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Genetic variants in Cell Adhesion Molecule 1 (*CADM1*): a validation study of a novel endothelial cell venous thrombosis risk factor

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Abstract

Introduction—In a protein C deficient family, we recently identified a candidate gene, *CADM1*, which interacted with protein C deficiency in increasing the risk of venous thrombosis (VT). This study aimed to determine whether *CADM1* variants also interact with protein C pathway abnormalities in increasing VT risk outside this family.

Materials and methods—We genotyped over 300 *CADM1* variants in the population-based MEGA case-control study. We compared VT risks between cases with low protein C activity (n=194), low protein S levels (n=23), high factor VIII activity (n=165) or factor V Leiden carriers (n=580), and all 4004 controls. Positive associations were repeated in all 3496 cases and 4004 controls.

Results—We found 22 variants which were associated with VT in one of the protein C pathway risk groups. After mutual adjustment, six variants remained associated with VT. The strongest evidence was found for rs220842 and rs11608105. For rs220842, the odds ratio (OR) for VT was 3.2 (95% CI 1.2–9.0) for cases with high factor VIII activity compared with controls. In addition, this variant was associated with an increased risk of VT in the overall study population (OR: 1.5,

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Conflict of interest statement

Stock ownership, Haematologic Technologies, Inc., Essex Jct., VT, USA (EGB)

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95% CI 1.0–2.2). The other variant, rs11608105, was not associated with VT in the overall study population (OR: 1.0, 95% CI 0.8–1.1), but showed a strong effect on VT risk (OR: 21, 95% CI 5.1–88) when combined with low protein C or S levels.

Conclusions—In a population-based association study, we confirm a role for *CADM1* variants in increasing the risk of VT by interaction with protein C pathway abnormalities.

Keywords

venous thrombosis; genetic variation; *CADM1*; protein C pathway

Introduction

We have identified a candidate gene, cell adhesion molecule 1 (*CADM1*), which appears to interact with protein C deficiency to increase the risk of venous thrombosis in an extended French Canadian family with type I protein C deficiency due to a *PROC* 3363C insertion (“Vermont family”)[1]. The 300kb *CADM1* gene is also known as nectin-like protein 2 (*NECL2*), tumor suppressor in lung cancer 1 (*TSLC1*), synapse cell adhesion molecule (*SynCAM1*), spermatogenic immunoglobulin super family (*SgIGSF*), and immunoglobulin super family 4 (*IGSF4*) [2–6]. *CADM1*, an immunoglobulin cell adhesion molecule involved in binding interactions supporting intercellular adhesion, has been best characterized as a constitutive cell-cell adhesion molecule in epithelial cells and at neuronal synapses [4, 5].

In the Vermont family study, several single nucleotide variants (SNVs) in *CADM1* showed a strong association with venous thrombosis in interaction with protein C deficiency [1]. For example, among protein C deficient family members, carriers of the rs6589488 minor allele had a 17- fold increased risk of venous thrombosis (OR 17, 95% CI 13.5–21.4) compared with homozygous major allele carriers. Subsequent *CADM1* gene expression assays, using blood outgrowth endothelial cells cultured from family members, showed a decreased expression compared with controls, lending phenotypic support to the SNV associations. We also demonstrated *CADM1* in endothelial cells, where it appears to be selectively involved in endothelial cell migration, suggesting a role in maintenance of endothelial barrier function [1,7].

Activated Protein C, bound to the endothelial protein C receptor (APC-EPCR) on the endothelial membrane, mediates endothelial barrier enhancement through activation of protease activated receptor 1 (PAR-1) and the sphingosine-1-phosphate-receptor-1 (S₁P₁) pathways [8–12]. This APC-EPCR mediated activation of PAR-1 and S₁P₁ leads to activation of endothelial Rac1 and the cytoskeletal rearrangements associated with endothelial barrier enhancement [10, 11, 13]. The *CADM1* pathway [14], which is associated with migration and adhesion in epithelial cells, appears to mediate this epithelial cell behavior, in part, through regulating small Rho-GTPases including Rac1 [15, 16]. This suggests that our observation of a strong interaction between the *CADM1* and protein C genes in increasing thrombosis risk in the Vermont family may be related to a shared common signalling pathway involving the small Rho-GTPases. Thus, the *CADM1* pathway interaction with the protein C system may represent a novel biological pathway conferring

increased risk for venous thrombosis at the level of the vessel wall due to impaired maintenance of endothelial barrier function.

In order to validate the association between *CADM1* and thrombosis observed in the Vermont family study, we investigated *CADM1* gene variants in the Multiple Environmental and Genetic Assessment of risk factors for venous thrombosis (MEGA study), a case-control study on venous thrombosis including over 4000 patients and 4000 controls. To study the effect of *CADM1* variants on thrombosis risk, we primarily focused on subsets of thrombosis patients with protein C pathway abnormalities (i.e. low levels of protein C or S, high factor VIII levels, and the factor V Leiden variant) as *CADM1* variants were found to interact with protein C deficiency in the Vermont family study[1]. Protein S interacts closely with protein C in the inactivation of the procoagulant factors Va and VIIIa [17], and synergistic effects of *CADM1* with protein C deficiency might therefore also occur with protein S deficiency, high levels of factor VIII, or activated protein C resistance due to factor V Leiden (*F5*, rs6025).

Material and methods

Study population

The MEGA study [18,19] is a population-based case-control study. Consecutive patients aged 18 to 70 years with a first venous thrombosis of the leg or arm, or with a pulmonary embolism were recruited from 6 anticoagulation clinics in the western part of the Netherlands between 1999 and 2004. Partners of patients, as well as additional individuals recruited by random digit dialling and frequency-matched on age and sex, were invited as control subjects. All participants received a standardized questionnaire about risk factors for venous thrombosis. A blood sample was taken approximately 3 months after discontinuation of anticoagulant therapy (usually 3–12 months after the diagnosis of venous thrombosis), or after a year when patients continued their anticoagulant therapy, and from control subjects. Participants who refused to or were unable to provide a blood sample and patients and their partners included after June 1, 2002 were offered the option of providing a buccal swab sample for DNA. Exclusion criteria were previous venous thrombosis (patients and controls), no venous thrombosis (patients, after checking hospital records), age younger than 18 or older than 70, severe psychiatric problems, inability to speak Dutch and, for genetic and blood sample analysis, poor sample quality. For the present analysis, we only included individuals from North- or Western European origin (90%), which was assessed by self-reported country of birth of the parents, in order to avoid population stratification. This left 1970 patients and 2490 control subjects (n=4460) with a plasma and DNA sample and another 1526 patients and 1514 control subjects (n=3040) with only a DNA sample eligible for analysis.

Protein C pathway abnormalities

We selected individuals with protein C pathway abnormalities, i.e., low protein C activity, low protein S levels, high factor VIII activity levels, or factor V Leiden carriership. The protein C, protein S and factor VIII abnormalities were not individually diagnosed, but instead we used clinically relevant cut-off levels to categorize individuals as abnormal. Low

protein C activity was defined by taking the lower limit of normal (67% of normal in our laboratory) as cut-off point. When individuals were on oral anticoagulant therapy at time of blood draw, we calculated the expected protein C activity relative to factor VII activity by linear regression according to a method described by O'Brien et al [20]. The observed levels were classified as "low" when the observed/expected ratio was below the geometric mean minus 2 standard deviations as calculated among control subjects. Of 1959 patients and 2471 control subjects with protein C (and factor VII) measurements, 194 patients (10%; mean protein C activity 43% of normal; range 19–66) and 28 control subjects (1%; mean protein C activity 42% of normal; range 30–62) had low protein C activity. Of these 194 patients and 28 control subjects, 178 patients and 21 controls were on oral anticoagulant therapy at the time of the blood draw.

Similarly to the selection of individuals with low protein C, we selected low protein S individuals by selecting total protein S levels below the lower limit of normal (67% of normal) for individuals not on oral anticoagulant therapy at the time of the blood draw and calculated protein S levels relative to factor II for patients using oral anticoagulant therapy at the time of the blood draw. Of the 1828 patients and 2252 control subjects with protein S (and factor II) measurements, 23 patients (1%; mean protein S level 58% of normal; range 32–66) and 26 controls (1%; mean protein S level 60% of normal; range 45–67) had low protein S levels. Of these 33 patients and 28 controls, 3 patients and none of the controls were on oral anticoagulant therapy at the time of the blood draw.

High factor VIII was defined as activity levels higher than the geometric mean plus 2 standard deviations as calculated among control subjects, which was 204 IU/ml. In total, 165 (8%) of 1969 patients and 51 (2%) of 2488 control subjects with factor VIII levels available had high factor VIII activity levels.

For the factor V Leiden subgroup analysis we selected 580 (17%) patients and 219 (5%) control subjects who carried the variant from among 3493 patients and 4000 control subjects with factor V Leiden genotypes available.

Laboratory analysis

Collection and processing of blood and buccal swab samples, subsequent DNA isolation and genotyping of factor V Leiden variant have been described previously [18]. Measurements of protein C activity were performed with a chromogenic assay and factor II, VII and VIII activity measurements were based on clotting time assays using immune-depleted plasma, deficient for the factor under study. These measurements were performed on a STA-R coagulation analyzer following the instructions of the manufacturer (Diagnostica Stago, Asnières, France). Total protein S levels were measured by an enzyme-linked immunosorbent assay (ELISA, Diagnostica Stago, Asnières, France). The mean intra- and inter-assay coefficients of variation in our laboratory were 1.4% and 3.5%, respectively, for protein C, 2.7% and 4.2% for factor II, 3.4% and 4.0% for factor VII, 3.6% and 8.9% for factor VIII and 5.0% and 3.5% for protein S. All measurements were performed on a single blood draw.

SNV Selection

We selected 364 SNVs throughout *CADM1* and 2kb downstream and 10kb upstream of the gene in order to include conserved elements which may play a regulatory role (chr11:114,543,000-114,893,000, NCBI B36 assembly). From the *CADM1* SNVs that were genotyped in the European HapMap population, we chose 86 tagging SNVs with minor allele frequency (MAF)>0.01 by pairwise tagging ($r^2>0.8$) as implemented in Haploview [21]. From the HapMap list we added 42 SNVs from blocks with multiple SNVs for redundancy and 29 SNVs in regions where the distance between adjacent SNVs was largest. In addition, we selected 99 SNVs that had not been genotyped by HapMap but were validated in dbSNP and 108 SNVs that we identified by resequencing the region in the Vermont family. Of 364 SNVs selected for genotyping, 47 were excluded because of poor assay performance, 3 SNV assays were excluded because of atypical clustering and 30 were not polymorphic in the MEGA study population, which left 284 SNVs for statistical analysis. Genotyping was performed at the Johns Hopkins University through the NHLBI Genotyping and Resequencing Service. Genotyping quality was assessed by establishing the call rate (>99%) and the Hardy-Weinberg equilibrium of each SNV.

Statistical analysis

The primary analysis was to compare allele frequencies between patients with specific abnormalities in the protein C pathway (i.e. low protein C, low protein S, high factor VIII or factor V Leiden) and all control subjects. The choice for taking all control subjects as a reference group was made because few control subjects had low protein C activity or low protein S levels.

Odds ratios (OR) and 95% confidence intervals (95% CI) were computed using logistic regression for an additive genetic model. The reference allele was the most prevalent (major) allele in the total study population and the OR was calculated per additional minor allele copy. Variants that were associated with venous thrombosis in the primary analysis (one of the subgroups of protein C pathway abnormalities versus all controls) with p-value <0.05 were further studied. Next, linkage disequilibrium (LD) between SNVs of interest was studied in Haploview [20]. Of the variants that were in strong linkage disequilibrium, defined as r^2 of 0.7 or higher, we selected the variant with the highest allele frequency in controls for follow-up. To assess the causal effects of the SNVs, we mutually adjusted the associations by entering all positive variants into a conditional logistic regression model. Positive associations were repeated in the overall MEGA study (3496 cases and 4004 controls) and studied for the joint effects of the variants and the protein C pathway abnormality under study.

With more than 250 variants tested for association with venous thrombosis in each subgroup, the chance of false positive findings is substantial. In order to decrease the chance of false-positive reporting, we calculated an FDR-adjusted q-value[22].

Results

Characteristics of the study population are presented in Table 1. We studied 284 variants in four subgroups of venous thrombosis patients with a protein C pathway abnormality, i.e., patients with low protein C activity (N=194), patients with low protein S levels (N=23), patients with high FVIII activity (N=165) and patients carrying the FV Leiden polymorphism (N=580), and all controls (N=4004). The subgroups were not mutually exclusive, i.e., 72 patients (12%) had multiple abnormalities in the protein C pathway.

Associations between *CADM1* variants and VT within protein C pathway subgroups

For all 284 variants, allele frequencies among all MEGA study patients and all MEGA study controls are listed in Supplementary Table 1. Twelve of the 284 variants were monomorphic among control subjects and eight were monomorphic among patients of the overall MEGA study. In addition, several variants were monomorphic in one of the subgroups of patients with a protein C pathway abnormality: 16 variants among patients with low protein C activity, 46 variants among patients with low protein S levels, 14 variants among patients with high factor VIII activity and 17 variants among patients carrying factor V Leiden. These variants could not be studied.

During the first stage of the analysis, we identified 22 *CADM1* variants that were associated with venous thrombosis (p -value<0.05) in one of the subgroups of patients with a protein C pathway abnormality and all controls (Table 2). One variant was associated with venous thrombosis in the low protein C subgroup, nine variants in the low protein S subgroup, six variants in the high factor VIII subgroup, and seven variants in the factor V Leiden subgroup (Table 2). Only one variant (rs11608105) was associated with venous thrombosis in multiple subgroups, i.e. the low protein C subgroup (OR 1.57, 95% CI 1.05–2.34) and the low protein S subgroup (OR 2.98, 95% CI 1.27–7.02). To correct for multiple testing, we calculated FDR-adjusted q -values after which none of the variants remained associated with venous thrombosis (Table 2).

Next, we studied linkage disequilibrium between the positive variants. Of the 22 variants, four pairs of variants were in strong linkage disequilibrium (Figure 1; $r^2 = 0.7$). Of each pair of variants, the variant having the highest risk allele frequency among controls was selected for the remaining analyses. To study the causal effects of the positive variants on venous thrombosis, we entered all positive variants within each subgroup in a logistic regression model. In the subgroup of protein S, two variants remained associated with venous thrombosis, i.e., rs11608105 and rs45520832 (Table 3; OR 3.54, 95% CI 1.46–8.60 and OR 22.1, 95% CI 2.35–208, respectively). In addition, two variants, i.e., rs11215504 and rs220842, remained associated with venous thrombosis in patients with high factor VIII activity (OR 1.89, 95% CI 1.24–2.88 and OR 3.23, 95% CI 1.17–8.97, respectively). In the patients that carried FV Leiden, another two variants, i.e., rs45608938 and rs45545346, remained associated with a decreased risk of venous thrombosis (Table 3; OR 0.71, 95% CI 0.52–0.97 and OR 0.53, 95% CI 0.30–0.93 respectively).

Associations to venous thrombosis in overall MEGA study

We further investigated the six variants, which remained associated with venous thrombosis after mutual adjustment, in the overall MEGA study population in order to study the effect on venous thrombosis independently of the protein C pathway abnormalities. We observed a weak association between rs220842 and venous thrombosis (OR 1.49, 95% CI 0.99–2.24) and between rs11215504 and venous thrombosis (OR 1.14, 95% CI 0.98–1.33). The other four variants were not associated with venous thrombosis in the overall MEGA study population (Table 4).

Joint effect of *CADM1* variants and protein C pathway abnormalities

We studied the joint effect of the thrombosis associated variants and the protein C pathway abnormalities by using homozygous major allele carriers without the protein C pathway abnormality under study as a reference for the odds ratio (Table 5). The combination of carrying variant rs11608105 and having low protein C or protein S levels was associated with a 21-fold increased risk (95% CI 5.08–88.8) of venous thrombosis. Compared with non-carriers having low protein C or S levels, the risk of venous thrombosis was a 4-fold increased (95% CI 1.00–18.7) in carriers of the risk allele with low protein C or S levels.

Similar to findings in the overall MEGA study population, variant rs220842 was associated with an increased risk of venous thrombosis (OR 1.88, 95% CI 1.07–3.31; Table 5) in individuals without high factor VIII activity. The joint effect of the variant and high factor VIII activity could not be studied as only patients and no controls with high factor VIII activity carried the variant (n=5; Table 5). Furthermore, having high factor VIII activity and carrying the risk allele of variant rs11215504 was associated with a 6.5-fold increased risk of venous thrombosis. This exceeded the risk for rs11215504 or the defect alone (Table 5) albeit with a wide confidence interval due to the small number of carriers with also a defect (95% CI 2.48–17.1). For the other positive variants, no clear joint effect with a protein C pathway abnormality could be calculated (rs45520832) or was observed (rs45608938, rs45545346) (Table 5).

Discussion

In this study we aimed to validate the *CADM1* gene, encoding cell adhesion molecule 1, as a gene involved in the etiology of venous thrombosis. We identified this gene as a candidate risk gene in the Vermont family [1]. The thrombosis association was most pronounced among individuals in this family with both variation in *CADM1* and protein C deficiency. To confirm the interaction of protein C deficiency and *CADM1* variants in increasing the risk of thrombosis, we studied 284 variants in *CADM1* in the population-based MEGA study. We performed analyses mainly by comparing thrombosis cases with protein C pathway abnormalities, i.e. low protein C or S levels, high factor VIII activity or factor V Leiden, with all controls.

For six variants in the *CADM1* gene, a consistent association with venous thrombosis was observed in one of the subgroups of protein C pathway abnormalities. Within individuals with low protein C or S levels, rs11608105 showed a 21-fold increased risk of venous

thrombosis. Another variant (rs220842) was associated with venous thrombosis in the overall MEGA population and was only present in patients, and not in control subjects, with high factor VIII activity. Whether the variants are causal or are in linkage disequilibrium with unmeasured causal variants is not known. Our results suggest independent effects for the two variants. Both variants lie in intron 1, which comprises 240 kb of the 300 kb *CADMI* gene. There are a number of transcription factor binding sites and regulatory elements in intron 1. Examination of the 500 bp sequence flanking the variants revealed the occurrence of conserved elements (across 37 mammals) and open chromatin regions (DNase I hypersensitivity assay) [23]. This suggests that epigenetic control may be the underlying functional mechanism by which these variants exert their effect on venous thrombosis.

One of the drawbacks of our study is the relatively low number of individuals per protein C pathway abnormality subgroup, which decreased our power to detect effects for *CADMI* variants. In addition, testing multiple SNVs for association with venous thrombosis increases the chance of false-positive associations. We therefore calculated FDR-adjusted q-values, after which we were no longer able to detect an association between the *CADMI* variants and venous thrombosis. We sought support for our hypothesis through addressing the association between venous thrombosis and *CADMI* variants in not only patients with low protein C levels, but also in other subgroups of patients with protein C pathway abnormalities. Although there was some overlap in patients within the protein C pathway subgroups, we observed almost no overlap in the thrombosis-associated *CADMI* variants across the subgroups of protein C pathway abnormalities. Only one variant (rs11608105) was found to be associated with venous thrombosis in multiple subgroups, in this case in patients with low protein C and S levels. In some cases, the direction of the odds ratio for venous thrombosis risk of the positive *CADMI* variant differed across the protein C pathway abnormalities (Supplementary Table 2). Taken together, this may suggest that genetic variation in *CADMI* interacts only with single or specific factors within the protein C pathway.

Another drawback of our study is that the protein C and protein S deficiencies were not individually diagnosed, but we determined levels below clinical cut-offs using a single test. Therefore, the prevalence of the protein C pathway abnormalities may vary and some misclassification may have occurred. It is unlikely though to have affected the comparisons on a group level. In addition, as in all case-control studies, we cannot rule out that the thrombotic event itself influenced the coagulation factor levels, in particular the levels of the acute phase reactant factor VIII. However, the median time between blood draw and thrombotic event was 10 months and we did not observe any difference between the mean FVIII levels of blood samples drawn less than 6 months after the thrombotic event and blood samples drawn 6 or more months after the thrombotic event (mean levels of 134.9 and 132.7 IU/ml, respectively).

We identified several variants of which the risk allele was carried by patients or control subjects only. These might be involved as risk or protective alleles for venous thrombosis when co-occurring with a protein C pathway abnormality. However, since these variants were rare and the number of individuals was low, we are not able to draw conclusions about these variants.

The variant that was most strongly associated in the French Canadian family study, rs6589488, was not associated in the overall MEGA study (OR 1.07, 95% CI 0.98–1.17) nor in one of the subgroups of protein C pathway abnormalities (Supplementary Table 3). Linkage disequilibrium, as determined by r^2 , with the variants consistently associated with venous thrombosis in our analysis (listed in Table 2) was low (<0.15). One explanation for the lack of a clear effect of rs6589488 in the current study is that the variants in the family study are rare mutations, private to this family or the French Canadian population. The results found in the current case-control study for a joint thrombophilic effect of *CADMI* variants with protein C deficiency, protein S deficiency, or high factor VIII levels does suggest though that the *CADMI* pathway might play a role in the biology of hemostasis in the general population as well. The *CADMI* pathway links to the actin cytoskeleton and in the cancer literature its oncogenic effect is due to variants in *CADMI* as well as downstream proteins [24–27]. Analysis of genes of downstream members of the *CADMI* pathway might identify additional novel risk factors for venous thrombosis. Another possibility is that mutations in the gene for protein C (*PROC*) itself affect the interaction between *CADMI* and protein C pathway. However, this would involve an indirect interaction between the downstream pathways associated respectively with the Endothelial Cell Protein C receptor and *CADMI*, as there is no evidence for a direct interaction of protein C with *CADMI*.

In conclusion, this study found some evidence of a joint effect of genetic variation in *CADMI* and protein C pathway abnormalities on the risk of venous thrombosis. This study aimed to validate a previous genetic study in a large thrombophilic family study, but could not replicate the specific associations observed in the family. Therefore, further study of the *CADMI* pathway is needed to determine whether abnormalities of the *CADMI* pathway link the risk for venous thrombosis to the vessel wall.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Highlights

- We genotyped over 300 variants in *CADMI* in a population-based case-control study
- We compared VT risk between cases with a protein C pathway defect and controls
- One variant increased the risk of VT when combined with low protein C or S levels
- One variant showed an increased risk of VT in the overall study population
- We confirmed a joined role for *CADMI* and protein C pathway abnormalities in VT risk

Figure 1. Linkage disequilibrium between *CADMI* variants which were associated with venous thrombosis in the univariable analysis of specific protein C pathway abnormality groups. Linkage equilibrium between the variants, as calculated in the controls, is expressed as r^2 .

Table 1
Characteristics of the MEGA study population

Low protein C was defined as activity levels below 67% of normal or when on anticoagulant treatment relative to factor VII (see methods). Similarly, low protein S was defined as activity levels below 67% of normal or when on anticoagulant treatment relative to factor II (see methods). High factor VIII was defined as activity levels higher than the geometric mean plus two standard deviations among controls (see methods).

	Patients (N=3496)	Controls (N=4004)
Men (%)	1633 (46.7)	1892 (47.3)
Mean age (SD)	49.18 (12.81)	48.40 (12.36)
FVL carrier (%)	580 (16.60)	219 (5.48)
Plasma available	1970	2490
Low protein C (%)	194 (9.90)	28 (1.13)
Low protein S (%)	23 (1.26)	26 (1.15)
High factor VIII (%)	165 (8.38)	51 (2.05)

Table 2
Associations with venous thrombosis in the different subgroups

In the univariable analysis, 22 variants were associated with venous thrombosis. The risk allele frequency was calculated in the subgroup of cases in which the variant was identified and in the overall controls.

		Risk allele frequency, %				FDR	
		Patients	Controls	OR	95% CI	p-value	q-value
Low protein C patients							
rs11608105	7.22	4.72	1.57	1.05–2.34	0.026	1	
Low protein S patients							
rs4938182	32.6	19.8	1.95	1.05–3.63	0.034	0.756	
rs4450197	8.70	2.04	4.95	1.67–14.7	0.004	0.333	
rs10128746	13.0	3.63	4.40	1.75–11.1	0.002	0.333	
rs11215418	10.9	3.62	3.37	1.29–8.83	0.013	0.371	
rs45595941	4.35	0.70	6.71	1.54–29.3	0.011	0.371	
rs45616036	4.35	0.84	5.03	1.25–20.3	0.023	0.575	
rs11608105	13.0	4.72	2.98	1.27–7.02	0.013	0.371	
rs45520832	2.17	0.11	20.1	2.45–166	0.005	0.333	
rs45583332	4.35	0.70	6.71	1.54–29.3	0.011	0.371	
High factor VIII patients							
rs10891823	9.47	6.48	1.48	1.02–2.16	0.040	0.999	
rs11215504	7.58	4.35	1.79	1.18–2.73	0.006	0.750	
rs11215515	7.10	4.26	1.75	1.14–2.68	0.010	0.833	
rs11215458	5.62	3.65	1.61	1.00–2.60	0.050	0.999	
rs220842	1.52	0.51	3.02	1.18–7.74	0.022	0.999	
rs10891856	9.47	5.75	1.75	1.21–2.55	0.003	0.750	
Factor V Leiden patients							
rs12577709	15.9	13.6	1.19	1.01–1.42	0.041	0.988	
rs45545346	1.73	3.42	0.50	0.32–0.79	0.003	0.741	
rs45608938	3.89	5.42	0.71	0.52–0.97	0.032	0.988	
rs17443832	3.97	5.38	0.73	0.54–0.99	0.045	0.988	
rs45578937	5.10	7.07	0.71	0.54–0.93	0.014	0.865	

Risk allele frequency, %		OR	95% CI	p-value	q-value
Patients	Controls				
rs45458294	4.84	6.92	0.68	0.52–0.91	0.008
rs314497	7.84	5.86	1.37	1.08–1.73	0.009

Table 3
Mutually adjusted associations with venous thrombosis in the different subgroups

When including the positive associations per subgroup together in a logistic regression model, six variants remained associated with venous thrombosis.

	Risk allele frequency, %			
	Patients	Controls	OR	95% CI
Low protein C patients				
rs11608105	7.22	4.72	1.57	1.05–2.34
Low protein S patients				
rs4938182	32.6	19.8	1.60	0.79–3.22
rs4450197	8.70	2.04	1.22	0.21–7.25
rs10128746	13.0	3.63	3.04	0.72–13.0
rs45616036	4.35	0.84	1.92	0.07–51.9
rs11608105	13.0	4.72	3.54	1.46–8.60
rs45520832	2.17	0.11	22.1	2.35–208
rs45583332	4.35	0.70	4.27	0.15–124
High factor VIII patients				
rs10891823	9.47	6.48	1.13	0.59–2.14
rs11215504	7.58	4.35	1.89	1.24–2.88
rs11215515	7.10	4.26	1.16	0.56–2.40
rs11215458	5.62	3.65	1.03	0.42–2.50
rs220842	1.52	0.51	3.23	1.17–8.97
rs10891856	9.47	5.75	1.60	0.89–2.85
Factor V Leiden patients				
rs12577709	15.9	13.6	1.09	0.90–1.32
rs45545346	1.73	3.42	0.53	0.30–0.93
rs45608938	3.89	5.42	0.71	0.52–0.97
rs45578937	5.10	7.07	0.93	0.66–1.30
rs314497	7.84	5.86	1.27	0.98–1.65

Table 4

Associations with venous thrombosis in MEGA overall study population.

<i>CADMI</i> variants	Risk allele frequency, %		OR	95% CI
	Patients	Controls		
rs11608105	4.57	4.72	0.97	0.83–1.13
rs45520832	0.14	0.11	1.27	0.52–3.13
rs11215504	4.94	4.35	1.14	0.98–1.33
rs220842	0.76	0.51	1.49	0.99–2.24
rs45608938	5.33	5.42	0.98	0.85–1.13
rs45545346	3.13	3.42	0.92	0.77–1.09

Table 5
Combined associations for *CADMI* SNVs with protein C pathway abnormalities and venous thrombosis

REF = reference.

<i>CADMI</i> variants	Pathway defect	Patients, N	Controls, N	OR	95% CI
rs11608105	No PC/PS	1503	1996	1(REF)	
rs11608105	Yes PC/PS	123	206	0.79	0.63–1.00
rs11608105	No PC/PS	181	49	4.91	3.55–6.77
rs11608105	Yes PC/PS	32	2	21.3	5.08–88.8
rs45520832	No PS	1798	2219	1(REF)	
rs45520832	Yes PS	5	3	2.06	0.49–8.62
rs45520832	No PS	22	26	1.04	0.59–1.85
rs45520832	Yes PS	1	0	NA	NA
rs220842	No FVIII	1775	2416	1(REF)	
rs220842	Yes FVIII	29	21	1.88	1.07–3.31
rs220842	No FVIII	160	51	4.27	3.10–5.89
rs220842	Yes FVIII	5	0	NA	NA
rs11215504	No FVIII	1638	2222	1(REF)	
rs11215504	Yes FVIII	166	215	1.05	0.85–1.30
rs11215504	No FVIII	141	46	4.16	2.96–5.84
rs11215504	Yes FVIII	24	5	6.51	2.48–17.1
rs45608938	No FVL	2591	3379	1(REF)	
rs45608938	Yes FVL	318	397	1.04	0.89–1.22
rs45608938	No FVL	533	198	3.51	2.96–4.17
rs45608938	Yes FVL	45	19	3.09	1.80–5.29
rs45545346	No FVL	2721	3524	1(REF)	
rs45545346	Yes FVL	192	255	0.98	0.80–1.18
rs45545346	No FVL	559	210	3.45	2.92–4.07
rs45545346	Yes FVL	20	9	2.88	1.31–6.33