05, 5.44]	2794	0.50	[-3.44, 4.60]
Plasminogen concentration (%)[§]	704	-0.19	[-1.92, 1.54]	711	-0.74	[-2.82, 1.34]	710	0.24	[-1.88, 2.36]
α2-antiplasmin concentration (%)[§]	704	0.25	[-0.92, 1.41]	711	-0.57	[-1.97, 0.84]	710	0.52	[-0.92, 1.95]
TAFI activity (%)[§]	703	-0.27	[-2.15, 1.61]	710	1.29	[-0.95, 3.53]	709	1.22	[-1.06, 3.51]

[a.] Natural logarithm transformed, with the beta estimated as the percentage change in the factor measures per unit (μg/mL) in genetically determined CETP concentration.
[§]TFPI: Tissue factor pathway inhibitor; ETPLP: Endogenous thrombin potential (area under the curve) obtained at low tissue factor concentration; t-PA: Tissue plasminogen activator; PAI-1: Plasminogen activator inhibitor; TAFI: Thrombin activatable fibrinolysis inhibitor.

SUPPLEMENTAL TABLE 2. The association of CETP concentration determined SNPs to the first event of VT by additive models

	rs247616 (allele-C)				rs12720922 (allele-A)				rs1968905 (allele-G)			
	N_{case}	$N_{control}$	OR	95%CI	N_{case}	$N_{control}$	OR	95%CI	N_{case}	$N_{control}$	OR	95%CI
All participants												
All participants	3140	3974	0.96	[0.89,1.03]	3656	4539	1.04	[0.96, 1.13]	3653	4554	0.98	[0.91, 1.06]
Stratified by sex												
Men only	1421	1878	1.01	[0.91, 1.12]	1641	2126	1.12	[0.99, 1.27]	1638	2129	0.98	[0.87, 1.11]
Women only	1719	2096	0.92	[0.84, 1.02]	2015	2413	0.98	[0.88, 1.10]	2015	2425	0.99	[0.89, 1.10]
Provoked/unprovoked VT												
Provoked VT only	2160	3974	0.97	[0.90, 1.05]	2515	4539	1.05	[0.96, 1.15]	2512	4554	1.01	[0.92, 1.10]
Unprovoked VT only	911	3974	0.91	[0.82, 1.02]	1060	4539	1.01	[0.89, 1.15]	1061	4554	0.94	[0.83, 1.06]
PE/DVT												
PE&DVT	311	3974	0.99	[0.84, 1.18]	349	4539	1.01	[0.82, 1.24]	352	4554	1.16	[0.94, 1.42]
DVT only	1831	3974	0.95	[0.87, 1.03]	2142	4539	1.05	[0.95, 1.15]	2139	4554	0.98	[0.90, 1.08]
PE only	998	3974	0.96	[0.87, 1.07]	1165	4539	1.03	[0.91, 1.16]	1162	4564	0.93	[0.83, 1.05]