Complement Activation Assessed by the Plasma Terminal Complement Complex and Future Risk of Venous Thromboembolism

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ABSTRACT

Background: Growing evidence support a link between key components of the complement system and risk of venous thromboembolism (VTE). However, it remains uncertain whether activation of the complement system, assessed by the soluble terminal C5b-9 complement complex (plasma TCC), is associated with future risk of incident VTE.

Aims: To investigate the association between plasma levels of TCC and future risk of incident VTE in a nested case-control study, and to explore genetic variants associated with plasma levels of TCC using protein quantitative trait loci (pQTL) analysis of exome sequencing data.

Methods: The study population consisted of 415 VTE cases and 848 age- and sex-matched controls who were sampled from a population-based cohort, the Tromsø study. Logistic regression models were used to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for VTE across quartiles of plasma levels of TCC. Whole exome sequencing was conducted in a subgroup (355 VTE patients and 354 controls) using the Agilent SureSelect 50Mb capture kit.

Results: The risk of VTE increased across quartiles of plasma TCC levels, particularly for unprovoked VTE (p for trend 0.03). Participants with plasma TCC in the highest quartile (>1.40 CAU/mL) had an OR for unprovoked VTE of 1.83 (95% CI 1.02-3.38) compared to those with plasma TCC in the lowest quartile (≤0.80 CAU/mL) in analyses adjusted for age, sex and body mass index. The OR for VTE by high plasma TCC was substantially higher in individuals with short time between blood sampling and VTE event. We found no significant association between genome-wide and complement-related gene variants and plasma levels of TCC.

Conclusions: Our findings showed that plasma levels of TCC are independent of gene regulation and that high levels of plasma TCC are associated with VTE risk, and unprovoked events in particular.

Key words: Terminal complement complex, complement system, whole exome sequencing, protein quantitative trait loci (pQTL) analysis, venous thromboembolism

ESSENTIALS

- It is not known whether complement activation, assessed by plasma levels of TCC, is associated with VTE
- Genome-wide and complement-related gene variants were not associated with plasma levels of TCC
- High plasma levels of TCC were associated with future risk of VTE, particularly unprovoked events
- The risk of VTE by plasma TCC increased substantially with shorter follow-up time in the analysis.

INTRODUCTION

Venous thromboembolism (VTE) is a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE). VTE is a complex disease, affecting 1-2 per 1000 individuals annually (1, 2), with serious short- and long-term complications, including recurrence and death (3, 4). The incidence of VTE has slightly increased during the last two decades (5, 6), and it is likely that the incidence will continue to rise since the prevalence of major risk factors for VTE, such as high age, obesity and cancer, are increasing in the population (7-9). VTE has become a major challenge to public health and healthcare systems due to frequent hospitalizations, monitoring of treatment to avoid bleeding complications, severe complications and a high mortality rate after 30 days (10). It is therefore pivotal to identify novel biomarkers and unravel underlying disease mechanisms of VTE in order to facilitate targeted prevention and treatment.

The complement system is an important part of the innate immune system organized in a cascade of proteolytic serine proteases with a number of biological functions (11). Growing evidence from observational and animal studies suggest that the complement system is involved in the early steps of the pathogenesis of VTE, consistent with an extensive cross-talk between the complement and hemostatic systems (12). Complement factor C3 is an acute-phase reactant and a central component in activation of the complement system (13). In a large population-based cohort, subjects with plasma complement C3 levels in the highest tertile had a 58% higher risk of VTE than those in the lowest tertile, and the risk estimate declined to 31% after further adjustment for C-reactive protein (CRP) and body mass index (14). However, it is not known whether complement C3 is a marker or a mediator of VTE risk.

Activation of any of the three complement pathways merge at C3 which subsequently activates C5 and the terminal complement pathway with final formation of C5a and the terminal C5b-9 complement complex (TCC) (15). The TCC exists in two forms, surface-bound C5b-9 forming the membrane attack complex, and a soluble form where C5b-9 binds to S-protein and clusterin and becomes non-lytic (16). Soluble TCC is generally accepted as a marker of *in vivo* complement activation and can be measured in plasma (17, 18).

No previous study has, to the best of our knowledge, investigated the association between plasma levels of TCC and future risk of VTE in individuals recruited from the general population. Therefore, the aims of the present study were; (i) to investigate whether plasma levels of TCC were associated with incident VTE in a nested case-control study, (ii) to explore whether genetic variants were associated with plasma levels of TCC using protein quantitative trait loci (pQTL) analysis of exome sequencing data, and (iii) investigate whether identified gene variants (if present) were associated with risk of VTE.

METHODS

Study population

The Tromsø Study is a single-center, population-based cohort, with repeated health surveys of inhabitants of Tromsø, Norway. All inhabitants aged ≥25 years living in the municipality of Tromsø, Norway were invited to participate in the fourth survey, conducted in 1994/95. A total of 27,158 subjects participated (77% of those invited), and were followed from the date of inclusion until an incident VTE, migration, death, or end of follow-up (September 1, 2007). All first lifetime VTE events were identified using the hospital discharge diagnosis registry, the autopsy registry and the radiology procedure registry at the University Hospital of North Norway (UNN), which is the sole hospital in the Tromsø region. Trained personnel adjudicated and recorded each VTE by extensive review of medical records. A VTE was confirmed if presence of signs and symptoms of PE or DVT were combined with objective confirmation by radiological procedures (i.e. compression ultrasonography, venography, spiral computed tomography, perfusion-ventilation scan, pulmonary angiography) or autopsy, and resulted in treatment initiation (unless contraindications were specified) as previously described (19). A VTE occurring in the presence of one or more provoking factors was classified as provoked. The following were regarded as provoking factors: surgery or trauma (within eight weeks before the event), acute medical conditions (acute myocardial infarction, acute ischemic stroke, acute infections), immobilization (bed rest> 3 days or confinement to wheelchair within the last 8 weeks, or long distance travel ≥4 hours within the last 14 days), or other factor specifically described as provoking by a physician in the medical record (e.g. intravascular catheter).

During the follow-up period (1994-2007), 462 individuals experienced a VTE event. For each case, two age- and sex-matched controls, who were alive at the index date of the VTE event, were randomly sampled from the source cohort (n=924). In total, 47 cases and 76 controls did

not have available plasma samples of sufficient quality for the analyses. Thus, our final nested case-control study consisted of 415 cases and 848 controls.

Baseline Measurements

Height (to the nearest cm) and weight (to the nearest 0.5 kg) were measured in participants wearing light clothing and no shoes. Body mass index (BMI) was calculated as weight divided by the square of height in meters (kg/m^2). A self-administered questionnaire was used to collect detailed baseline information on smoking status and previous cardiovascular events (stroke, angina pectoris, TIA, and MI).

Blood and DNA samples

Non-fasting blood was collected from an antecubital vein into 5 mL vacutainers (Becton Dickinson, Meylan Cedex, France) containing EDTA (K_3-EDTA 40µL, 0.37mol/L per tube) as an anticoagulant. Platelet poor plasma (PPP) was prepared by centrifugation at 3000x*g* for 10 minutes at room temperature, after which the supernatant was transferred into cryovials (Greiner laboratechnik, Nürtringen, Germany) in 1 mL aliquots and stored at -80°C until further analysis. DNA isolated from blood was stored at the National CONOR Biobank (20).

Exome sequencing

Whole exome sequencing at high-coverage (≈100x) was conducted in a subgroup of the study population (355 VTE patients and 354 control subjects) using the Agilent SureSelect 50Mb capture kit. The resulting genotypes were effectively filtered (21) and imputations were carried out as previously described in detail (22).

Measurement of the soluble Terminal Complement Complex

Plasma samples were thawed on crushed ice, and plasma levels of TCC were measured using an in-house enzyme-linked immunosorbent assay (ELISA) as described previously (23). In brief, ELISA plates (Nunc, Immunoplate II, Copenhagen, Denmark) were coated with the monoclonal antibody, aE11 specific for a neoantigen exposed in C9 after activation and incorporation into TCC, the poly (C9) unit of the TCC. Biotinylated anti-C6 monoclonal antibody (9C4) was used as detection antibody and Streptavidin horseradish peroxidase (GE Healtcare UK) was added as the final step. Optical density was measured at 405 nm (Dynatech MR580, Dynatech Laboratories Inc., Alexandria, Va., USA). Results are given in Complement Arbitrary Units (CAU)/mL.

Statistical analysis

Statistical analyses were carried out using R version 3.4.4 (The R Foundation for Statistical Computing c/o Institute for Statistics and Mathematics, Vienna, Austria). Plasma TCC was categorized according to quartile cut offs in the control population (≤0.80, 0.80-1.04, 1.04-1.40, >1.40 CAU/ml). Means and proportions of baseline characteristics across categories of TCC were calculated using descriptive statistics. Logistic regression models were used to calculate odds ratios (OR) for VTE with 95% confidence intervals (CI) according to quartiles of TCC adjusted for age, sex and BMI. We did not adjust for CRP since CRP is likely to be in the causal pathway of complement activation and VTE. The lowest quartile of TCC was used as the reference group. We also calculated the *P* value for linear trend of ORs across increasing quartiles of TCC.

As the follow-up time in the source cohort was long (more than 12 years for many persons), the results based on baseline TCC measurements could be influenced by regression dilution

bias. To investigate this, we performed analyses where we restricted the maximum time from blood sampling in Tromsø 4 to the VTE events, while keeping all controls in the analyses. The logistic regression analyses on time restrictions were set to require at least 10 VTE events, and ORs were generated at every 0.1 year increase in time since blood sampling and plotted as a function of this maximum time.

The 1.034.910 genome-wide variants derived from the whole-exome-sequencing were used to investigate whether plasma TCC was regulated by any alternative genetic variants. This pQTL analysis was performed both in a genome-wide setting, and restricted to the loci within ±500 kb of the different genes involved in the complement system (Supplementary Table 1). To adjust for multiple testing, the commonly used significance threshold of $5x10^{-8}$ was used in the genome-wide setting. As the cis analysis in total contained 11.806 variants, a Bonferroni-based adjustment for multiple testing corresponded to a significance threshold of $0.05/11.806 = 4.23x10^{-6}$. The plasma TCC values were transformed to follow a perfect standard normal distribution before entering the QTL analysis. The QTL analysis was done by the EPACTS (Efficient and Parallelizable Association Container Toolbox) software (24). The EMMAX (Efficient Mixed Model Association eXpedited) (25) test within EPACTS was used, which uses a mixed model to test for associations between a quantitative trait (TCC in this study) and genetic variants while adjusting for covariates and genetic relatedness structure in the cohort.

RESULTS

The distribution of baseline characteristics across quartiles of plasma TCC is shown in Table 1. The mean age, sex distribution, and mean BMI were essentially similar across quartiles of plasma TCC. The proportion of subjects with a history of cardiovascular disease was slightly lower and the proportion of smokers higher in the lowest TCC quartile. Plasma levels of hsCRP increased as expected across quartiles of TCC from 1.32±1.08mg/L in the lowest quartile to 1.96±1.65mg/L in the highest quartile.

The characteristics of the VTE patients are shown in Table 2. The mean age at the time of VTE was 67.4 years, and 48.4% of the cases were men. In total, 37.8% of the VTE events were PEs and 62.2% were DVTs, and 42.2% of the cases were unprovoked. The most common provoking factors were surgery/trauma (22.4%) and active cancer (21.4%).

The ORs of VTE across quartiles of plasma TCC levels are shown in Table 3. For overall VTE, the OR increased across quartiles of plasma TCC (*p* for trend: 0.06), and subjects with plasma TCC >1.40 CAU/ml had a 35% higher risk of VTE compared to those with TCC ≤0.80 CAU/ml (OR 1.35; 95% CI: 0.97-1.88). In subgroup analyses restricted to unprovoked VTE, the OR for the upper (>1.40 CAU/ml) versus the lower quartile of plasma TCC (≤0.80 CAU/ml) was 1.74 (95% CI: 1.10-2.78), and there was a statistically significant linear trend in the ORs across increasing quartiles of TCC (*p* for trend: 0.02). When restricting the analysis to provoked VTE, no difference in risk estimates of VTE across quartiles of TCC was observed. Further adjustment for BMI did not influence the estimates (Table 3). To consider the possibility of underestimating ORs due to regression dilution bias, we estimated ORs for VTE among subjects with high (highest quartile) versus low (lowest quartile) plasma TCC as a function of time between blood sampling and the VTE events (Figure 1). The OR for VTE

by high plasma TCC was substantially higher with shortened time between the blood sampling and the VTE events.

The ORs for DVT and PE across quartiles of plasma TCC are shown in Table 4. The OR for DVT increased across quartiles of plasma TCC (*p* for trend: 0.06). Subjects with plasma TCC values >1.40 CAU/ml had a 38% higher risk of DVT compared to those with TCC ≤0.80 CAU/ml (OR 1.38; 95% CI: 0.93-2.06). Although not statistically significant, the OR of unprovoked PE was higher in subjects with plasma TCC in the highest quartile compared to those in the lowest quartile (OR:1.58, 95% CI: 0.82-3.10). The estimates did not change after adjustment for BMI (Table 4). The ORs for DVT and PE, respectively, among subjects with high (highest quartile) versus low (lowest quartile) plasma TCC as a function of time between blood sampling and VTE were then calculated (Figure 2). The ORs for DVT and PE showed essentially similar patterns as the ORs for overall VTE (Figure 1, left panel), and increased substantially with shortened time between blood sampling and the respective events.

To identify genetic variation associated with plasma levels of TCC, we tested for association between genome-wide and complement-related variants and plasma levels of TCC. Figure 3 shows a modified Manhattan plot based on the results of the pQTL analysis with the genome-wide variants marked by grey circles and the complement-related analysis (variants within ±500 kb of the genes involved in the complement system) marked by blue triangles. Age, sex, BMI, and VTE status were included as covariates in the models. No gene variant had a *p* value that was statistically significant neither in the genome-wide nor in the complement-related analysis.

DISCUSSION

We investigated the association between complement activation, assessed by plasma levels of TCC, and future risk of VTE in a population-based nested case-control study. The risk of VTE increased across quartiles of plasma TCC, and subjects with plasma TCC >1.40 CAU/ml (highest quartile) had a 35% higher risk of overall VTE and 74% higher risk of unprovoked VTE compared to those with TCC ≤0.80 CAU/ml (lowest quartile). The ORs for VTE by plasma TCC increased substantially with shortened time between blood sampling and the VTE events. Further, we found no significant association between genome-wide and complement-related gene variants and plasma levels of TCC. Our findings indicate that plasma levels of TCC are independent of gene regulation, and that high levels of plasma TCC are associated with VTE risk, and unprovoked events in particular.

A few studies have investigated the link between complement factors and the risk of VTE. In a large population-based cohort (the Copenhagen General Population Study), 80 517 Danes were followed on average 4.9 years in which 1176 developed VTE. Subjects with plasma complement C3 levels in the highest tertile had a 58% higher risk of VTE compared to those in lowest tertile, which declined to 31% higher risk of VTE after additional adjustment for CRP and BMI (14). Even though the risk estimate was attenuated by adjustment for CRP and BMI, the remaining risk and the pivotal role of complement C3 (14), may suggest that complement C3 is not merely a marker, but a mediator of VTE risk. The latter concept is supported by experiments in C3- and C5-deficient mice which had a lower incidence of VTE and reduced thrombus size compared to wild-type mice in a tissue factor (TF)-dependent model of flow restriction-induced VTE (26). Recently, we reported that high activity of the classical complement pathway was associated with unprovoked VTE in a small case-control study (27). Furthermore, systemic lupus erythematosus (SLE) patients with a history of VTE

had increased deposition of C1q, C3, and C4d on platelets compared to SLE patients without VTE, suggesting that activation of the classical pathway of the complement system may play a role in the pathogenesis of VTE in SLE patients (28). The authors speculated that the effect was mediated by formation of TCC at the surface membrane of platelets. Accordingly, we found that soluble TCC, the end stage of complement activation, measured in plasma was associated with increased risk of VTE, and especially unprovoked VTE. In contrast to plasma TCC and other activation products, which directly reflect complement activation, plasma levels of individual complement components like C3 is not a reliable indicator of the degree of complement activation *in vivo*. Thus, our study strongly support the concept that the degree of complement activation, and not merely concentrations of parent molecules per se, is related to future risk of VTE.

Our study is, to the best of our knowledge, the first to provide actual evidence for an association between complement activation, assessed by plasma TCC, and future risk of VTE. In prospective studies, the biomarker levels are usually measured at the time of inclusion and related to an outcome that occur several years later. Plasma levels of modifiable biomarkers are expected to change over time. Fluctuations in exposure during follow-up will lead to a phenomenon called regression dilution bias (29), which results in an underestimation of the true association between exposure and outcome. Accordingly, we found that the risk of VTE by plasma levels of TCC declined substantially with time between blood sampling and VTE.

In our study, genome-wide and complement-related gene variants showed no significant association with plasma levels of TCC. These findings strongly suggest that the plasma levels of TCC are determined by environmental rather than genetic factors. Autopsy- and imaging

studies indicate that venous thrombi originate in the valvular sinuses of large veins. The milieu in the valvular sinuses is characterized by severe hypoxia and stasis (30, 31), conditions known to induce cellular immune responses. As the complement system is activated by hypoxic cells and tissues (32), it is reasonable to assume that complement is also activated in the hypoxic milieu in the valvular sinuses. It may be speculated that subjects susceptible to VTE exhibit a certain pathophysiological milieu in the valvular sinuses which makes them prone to complement activation and thereby development of VTE. Alternatively, other inflammatory conditions associated with increased plasma levels of TCC (33, 34) may be differentially distributed between subjects who will and will not develop VTE. However, because the inflammatory response, in our study assessed by plasma C-reactive protein (CRP) levels, secondary to such conditions would be in the causal pathway between plasma TCC and VTE risk, it would be methodological incorrect to adjust for plasma CRP levels in the statistical analyses. In fact, complement activation is upstream of inflammatory responses such as CRP formation (35), but when CRP is produced and bound to surfaces it is able to activate the classical pathway of the complement system (36, 37).

A clear temporal sequence between exposure and outcome, such as in our nested case-control study, is a prerequisite to establish plasma levels of TCC as a risk factor of VTE. Circumstantial evidence support the concept that complement activation, assessed by plasma TCC, may be a mediator of VTE risk through platelet- and coagulation activation since it reflects the degree of membrane inserted C5b-9. First, incorporation of the C5b-9-complex into the cell membrane activates platelets and results in (i) the exposure of negatively charged phosphatidylserine (PS) (38) which may assembly and amplify coagulation reactions (39), (ii) the formation of procoagulant microvesicles (MVs) (40) which is associated with risk of VTE (41), and (iii) secretion of procoagulant granules from the cytoplasm of platelets (42).

Second, the complement system has direct procoagulant activities, including the ability to cleave and activate coagulation factors (44) and increase TF expression in various cell types (43, 44). In addition, C5a has the ability to upregulate the synthesis and release of plasminogen activator inhibitor-1 (PAI-1), the main endogenous inhibitor of the fibrinolytic system, from mast cells and basophils (43), which will further shift the prothrombotic-antithrombotic balance in favor of a prothrombotic condition (44).

Strengths of our study include the recruitment of VTE patients from a population-based cohort and age- and sex-matched apparently healthy controls from the same source population. It is a large prospective study, with robust measurements of plasma TCC. The fact that blood samples were collected before VTE makes it possible to make assumptions on the direction of the association between exposure (complement activation) and outcome (VTE). Some limitations of the study need to be considered. The blood samples, in which the analysis of plasma TCC was conducted, were drawn in 1994/95 and stored at -80°C for up to 22 years. The long storage time could potentially affect the plasma levels of TCC. However, it is unlikely that this would change the results, as this storage-effect would be similar in all samples. Additionally, plasma TCC was only measured at baseline, and changes in TCC levels during up to 12 years of follow-up could result in underestimation of the true association (45). Accordingly, we found that the ORs for VTE by plasma TCC decreased substantially with prolonged time between blood sampling and the VTE events. Finally, plasma TCC is a very stable activation product compared to upstream activation products (23). Plasma levels of TCC remains unchanged after several freezing and thawing cycles (23) and storage at -70°C for 10 years (unpublished data).

In conclusion, results from our nested case-control study imply that complement activation, assessed by plasma levels of TCC, is associated with increased risk of VTE, and unprovoked events in particular. Genome-wide and complement-related gene variants were not associated with plasma levels of TCC, suggesting that local or systemic environmental factors are the dominating determinants of complement activation leading to increased plasma TCC. Functional studies are warranted to investigate the molecular mechanisms behind the association between plasma TCC and VTE risk.

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Tables and figures

Table 1: Characteristics across quartiles of plasma levels of the terminal complement complex (TCC)

	Plasma TCC (CAU/ml)			
	≤0.8 (n=313)	**<0.80-1.04]** (n=297)	**<1.04-1.40]** (n=322)	**>1.40** (n=331)
Age, years	58.7±13.5	61.3±12.8	61.4±14.0	59.8±14.4
Sex, % men (n)	50.8 (159)	48.1 (143)	42.2 (136)	48.0 (159)
BMI, kg/m^2	26.3±4.2	26.6±4.0	26.2±4.3	26.6±4.4
hsCRP, mg/L	1.32±1.08	1.50±1.28	1.71±1.30	1.96±1.65
CVD*	13.7 (43)	17.5 (52)	16.1 (52)	15.1 (50)
Daily smoking	34.2 (107)	30.3 (90)	32.9 (106)	27.5 (91)

*Self-reported history of cardiovascular disease (myocardial infarction, angina, stroke)

Table 2: Characteristics of the VTE events (n=415)

	% (n)
Age at VTE (years)	67.4±13.6
Sex (males)	48.4 (201)
Deep vein thrombosis	62.2 (258)
Pulmonary embolism	37.8 (157)
Unprovoked VTE	42.2 (175)
Provoked VTE	57.8 (240)
Surgery/Trauma	22.4 (93)
Active cancer	21.4 (89)
Acute medical condition	15.4 (64)
Immobilization	17.8 (74)
Other factor	3.9 (16)

Table 3. Odds ratios (OR) with 95% confidence intervals (CI) for venous thromboembolism (VTE) according to quartiles of plasma levels of the terminal complement complex (TCC)

Quartiles of plasma TCC (CAU/ml)	**Controls**	**Cases**	**Model 1 OR (95% CI)**	**Model 2 OR (95% CI)**
All VTE				
≤0.80	220	93	Ref	Ref
0.80-1.04	204	93	1.08 (0.76-1.53)	1.06 (0.75-1.51)
1.04-1.40	213	109	1.22 (0.87-1.71)	1.23 (0.88-1.73)
>1.40	211	120	1.35 (0.97-1.88)	1.33 (0.96-1.86)
p for trend			0.06	0.06
Per 1 SD increase	848	415	1.09 (0.97-1.22)	1.08 (0.97-1.22)
Unprovoked VTE				
≤0.80	220	35	Ref	Ref
0.80-1.04	204	39	1.23 (0.75-2.02)	1.17 (0.71-1.94)
1.04-1.40	213	43	1.31 (0.81-2.15)	1.34 (0.82-2.19)
>1.40	211	58	1.74 (1.10-2.78)	1.72 (1.08-2.75)
p for trend			0.02	0.02
Per 1 SD increase	848	175	1.13 (0.99-1.29)	1.13 (0.99-1.29)
Provoked VTE				
≤0.80	220	58	Ref	Ref
0.80-1.04	204	54	0.99 (0.65-1.51)	0.98 (0.64-1.49)
1.04-1.40	213	66	1.17 (0.78-1.74)	1.15 (0.77-1.73)
>1.40	211	62	1.11 (0.74-1.67)	1.11 (0.74-1.67)
p for trend			0.47	0.47
Per 1 SD increase	848	240	1.03 (0.88-1.19)	1.04 (0.88-1.20)

Model 1: adjusted for age and sex

Model 2: adjusted for age, sex and body mass index

Table 4. Odds ratios (OR) with 95% confidence intervals (CI) for deep vein thrombosis (DVT) and pulmonary embolism (PE) according to quartiles of plasma levels of the terminal complement complex (TCC)

Quartiles of plasma TCC (CAU/ml)	Controls	Cases	Model 1 OR (95% CI)	Model 2 OR (95% CI)
All DVT				
≤0.80	220	56	Ref	Ref
0.80-1.04	204	55	1.07 (0.70-1.62)	1.04 (0.68-1.58)
1.04-1.40	213	73	1.36 (0.91-2.02)	1.34 (0.90-2.00)
>1.40	211	74	1.38 (0.93-2.06)	1.36 (0.91-2.02)
p for trend			0.06	0.07
Per 1 SD increase	848	258	1.05 (0.90-1.20)	1.05 (0.90-1.21)
Unprovoked DVT				
≤0.80	220	19	Ref	Ref
0.80-1.04	204	22	1.28 (0.67-2.46)	1.22 (0.64-2.34)
1.04-1.40	213	28	1.58 (0.86-2.96)	1.59 (0.86-2.99)
>1.40	211	34	1.87 (1.05-3.45)	1.83 (1.02-3.38)
p for trend			0.03	0.03
Per 1 SD increase	848	103	1.05 (0.84-1.25)	1.06 (0.85-1.26)
Provoked DVT				
≤0.80	220	37	Ref	Ref
0.80-1.04	204	33	0.95 (0.57-1.59)	0.93 (0.56-1.55)
1.04-1.40	213	45	1.25 (0.78-2.02)	1.22 (0.75-1.97)
>1.40	211	40	1.12 (0.69-1.83)	1.12 (0.69-1.82)
p for trend			0.43	0.45
Per 1 SD increase	848	155	1.04 (0.86-1.22)	1.04 (0.86-1.22)
All PE				
≤0.80	220	37	Ref	Ref
0.80-1.04	204	38	1.10 (0.67-1.80)	1.07 (0.65-1.77)
1.04-1.40	213	36	1.01 (0.61-1.66)	1.04 (0.62-1.71)
>1.40	211	46	1.30 (0.81-2.09)	1.33 (0.82-2.15)
p for trend			0.35	0.28
Per 1 SD increase	848	157	1.12 (0.97-1.28)	1.12 (0.98-1.28)
Unprovoked PE				
≤0.80	220	16	Ref	Ref
0.80-1.04	204	17	1.16 (0.57-2.39)	1.10 (0.54-2.27)
1.04-1.40	213	15	0.99 (0.47-2.08)	1.00 (0.48-2.10)
>1.40	211	24	1.58 (0.82-3.10)	1.60 (0.83-3.15)
p for trend			0.23	0.20
Per 1 SD increase	848	72	1.19 (1.01-1.37)	1.19 (1.01-1.37)
Provoked PE				
≤0.80	220	21	Ref	Ref
0.80-1.04	204	21	1.05 (0.55-1.99)	1.02 (0.54-1.95)
1.04-1.40	213	21	1.01 (0.53-1.92)	1.03 (0.54-1.97)
>1.40	211	22	1.09 (0.58-2.05)	1.12 (0.59-2.11)
p for trend			0.83	0.74
Per 1 SD increase	848	85	1.02 (0.77-1.25)	1.03 (0.78-1.26)

Model 1: adjusted for age and sex

Model 2: adjusted for age, sex and body mass index

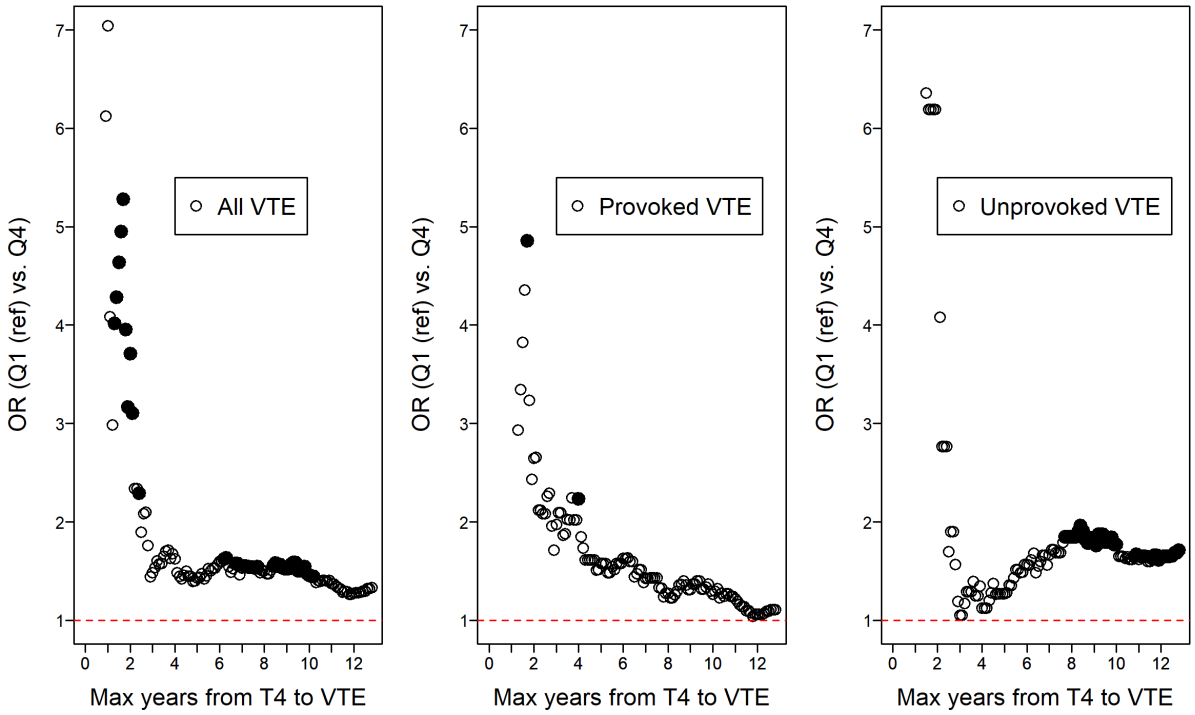

Figure 1: Plots of estimated OR for VTE as a function of time from blood sampling in Tromsø 4 (1994-95) and the VTE event (all, provoked and unprovoked events) in analyses adjusted for age, sex and BMI. Large, solid circles indicate ORs with *P*-values < 0.05.

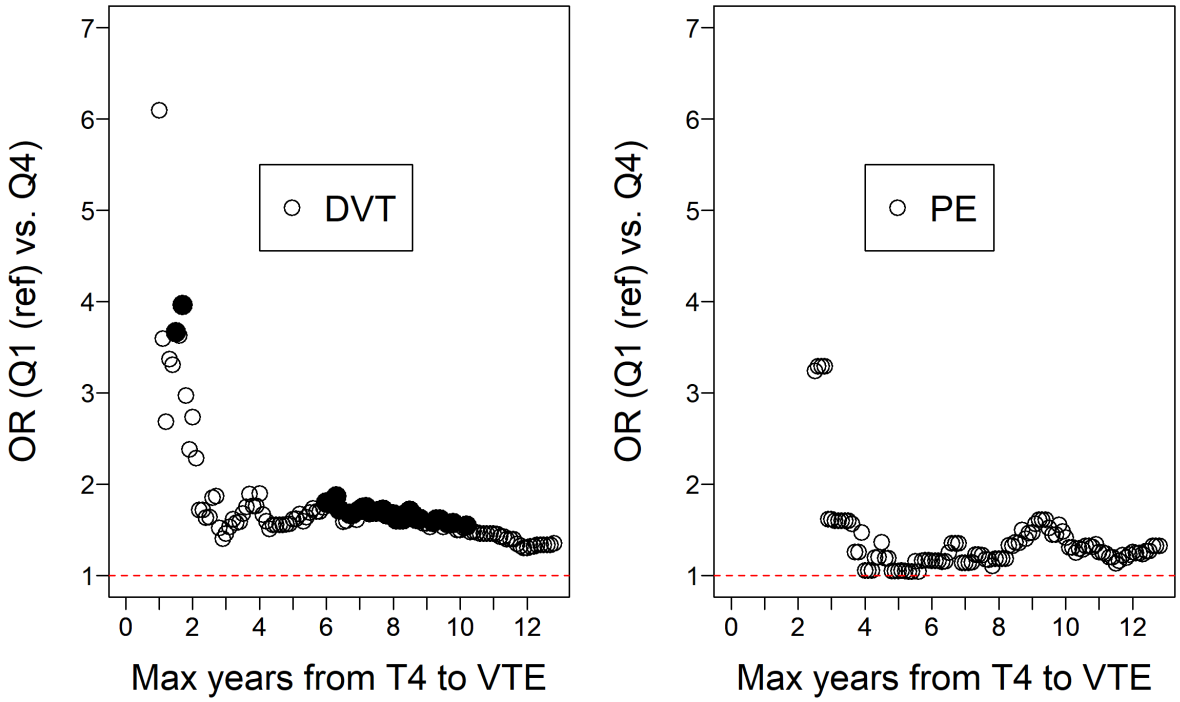

Figure 2: Plots of estimated OR for DVT and PE as a function of time from blood sampling in Tromsø 4 (1994-95) and event in analyses adjusted for age, sex and BMI. Large, solid circles indicate ORs with *P*-values < 0.05.

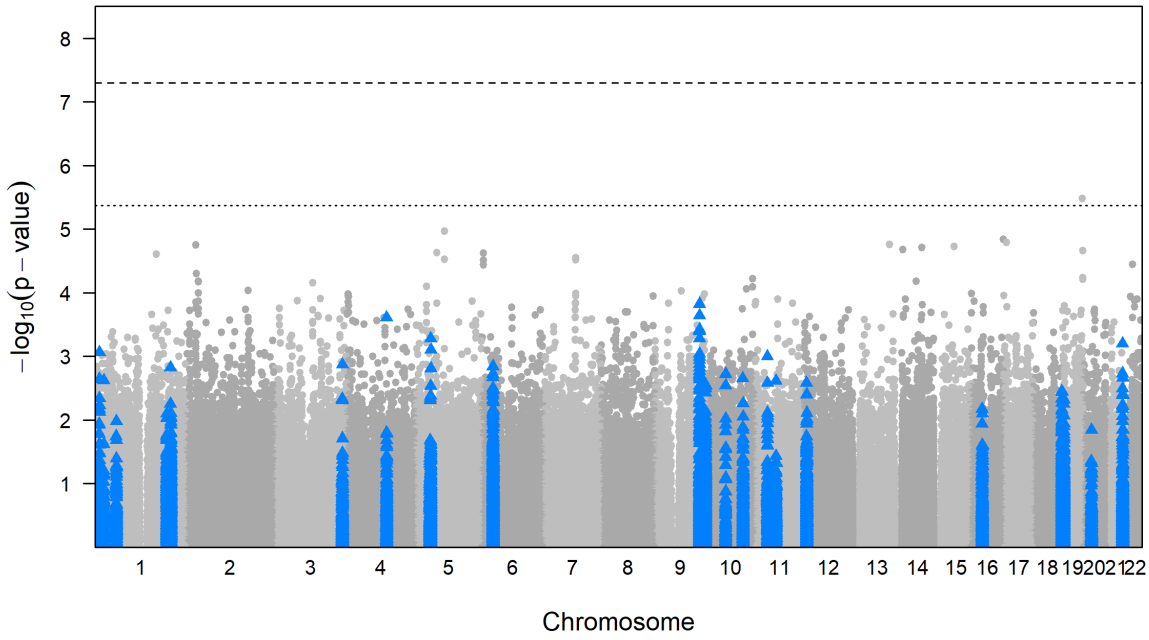

Figure 3: Modified Manhattan plot of QTL analysis results. The upper, dashed horizontal line indicates the $5x10^{8}$ *P* value significance threshold. As the cis analysis has 11.806 variants being tested, a strict Bonferroni-based control for multiple testing translates to a cis significance threshold of $-\log_{10} 0.00000423 = 5.37$. This is indicated by the lower, dotted horizontal line in the figure. The variants of the cis regions are marked with blue triangles.

Supplementary Table 1. Overview of the genes used in the quantitative trait loci (QTL) analysis when restricting the variants to loci within ±500 kb of the different genes involved in the complement system.

Name	**Main task**	**Pathway (mainly)**	**Gene**
C1q	Binds antigene:antibody complex and pathogens	Klassisk	*C1QA,B,C*
Mannose binding lectin (MBL)	Binds mannose on e.g. bacteria	MBL	*Mbl2*
C1r	Activating enzyme	Klassisk	*C1R*
C1s	Activating enzyme	Klassisk	C1S
C2b	Activating enzyme	Klassisk	C2
Bb	Activating enzyme	Alternativ	*CFB*
D	Activating enzyme	Alternativ	*CFD*
MASP-1	Activating enzyme	MBL	*MASP-1*
MASP-2	Activating enzyme	MBL	*MASP-2*
C3	Sentral protein	All	*C3*
C5	Sentral protein	All	*C5*
C4	Sentral protein	All	*C4A,B*
C4b	Binds to membranes and marks them for immune cells	All	
C3b	Binds to membranes and marks them for immune cells	All	
C5b	Protein in the TCC	All	*C5*
C6	Protein in the TCC	All	*C6*
C7	Protein in the TCC	All	*C7*
C8	Protein in the TCC	All	*C8,A,B,G*
C9	Protein in the TCC	All	*C9*
CR1	Receptor for complement proteins	All	*CR1*
CR2	Receptor for complement proteins	All	*CR2*
CR3	Receptor for complement proteins	All	*ITGB2*
CR4	Receptor for complement proteins	All	*ITGAX*
C1qR	Receptor for complement proteins	All	*CD93*
C1INH	One of the main regulators of the complement system	Alle	*SERPING1*
C4bp	Regulator	All	
MCP (CD46)	Regulator	All	*CD46*
DAF	Regulator	All	*CD55*
H	Regulator	All	*CFH*
I	Regulator	All	*CFI*
P	Regulator	All	
CD59	Regulator	All	*CD59*
CPN	Regulator	All	*CPN1*