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Elevated plasma concentration of complement factor C5 is associated with risk of future venous thromboembolism

Running head: High C5 levels associated with future thrombosis

Espen Waage Skjeflo[1,2], Sigrid Kufaas Brækkan[1,3], Judith Krey Ludviksen[2], Omri Snir[1], Kristian Hindberg[1], Tom Eirik Mollnes[1,2,4], John-Bjarne Hansen[1,3]

1 K. G. Jebsen - Thrombosis Research and Expertise Center (TREC), Department of Clinical Medicine, UiT - The Arctic University of Norway, Tromsø, Norway.

2 Research Laboratory, Nordland Hospital, Bodø, Norway.

3 Division of Internal Medicine, University Hospital of North Norway, Tromsø, Norway

4 Department of Immunology, Oslo University Hospital, University of Oslo, Norway.

Corresponding author: Espen Waage Skjeflo, espenwskjeflo@gmail.com, Department of Medicine, Nordland Hospital, P.O. box 1480, 8092 Bodø, Norway

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Key Points

- There was no association between genome-wide or C5-related gene variants and C5 levels. Plasma CRP and C5 showed a linear relationship
- Increased levels of complement factor C5 were positively associated with future venous thromboembolic events in a nested case-control study

Abstract

The role of complement in the pathogenesis of venous thromboembolism (VTE) is unclear.

We aimed to (i) investigate whether plasma complement component C5 levels are influenced by genetic variants or chronic inflammation, and (ii) investigate the association between plasma C5 and risk of future VTE in a nested case-control study with 415 VTE patients and 848 age- and sex-matched controls derived from the Tromsø study.

Plasma C5 levels were measured at inclusion. Odds ratios (ORs) with 95% confidence intervals (95% CI) for provoked and unprovoked VTE across tertiles of C5 concentrations were estimated using logistic regression. C-reactive protein (CRP) was adjusted for as a proxy for general inflammation. Whole exome sequencing and protein quantitative trait loci analyses were performed to assess genetic influence on C5 concentrations.

There was no association between genome-wide or C5-related gene variants and C5 levels. The association between plasma C5 levels and VTE risk displayed a threshold effect, where subjects with C5 levels above the lowest tertile had increased VTE risk. Subjects in tertile 3 (highest C5 levels) had an age and sex-adjusted OR of 1.45 (95% CI 1.07-1.96) compared to tertile 1 (lowest). This was more pronounced for unprovoked VTE (OR 1.70, 95% CI 1.11-2.60). Adjustments for body mass index and CRP had minor impact on risk estimates. The ORs increased substantially with shorter time between blood sampling and VTE event.

In conclusion, plasma C5 was associated with risk of future VTE. C5 levels were not genetically regulated and only slightly influenced by chronic inflammation.

Introduction

Venous thromboembolism, including deep vein thrombosis (DVT) and pulmonary embolism (PE), is a frequent disease with severe complications and a high mortality rate.[1,2] VTE is also a complex disease with several genetic and environmental risk factors involved.[3] The mechanisms by which venous thrombi initiate are not fully elucidated,[4] and there is still a need to identify risk factors and to unravel pathophysiological pathways with potential for targeted prevention of VTE.

During the last decades, inflammation and coagulation have been shown to be overlapping processes involving many humoral and cellular components of the vasculature.[5,6] The complement system is an important part of the innate immune system, and several points of intersection between the complement and coagulation system may potentially contribute to a prothrombotic phenotype upon complement activation.[5] A few prospective observational studies have investigated the association between individual components of the complement system and risk of VTE. In a large Danish population-based cohort,[7] subjects with plasma complement C3 levels in the highest tertile had a 30% higher risk of VTE compared with subjects in the lowest tertile. We recently showed that increasing levels of the final activation product of the complement cascade, the terminal complement complex C5b-9 (TCC), was associated with increased risk of VTE.[8] In addition, we reported that low levels of mannose-binding lectin, a major pattern recognition molecule of the lectin pathway of complement activation, was associated with a lower risk of VTE.[9]

Complement factor 5 (C5) is involved in hemostatic processes, and plays a central role in activation of the complement system as proteolytic cleavage of C5 culminates in the formation of the TCC.[10] C5 is involved in several hemostatic processes. Membrane-inserted TCC, the membrane attack complex (MAC), may lyse cells such as erythrocytes, increasing

the risk of thrombosis as seen in paroxysmal nocturnal hemoglobinuria, which is now treated with complement inhibitors, which partly reduces the risk for thrombosis.[11] The soluble part of the TCC may also insert into cell membranes without subsequent lysis, instead forming small pores and inducing proinflammatory responses via calcium influx[12]. This sublytic C5b-9 membrane insertion activates platelets,[13] induces exposure of procoagulant lipids such as phosphatidylserine (PS) on the surface of platelets and endothelial cells that are necessary for the assembly of the prothrombinase complex.[14,15] It induces the expression of adhesion molecules on endothelial cells and platelets,[16] and induces the secretion of vWF and proinflammatory cytokines.[17] In addition, activated C5 (C5a) has been shown to induce tissue factor expression on endothelial cells[18], and on monocytes in various disease states.[19]

Even though experimental studies suggest a role for C5 in the setting of venous thrombus formation, the impact of C5 as a risk factor for VTE in the general population remains unknown. In a murine inferior vena cava stenosis model of venous thrombosis, C5 deficient mice showed reduced fibrin formation, reduced exposure of negatively charged PS on adherent leukocytes, and reduced clot burden after 48 hours compared to wild-type controls.[20] To the best of our knowledge, no study has addressed whether C5 levels are influenced by gene variants or chronic inflammation. Therefore, the aim of this study was to investigate (i) whether plasma complement component C5 levels were influenced by genetic variants (using protein quantitative trait loci analysis of exome sequencing data) or chronic inflammation (assessed by C-reactive protein (CRP)), and (ii) explore the association between plasma C5 concentration and risk of future VTE.

Methods

Study population

The Tromsø Study is a single-center, population-based cohort, with repeated health surveys of inhabitants of Tromsø, Norway.[21] All inhabitants aged ≥25 years living in the municipality of Tromsø were invited to participate in the fourth survey, conducted in 1994–1995. 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 and the autopsy registry and the radiology procedure registry at the University Hospital of North Norway, 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 (proximal or distal) were combined with objective confirmation by radiological procedures (i.e., compression ultrasonography, venography, spiral computed tomography, perfusion-ventilation scan, and/or pulmonary angiography) or autopsy. All cases were treated unless anticoagulation therapy was contraindicated as previously described.[22] A VTE occurring in the presence of one or more of the following factors was classified as provoked: Surgery, trauma (within 8 weeks before the event), acute medical conditions (acute myocardial infarction, acute ischemic stroke, acute infections), immobilization (confined to bed >3 days or wheelchair within the past 8 weeks), or other factors specifically described as provoking by a physician in the medical record (e.g. intravascular catheterization).

During the follow-up period (1994–2007), 462 individuals experienced a VTE event. For each case, two age- and sex-matched controls (n=924), who were alive at the index date of the VTE event, were randomly sampled from the source cohort. 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 centimeter) 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 a detailed history of previous cardiovascular disease (CVD) events (stroke, angina pectoris and myocardial infarction) and cancer.

Plasma samples and storage

At inclusion in Tromsø 4 (1994-1995), non-fasting blood was collected from an antecubital vein into 5-mL vacutainers (Becton Dickinson, Le Pont de Claix, France) containing anticoagulant EDTA (K3-EDTA 40 μL, 0.37 mol/L per tube). Platelet poor plasma was prepared by centrifugation at 3000 x *g* for 10 min at room temperature and the supernatant was transferred to cryovials (Greiner Labortechnik, Nürtingen, Germany) in 1-mL aliquots and stored at -80°C. Plasma samples were thawed in a water bath at 37 °C for 5 min, followed by centrifugation for 2 min at 13 500 x *g* to obtain platelet-free plasma.

Quantification of CRP and C5

Plasma levels of high-sensitivity CRP were measured in duplicates using commercially available reagents by enzyme immunoassay (R&D Systems, Minneapolis, MN) in a 384-format using the combination of a SELMA (Jena, Germany) pipetting robot and a BioTek

(Winooski, VT) dispenser/washer (EL406). Absorption was read at 450 nm with a wavelength correction set to 540 nm using an EIA plate reader (Synergy H1 Hybrid, BioTek, Vinooski, VT). The intra-individual and inter-individual coefficients of variation (CVs) were 2.6% and 9.1%, respectively.

Complement component C5 was quantified according to the manufacturer's instructions of a commercially available sandwich ELISA (Abcam, Cambridge, UK). Optical densities were read at 450 nm with a wavelength correction for readings at 570 nm using an Infinite M200 PRO plate reader (Tecan Trading AG, Switzerland). The manufacturer reports intra-individual and inter-individual CVs of 5.0% and 8.6%, respectively. The reported average recovery rate was 101%.

Exome sequencing

Whole exome-sequencing was conducted on a subgroup of the study population consisting of 355 VTE patients and 354 controls. The Agilent SureSelect 50 Mb capture kit with a high average coverage of 100 reads was used. The exome sequenced genotypes were filtered[23] and imputed[24] as previously described.

Statistical analysis

Statistical analyses were carried out using R version 3.6.1. Plasma C5 was categorized according to tertile cutoffs in the control population (27-52, 52-60, 60-118 μg/mL) or used as a continuous variable. Means and proportions of baseline characteristics across tertiles of C5 were calculated using descriptive statistics. The correlation between C5 and CRP was determined by Pearson's correlation coefficient, linear regression and boxplots. Logistic

regression models were used to estimate odds ratios (OR) for VTE with 95% confidence intervals (CI) according to tertiles or per standard deviation (SD) of C5 adjusted for age, sex, BMI and CRP. We additionally performed a model with further adjustment for smoking, diabetes, history of CVD and cancer. The lowest tertile of C5 was used as the reference group. We also did subgroup analyses of provoked and unprovoked VTE, as well as of DVT and PE as outcomes.

Because the follow-up time in the source cohort was long (≥12 years for several individuals), the results based on baseline C5 measurement could be influenced by regression dilution bias.[25] To investigate this, we performed analyses restricted on the maximum time between blood sampling in Tromsø 4 and 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 time point a new VTE occurred and plotted as a function of this maximum time.

For the whole-exome data set, after filtering and imputation, there were 1 033 970 variants. These variants were checked for regulation of plasma C5 levels through a protein quantitative trait loci (pQTL) analysis. The pQTL analysis was performed on the whole genome as well as restricted to a cis-region ± 500 kb around transcription start/stop of the C5 gene. The cis-region contained 549 variants, which gave a Bonferroni-corrected p-value threshold of $0.05/549 = 9.1 \times 10^{-5}$ for the cis analysis. The commonly used 5×10^{-8} p-value threshold was used for the genome-wide analysis. The C5 plasma level data was transformed to a perfect standard normal distribution for the pQTL analysis and age, sex, BMI and VTE status were used as adjustment variables. The pQTL analyses were performed by the EPACTS (Efficient and Parallelizable Association Container Toolbox) software (Ann Arbor, MI) and the EMMAX (Efficient Mixed Model Association eXpedited)[26] test in EPACTS was used. EMMAX uses a mixed model to test for associations between quantitative traits and genetic

variants while allowing for traditional adjustment variables and adjustment for genetic relatedness in the data. Based on the 709 persons included, the pQTL analysis could detect SNPs explaining >1.1% of the variance in C5 levels with 80% power and a 5% significance level.

Results

The baseline characteristics of VTE-cases and controls are shown in Table 1. VTE patients had higher BMI (27.2 vs 26.1 kg/m^2) and higher proportion with history of cancer (6.2% vs 3.7%) than controls. The proportion with history of CVD was similar in cases and controls, and the proportion of smokers was lower in cases versus controls (29.5% vs 32.0%). The characteristics of study participants across tertiles of plasma C5 concentrations are shown in Supplementary table 1. Age, BMI, CRP, white blood cell count and thrombocyte levels, as well as the proportion of men and subjects with a history of CVD increased across tertiles of plasma C5 levels, whereas the proportion of smokers and subjects with history of cancer was similar in all three categories (Supplementary table 1).

C5 levels increased slightly across tertiles of plasma CRP levels (Figure 1). Correlation analysis (Pearson) revealed a significant correlation ($r=0.23$, $p=1.1 \times 10^{-16}$, $n=1258$), and linear regression revealed that C5 levels increased 2.1 µg/mL per 1 mg/L increase in CRP.

To identify any potential genetic regulation of plasma C5 levels we performed a pQTL analysis. Figure 2 shows the resulting Manhattan plot, with the cis-region variants marked by blue squares and the complement-related variants by purple triangles, after adjustment for age, sex, BMI, and VTE status. There was no statistically significant SNP in neither the genome-wide nor the cis analysis. The list of complement-related genes investigated in the pQTL is presented in Supplementary table 2.

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. Furthermore, 57.8% of the VTEs were classified as provoked, with surgery, trauma and/or active cancer as the most common provoking factors (Table 2). The mean time from blood sampling to VTE was 7.4 years.

The ORs for VTE, DVT, and PE across tertiles of C5 concentrations are shown in Figure 3. In the analyses of overall VTE, the OR was increased in the two highest tertiles of C5 concentrations, with an OR 1.48 (95% CI 1.10-1.98) in T2 and 1.45 (95% CI 1.07-1.96) in tertile (T3) when compared to T1 after adjustment for age and sex. Further adjustment for BMI (Model 2) slightly attenuated the OR to 1.46 (95% CI 1.09-1.97) for T2 versus T1 (Figure 3). A similar threshold effect was observed when comparing T2 and T1 for unprovoked and provoked VTE with OR 1.60, 95% CI: 1.06-2.44 and OR 1.39, 95% CI: 0.98-1.99, respectively. Likewise, for provoked VTE the threshold for risk increase appeared to be between T2 and T1 (OR for T2 vs. T1: 1.39, 95% CI: 0.98-1.99). Additional adjustment for CRP (Model 3) yielded an OR for overall VTE of 1.40 (95% CI 1.04-1.89) for T2 versus T1. The ORs from Model 3 in the subcategories were similar to those described for Model 2, with an OR for provoked VTE of 1.35 (95% CI 0.94-1.94), and an OR for unprovoked VTE of 1.50 (95% CI 0.99-2.31), when comparing T2 to T1 (Figure 3). Further adjustment for smoking, diabetes, history of CVD and cancer did not substantially change the risk estimates (Supplementary figure 1). The risk of VTE also increased per SD (12.5 μg/mL) increase in C5 levels, with an age- and sex-adjusted OR of 1.17 (95% CI 1.04-1.32) (Supplementary table 3).

To consider the possibility of underestimating ORs because of regression dilution bias, we estimated ORs for overall, unprovoked and provoked VTE among subjects with low (lowest tertile) versus high (highest tertile) plasma C5 as a function of time between blood sampling and VTE (Figure 4). The ORs were substantially higher when blood sampling was close to the VTE events, indicating substantial regression dilution over time. The ORs for overall VTE restricted to 2, 3 and 5 years of follow-up are shown in Figure 5. At 2 years, the age- and sex-adjusted OR for T3 versus T1 was 4.81 (95% CI 1.77-16.9), and at 5 years the corresponding OR was 1.87 (1.16-3.06). Boxplots showing the spread of C5 levels in all cases and controls

as well as in cases with different restrictions on follow-up time are shown in the Supplementary figure 2.

Discussion

In this study, we found that plasma C5 levels were slightly affected by inflammatory status, and that subjects with low (lowest tertile) plasma C5 levels had lower risk of future VTE. This threshold effect at tertile 2 was observed for all VTE outcomes (provoked/unprovoked and DVT/PE). Subjects with moderate or high C5 levels had increased VTE risk, particularly for unprovoked events, and PE in particular, compared to those with low C5 levels. The C5 levels were slightly affected by inflammatory status and the risk estimates were modestly attenuated when adjusted for BMI and CRP. Further adjustment for smoking status, although indicated to affect C5 levels[27], did not alter the risk estimates. Moreover, adjustment for diabetes, history of CVD and cancer did not affect the risk estimates. The ORs for VTE were substantially higher when VTE occurred within the first years after blood sampling, indicating regression dilution bias due to intra-individual fluctuation of C5 levels.[25] Our pQTL analysis revealed no association between genome-wide, complement-related or C5-related gene variants and plasma levels of C5. As our well-powered pQTL analysis found no significant SNPs, C5 plasma levels are unlikely to be considerably genetically regulated.

A clear temporal sequence between exposure and outcome, such as in our nested case-control study, is a prerequisite to establish whether plasma C5 concentration is a risk factor for VTE. No previous observational study has investigated the association between plasma C5 levels and risk of future VTE. Even though our finding is unchallenged, circumstantial evidence support a role of the complement system, and C5 in particular, in the pathogenesis of VTE. First, experimental studies have shown that pathological C5 activation induces TF expression in monocytes[19] and neutrophils,[28] induces the expression of adhesion molecules,[16,29] induces exposure of procoagulant PS at cell surfaces,[20] and promotes release of procoagulant extracellular vesicles;[15] all features which have been related to increased VTE risk.[30–33] Second, proteolytic cleavage of C5 culminates in the formation of the terminal complement complex

C5b-9, and high plasma levels of soluble C5b-9 have been associated with increased risk of VTE.[8] Third, in our study, moderate and high plasma C5 levels displayed the strongest risk estimates for unprovoked VTE events, indicating that moderate and high C5 levels alone, independent of provoking factors, provided sufficient strength to exceed an individual's threshold for thrombosis. Fourth, C5 deficient mice had reduced thrombus stability in a flow-restricted model,[20] which may explain why the risk of PE, and unprovoked PE in particular, was higher than for DVT in our study.

Our finding of an association between C5 levels and risk of VTE is in accordance with the results from a large Danish cohort showing that moderate and high levels of complement factor C3 were associated with increased risk of VTE.[7] In agreement with our findings, they found a threshold effect where subjects with C3 levels in the lowest tertile had lower risk of VTE, without any further concentration-dependent increased VTE risk at higher C3 levels.[7] Furthermore, the magnitude of the risk estimates for VTE were similar for C3 and C5 levels in the highest compared to the lowest tertiles. Furthermore, the risk estimates for VTE for tertile 1 vs. tertile 3 were comparable and they were stronger for PE than for DVT in the two studies.

Analyses of plasma components that fluctuate over time are expected to result in underestimations of the true risk in prospective studies with long follow-up, a phenomenon called regression dilution bias.[25] Accordingly, we demonstrated that the OR for VTE among subjects with high compared to low plasma C5 levels increased substantially with shorter time between blood sampling and VTE.

C5 has been assumed to be an acute phase protein similar to C3 and C4.[34] Therefore, we performed an analysis to determine the relationship between plasma CRP and C5 levels, and found a weak linear relationship. This suggest that inflammatory responses enhance C5 synthesis and release, similar to C3 and C4.[34] However, as a feedback mechanism of the initial

inflammatory response, CRP can bind to C1q and activate the classical pathway of the complement system.[35] Thus, CRP can be seen as both a confounder and as a mediator for the relationship between C5 levels and VTE. In our study, adjustment for CRP only slightly attenuated the ORs, indicating that the relationship between C5 and VTE was not explained by differences in CRP levels.

Strengths of this study include the temporal sequence of exposure and outcome in a sample recruited from the general adult population with validated VTE events. The study also has limitations. The study had somewhat limited power, and as the confidence intervals were wide and included unity for some categories, our results should be interpreted with caution. Moreover, changes in C5 levels during follow-up could result in underestimation of the OR, as indicated by the regression dilution plot showing higher ORs when analyses were restricted to the first years after follow-up. Blood samples were drawn in 1994-95 and stored at -80°C for up to 22 years. The long storage time could potentially affect the plasma C5 levels. However, as all samples were stored under the same conditions and for the same amount of time regardless of case-control status, the storage effect is assumed to be similar in cases and controls, and therefore unlikely to have influenced the relative differences.

In conclusion, the results from our nested case-controls study showed that C5 levels were slightly affected by chronic inflammation, and that medium and high C5 levels were associated with increased risk of future VTE, and unprovoked events in particular. No gene variants were found to regulate plasma C5, which may in part explain the substantial increased risk of VTE when the modifiable C5 levels were sampled close to the VTE event. Our findings provide further support for a role of individual components of the complement system in the pathogenesis of VTE.

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Conflict of interests

The authors have no conflicts of interest.

Author contributions

EWS analyzed the data, wrote, and revised the manuscript. JKL performed the laboratory analysis and revised the manuscript. KH, OS and SKB analyzed data and participated in the revision of the manuscript. TEM, SKB and JBH designed the study and participated in the writing and revision of the manuscript. All the authors read and approved the final manuscript.

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Tables

Table 1. Distribution of baseline characteristics for cases and controls. Values are means ± standard deviations or percentages with numbers in parentheses.

	Cases (n = 415)	**Controls (n = 848)**
C5 (μg/mL)	60.4 (12.4)	58.2 (12.5)
Age in years	60 ± 14	60 ± 14
Male sex	48.4 (201)	46.7 (396)
BMI (kg/m^2)	27.2 ± 4.5	26.1 ± 4.1
Smoking	29.5 (123)	32.0 (271)
Diabetes†	4.1 (17)	3.9 (33)
Cardiovascular disease†	15.7 (65)	15.6 (132)
Cancer†	6.2 (26)	3.7 (31)
hsCRP (mg/L)	1.7 ± 1.4	1.6 ± 1.4
White blood cell count, 10^9/L	7.2 ± 2.9	7.0 ± 1.8
Platelet count, 10^9/L	247 ± 57	244 ± 53

Abbreviations: BMI, body mass index; hsCRP, high-sensitivity C-reactive protein

†Self-reported history of diabetes, cancer or cardiovascular diseases (myocardial infarction, angina pectoris or stroke) at baseline.

Table 2. Characteristics of the venous thromboembolism (VTE) cases (n = 415). Values are means ± standard deviations or percentages with numbers in brackets.

	% (n)
Age at VTE (years)	67 ± 14
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)
Immobilization	18.1 (75)
Acute medical condition	15.4 (64)
Estrogens	7.7 (32)
Other provoking factor	3.9 (16)

Figure legends

Figure 1. Boxplots of C5 across tertiles of CRP. The horizontal lines of the grey box define the 25% (Q1), 50% (median) and 75% (Q3) percentiles. The whiskers extend up to min(max(C5), Q3+1.5*IQR) and down to max(min(C5), Q1-1.5*IQR)), where their interquartile range is IQR = Q3-Q1. Points outside the whiskers are plotted as open circles.

Figure 2. **Manhattan plot of pQTL analysis of C5 adjusted for age, sex and BMI.** The dashed lines indicate the genome-wide (upper) and cis-region (lower) p-value thresholds for significance. The blue squares mark variants in the ±500 kb cis-region around transcription start/stop of the C5 gene. The purple triangles mark variants in the complement-related genes.

Figure 3. **Forest plot showing odds ratios with 95% confidence intervals for venous thromboembolism (VTE) and subgroups of VTE across tertiles of complement component C5 plasma levels**. Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, body mass index. Model 3: Adjusted for age, sex, body mass index and CRP.

Figure 4. **Plots of estimated ORs for overall venous thromboembolism (VTE), unprovoked and provoked VTE as a function of maximum time since blood sampling in the fourth survey of the Tromsø Study (1994-1995) to VTE event (all controls are included in all analyses).** Analyses are adjusted for age, sex, BMI, and CRP. Large, blue, solid circles indicate ORs with P values < 0.05. Point estimates for 2, 3 and 5 years of follow-up are indicated and the number of events are given at the top of each plot.

Figure 5. **Forest plot showing odds ratios with 95% confidence intervals for venous thromboembolism (VTE) across tertiles of complement component C5 plasma levels at 2, 3 and 5 years of follow-up**. Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, body mass index. Model 3: Adjusted for age, sex, body mass index and CRP.

Supplementary figure 1: Forest plot showing odds ratios with 95% confidence intervals for venous thromboembolism (VTE) and subgroups of VTE across tertiles of complement component C5 plasma levels adjusted for age, sex, body mass index, CRP, smoking, diabetes, history of CVD and cancer.

Supplementary figure 2: Boxplot of C5 levels in all cases and controls as well as in cases at 2, 3 and 5 years of follow-up. The solid blue circles indicate means.

Supplementary tables

Supplementary table 1. Distribution of baseline characteristics according to tertiles of plasma levels of C5. Values are means ± standard deviations or percentages with numbers in parentheses.

	T1	T2	T3
Tertiles of C5 (μg/mL)	27-52	52-60	60-118
Number of people	32.9 (415)	34.0 (430)	33.1 (418)
Age in years	57 ± 15	60 ± 13	64 ± 12
Proportion males	43.6 (181)	48.8 (210)	49.3 (206)
BMI	25.8 ± 4.1	26.2 ± 3.9	27.2 ± 4.6
hsCRP	1.2 ± 1.2	1.6 ± 1.3	2.0 ± 1.5
Smoking	29.4 (122)	31.6 (136)	32.5 (136)
Diabetes	3.9 (16)	2.8 (12)	5.3 (22)
CVD	12.8 (53)	16.0 (69)	17.9 (75)
Cancer	5.0 (17)	6.1 (21)	6.1 (19)
Platelet count	242.5 ± 54.0	241.6 ± 49.3	250.1 ± 58.8
White blood cell count	6.7 ±1.7	7.1 ± 2.8	7.2 ± 1.9

Abbreviations: BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; CVD, cardiovascular disease

Self-reported history of cancer or cardiovascular diseases (myocardial infarction, angina pectoris or stroke) at baseline.

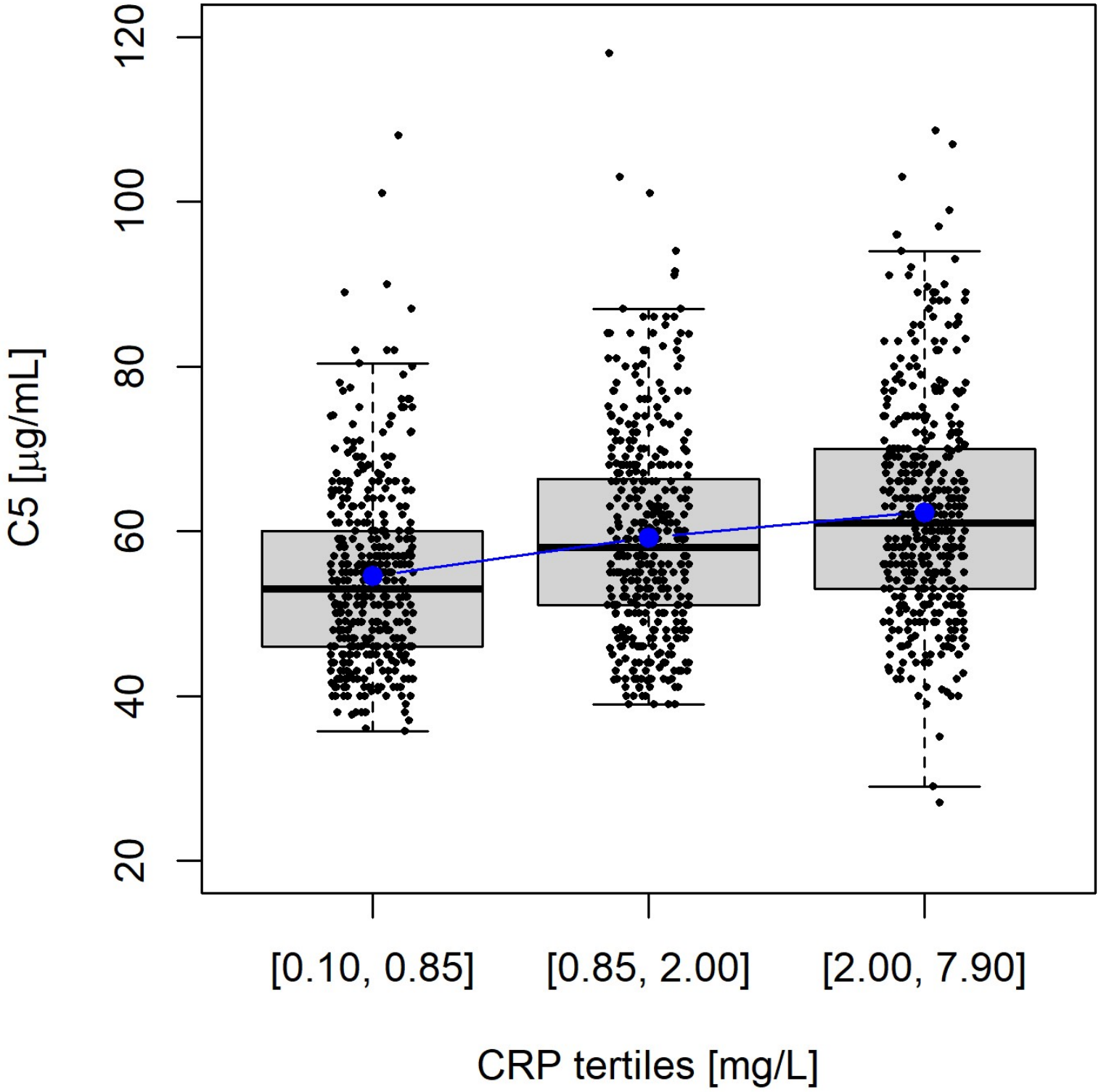
Supplementary Table 2: Complement-related genes investigated in the pQTL analysis and listed with purple triangles in the Manhattan plot presented in Figure 2 of the manuscript. The transcription start and stop positions are with respect to human reference genome hg19/GRCh37.

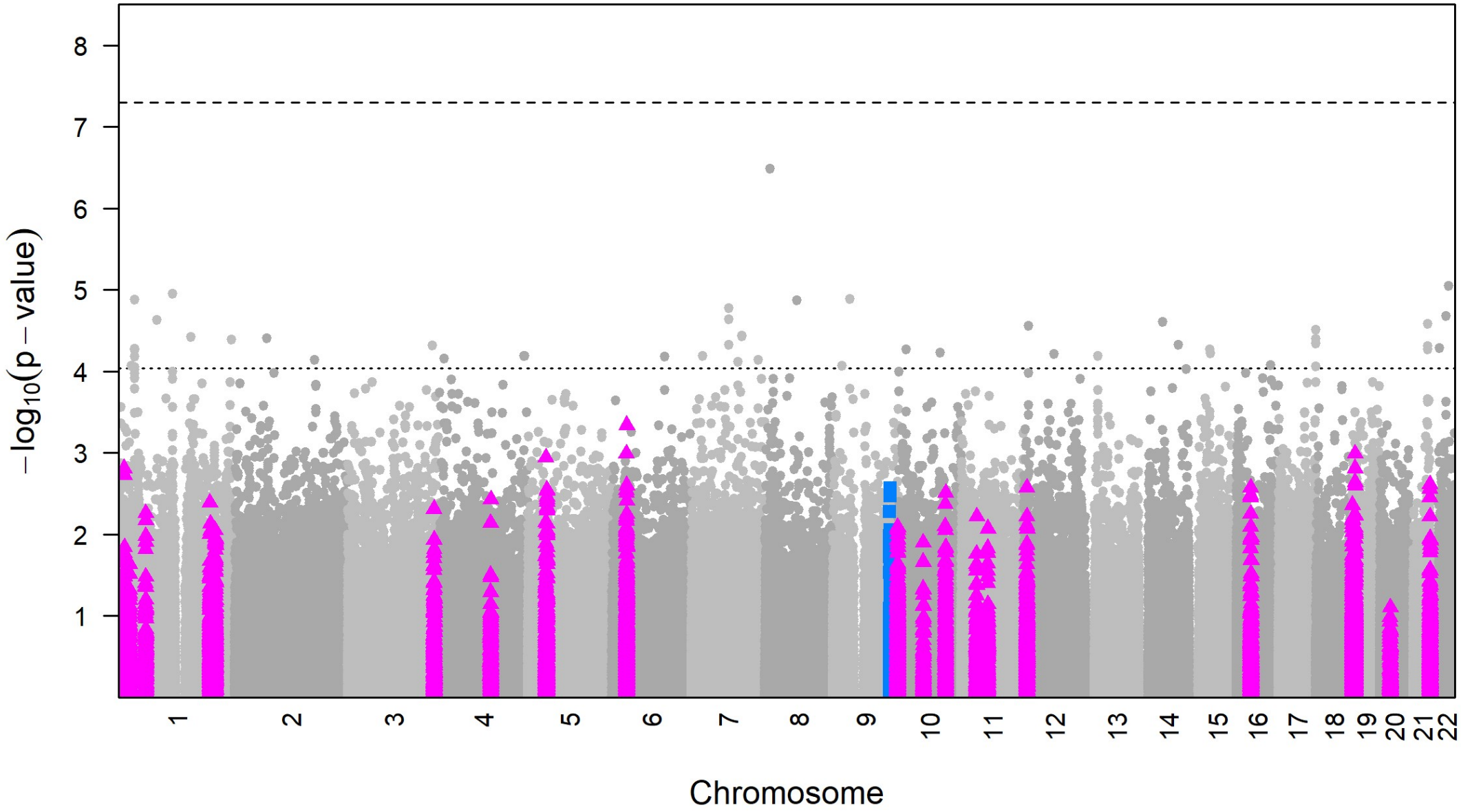
Gene	Chromosome	Start	Stop
MASP-2	1	11086580	11107290
C1QA	1	22962999	22966101
C1QC	1	22970123	22974603
C1QB	1	22979255	22988031
C8A	1	57320479	57383894
C8B	1	57394883	57431813
CFH	1	196621008	196716634
C4BPA	1	207277607	207318317
CD55	1	207494853	207534311
CR2	1	207627575	207663240
CR1	1	207669492	207813992
CD46	1	207925402	207968858
MASP-1	3	186935942	187009810
CFI	4	110661852	110723335
C9	5	39284364	39424970
C7	5	40909354	40983041
C6	5	41142336	41261540
C2	6	31865562	31913449
CFB	6	31895475	31919861
C4A	6	31949801	31970458
C4B	6	31982539	32003195
C5	9	123714616	123812554
C8G	9	139839698	139841426
Mbl2	10	54525140	54531460
CPN1	10	101801950	101841634
CD59	11	33719807	33757991
SERPING1	11	57364860	57382326
C1S	12	7096351	7178336
C1R	12	7187513	7245203
ITGAX	16	31366455	31394318
CFD	19	859453	863453
C3	19	6677715	6730573
CD93	20	23059986	23066977
ITGB2	21	46305868	46351904

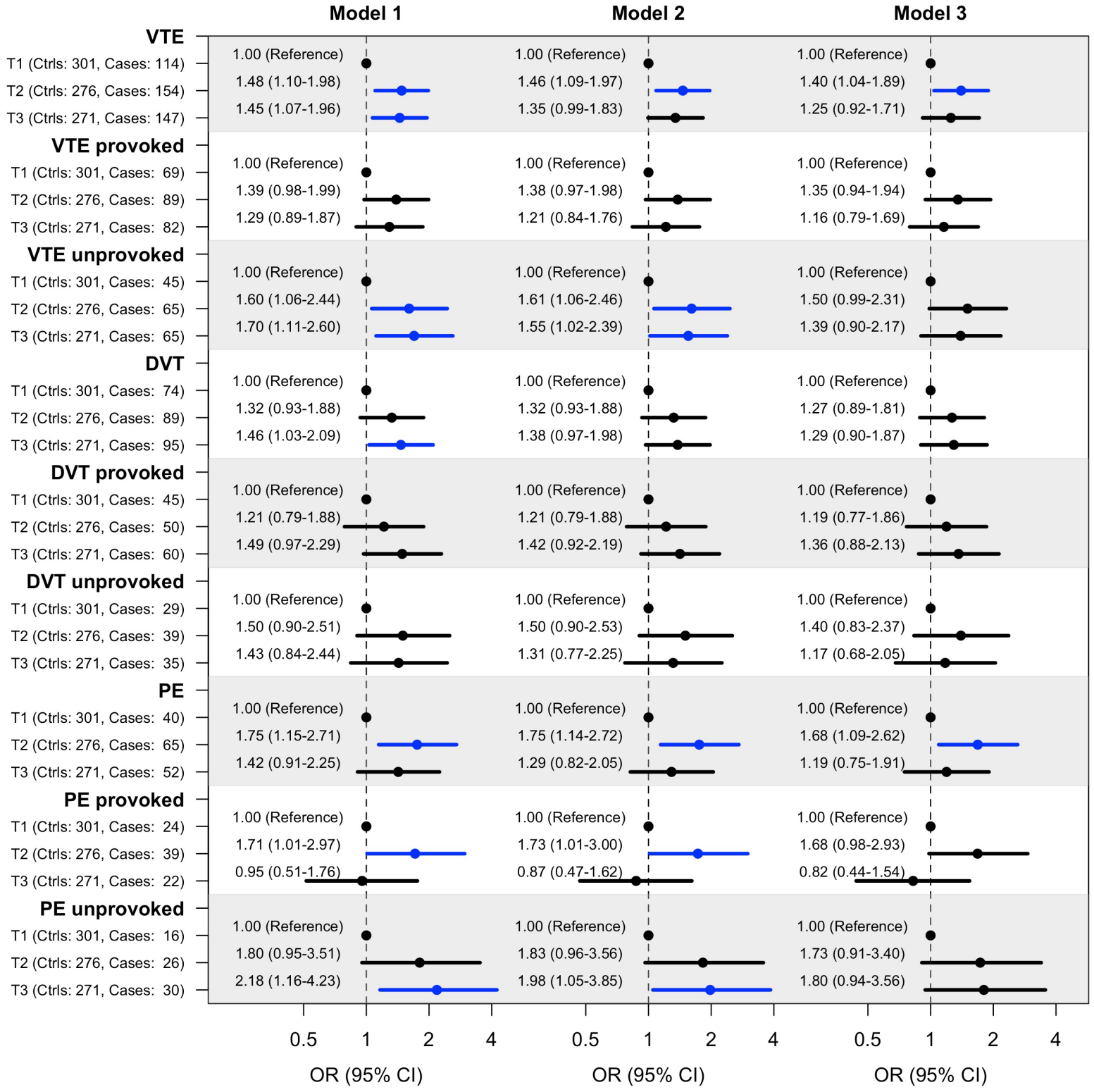
Supplementary Table 3: Odds ratios with 95% confidence intervals (in brackets) for unprovoked and provoked deep vein thrombosis (DVT) and pulmonary embolism (PE) per SD of plasma complement component C5 levels.

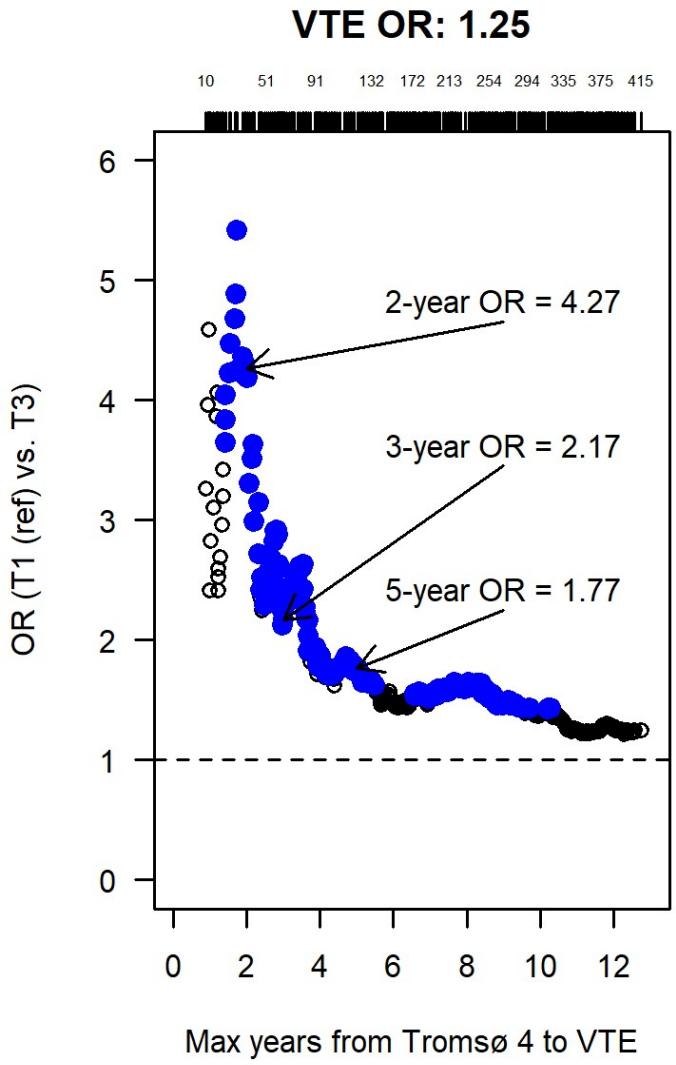
	Cases	Controls	Model 1 OR (95% CI)	Model 2 OR (95% CI)	OR Model 3 OR (95% CI)
VTE	415	848	1.17 (1.04,1.32)	1.14 (1.01,1.29)	1.11 (0.98,1.25)
Provoked VTE	240	848	1.18 (1.03,1.36)	1.14 (0.99,1.32)	1.12 (0.97,1.30)
Unprovoked VTE	175	848	1.16 (0.99,1.36)	1.13 (0.96,1.33)	1.08 (0.91,1.28)
DVT	258	848	1.17 (1.02,1.34)	1.15 (1.00,1.32)	1.12 (0.97,1.29)
Provoked DVT	155	848	1.21 (1.03,1.43)	1.19 (1.00,1.41)	1.17 (0.98,1.39)
Unprovoked DVT	103	848	1.10 (0.90,1.34)	1.08 (0.88,1.32)	1.04 (0.84,1.28)
PE	157	848	1.18 (1.00,1.40)	1.12 (0.95,1.33)	1.09 (0.91,1.29)
Provoked PE	85	848	1.13 (0.91,1.40)	1.06 (0.85,1.32)	1.04 (0.83,1.30)
Unprovoked PE	72	848	1.24 (0.98,1.56)	1.19 (0.94,1.51)	1.14 (0.89,1.46)

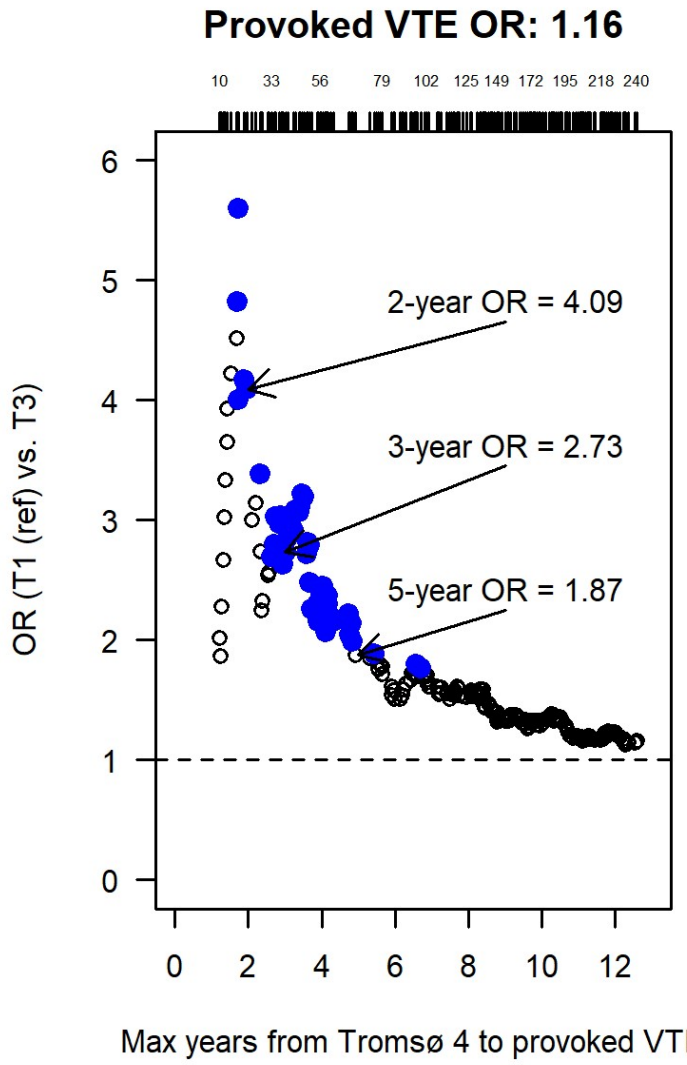
Model 1: Adjusted for age and sex. Model 2: Adjusted for age, sex, body mass index. Model 3: Adjusted for age, sex, body mass index and CRP. 1 SD=12.5 μg/mL.

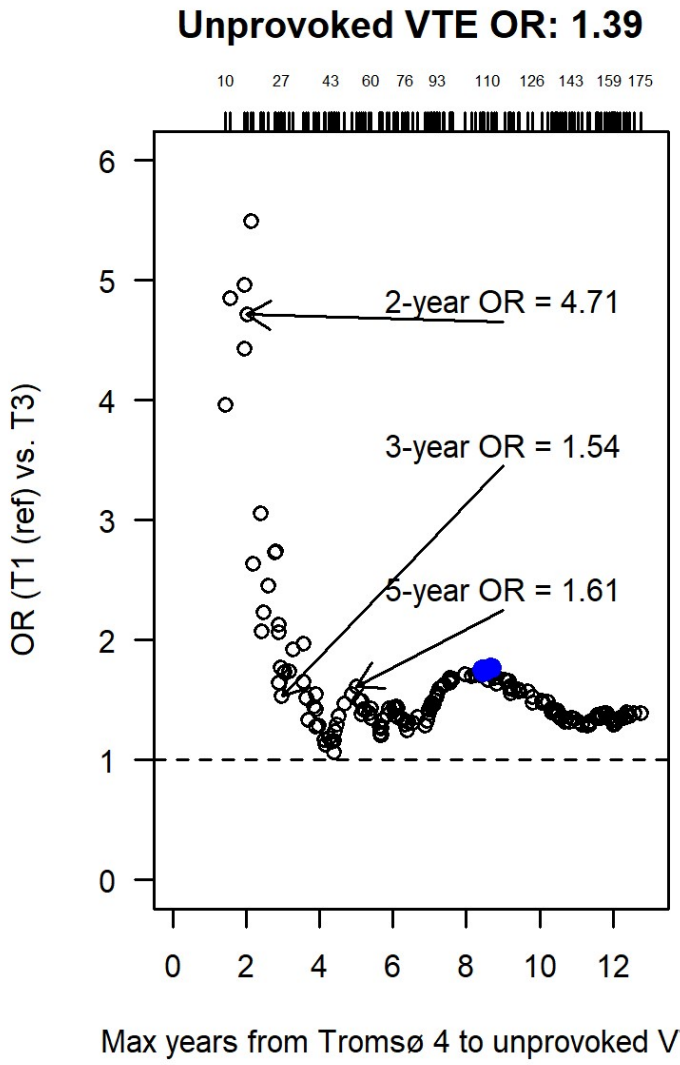

C5 [µg/mL]
120
100
80
60
40
20
[0.10, 0.85]
[0.85, 2.00]
[2.00, 7.90]
CRP tertiles [mg/L]

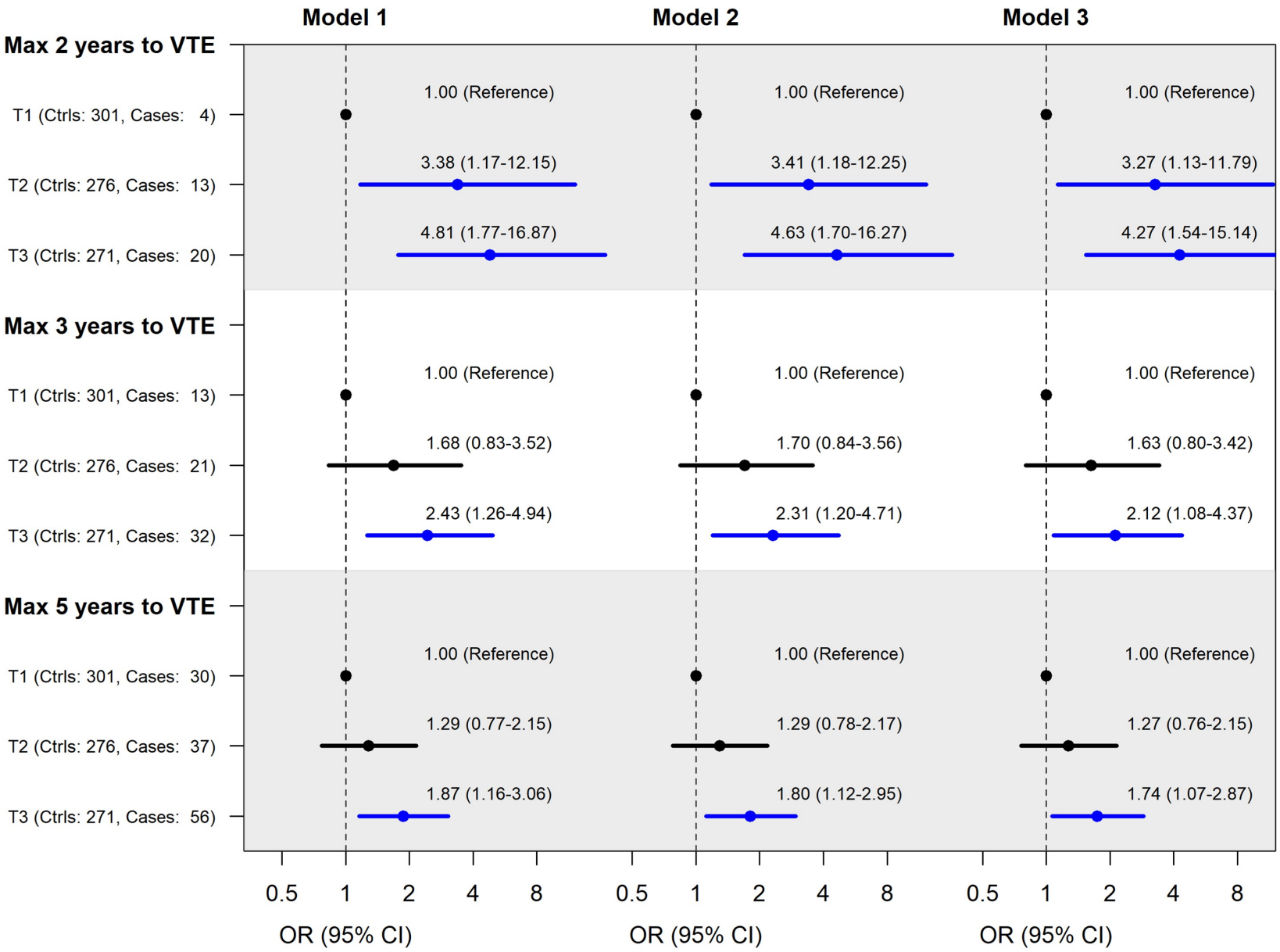

8
7
6
5
4
3
2
1
$-\log_{10}(p-value)$
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
Chromosome

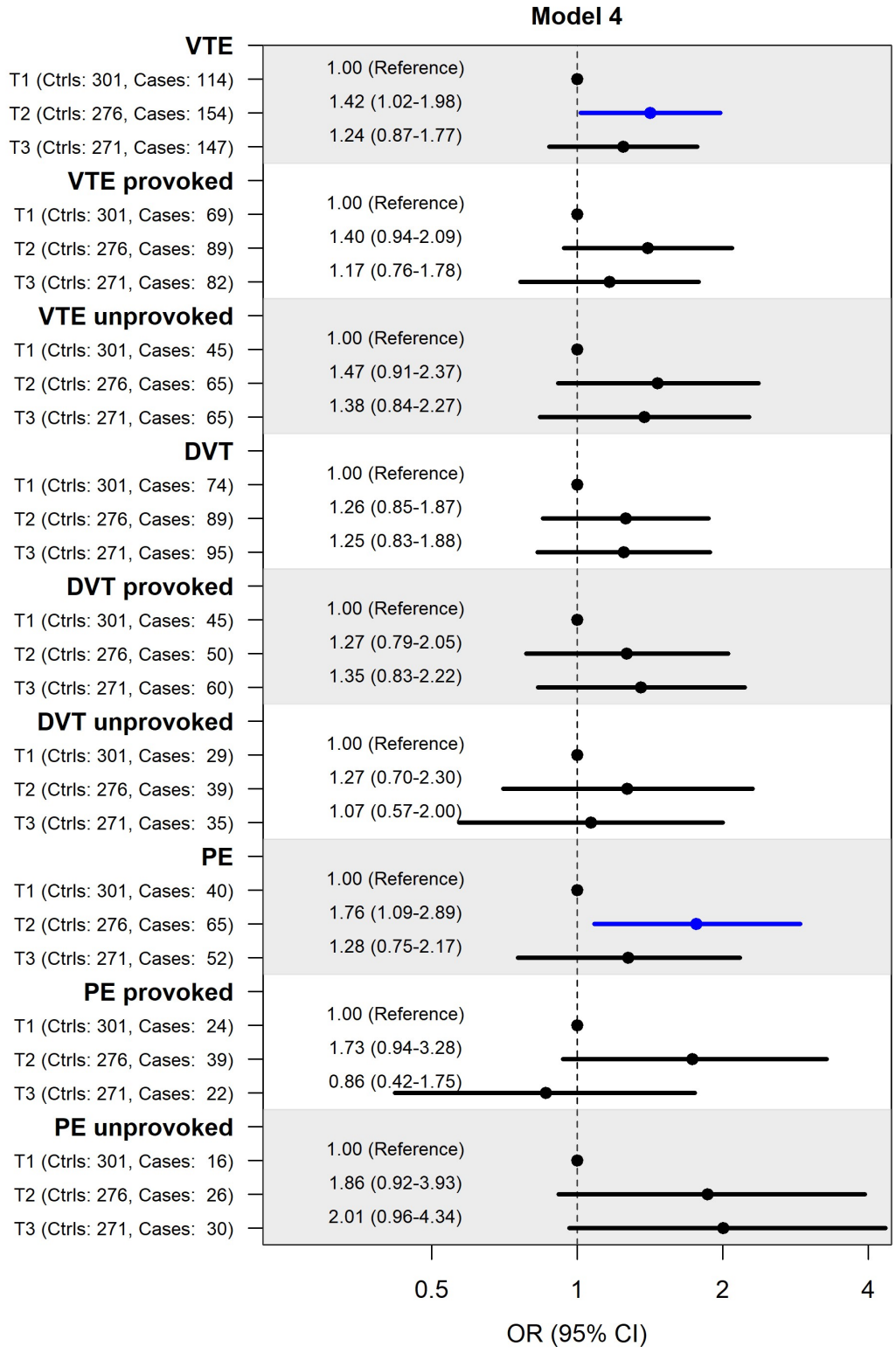

Model 1
Model 2
Model 3
VTE
T1 (Ctrls: 301, Cases: 114)
T2 (Ctrls: 276, Cases: 154)
T3 (Ctrls: 271, Cases: 147)
1.00 (Reference)
1.48 (1.10-1.98)
1.45 (1.07-1.96)
1.00 (Reference)
1.46 (1.09-1.97)
1.35 (0.99-1.83)
1.00 (Reference)
1.40 (1.04-1.89)
1.25 (0.92-1.71)
VTE provoked
T1 (Ctrls: 301, Cases: 69)
T2 (Ctrls: 276, Cases: 89)
T3 (Ctrls: 271, Cases: 82)
1.00 (Reference)
1.39 (0.98-1.99)
1.29 (0.89-1.87)
1.00 (Reference)
1.38 (0.97-1.98)
1.21 (0.84-1.76)
1.00 (Reference)
1.35 (0.94-1.94)
1.16 (0.79-1.69)
VTE unprovoked
T1 (Ctrls: 301, Cases: 45)
T2 (Ctrls: 276, Cases: 65)
T3 (Ctrls: 271, Cases: 65)
1.00 (Reference)
1.60 (1.06-2.44)
1.70 (1.11-2.60)
1.00 (Reference)
1.61 (1.06-2.46)
1.55 (1.02-2.39)
1.00 (Reference)
1.50 (0.99-2.31)
1.39 (0.90-2.17)
DVT
T1 (Ctrls: 301, Cases: 74)
T2 (Ctrls: 276, Cases: 89)
T3 (Ctrls: 271, Cases: 95)
1.00 (Reference)
1.32 (0.93-1.88)
1.46 (1.03-2.09)
1.00 (Reference)
1.32 (0.93-1.88)
1.38 (0.97-1.98)
1.00 (Reference)
1.27 (0.89-1.81)
1.29 (0.90-1.87)
DVT provoked
T1 (Ctrls: 301, Cases: 45)
T2 (Ctrls: 276, Cases: 50)
T3 (Ctrls: 271, Cases: 60)
1.00 (Reference)
1.21 (0.79-1.88)
1.49 (0.97-2.29)
1.00 (Reference)
1.21 (0.79-1.88)
1.42 (0.92-2.19)
1.00 (Reference)
1.19 (0.77-1.86)
1.36 (0.88-2.13)
DVT unprovoked
T1 (Ctrls: 301, Cases: 29)
T2 (Ctrls: 276, Cases: 39)
T3 (Ctrls: 271, Cases: 35)
1.00 (Reference)
1.50 (0.90-2.51)
1.43 (0.84-2.44)
1.00 (Reference)
1.50 (0.90-2.53)
1.31 (0.77-2.25)
1.00 (Reference)
1.40 (0.83-2.37)
1.17 (0.68-2.05)
PE
T1 (Ctrls: 301, Cases: 40)
T2 (Ctrls: 276, Cases: 65)
T3 (Ctrls: 271, Cases: 52)
1.00 (Reference)
1.75 (1.15-2.71)
1.42 (0.91-2.25)
1.00 (Reference)
1.75 (1.14-2.72)
1.29 (0.82-2.05)
1.00 (Reference)
1.68 (1.09-2.62)
1.19 (0.75-1.91)
PE provoked
T1 (Ctrls: 301, Cases: 24)
T2 (Ctrls: 276, Cases: 39)
T3 (Ctrls: 271, Cases: 22)
1.00 (Reference)
1.71 (1.01-2.97)
0.95 (0.51-1.76)
1.00 (Reference)
1.73 (1.01-3.00)
0.87 (0.47-1.62)
1.00 (Reference)
1.68 (0.98-2.93)
0.82 (0.44-1.54)
PE unprovoked
T1 (Ctrls: 301, Cases: 16)
T2 (Ctrls: 276, Cases: 26)
T3 (Ctrls: 271, Cases: 30)
1.00 (Reference)
1.80 (0.95-3.51)
2.18 (1.16-4.23)
1.00 (Reference)
1.83 (0.96-3.56)
1.98 (1.05-3.85)
1.00 (Reference)
1.73 (0.91-3.40)
1.80 (0.94-3.56)
0.5
1
2
4
OR (95% CI)

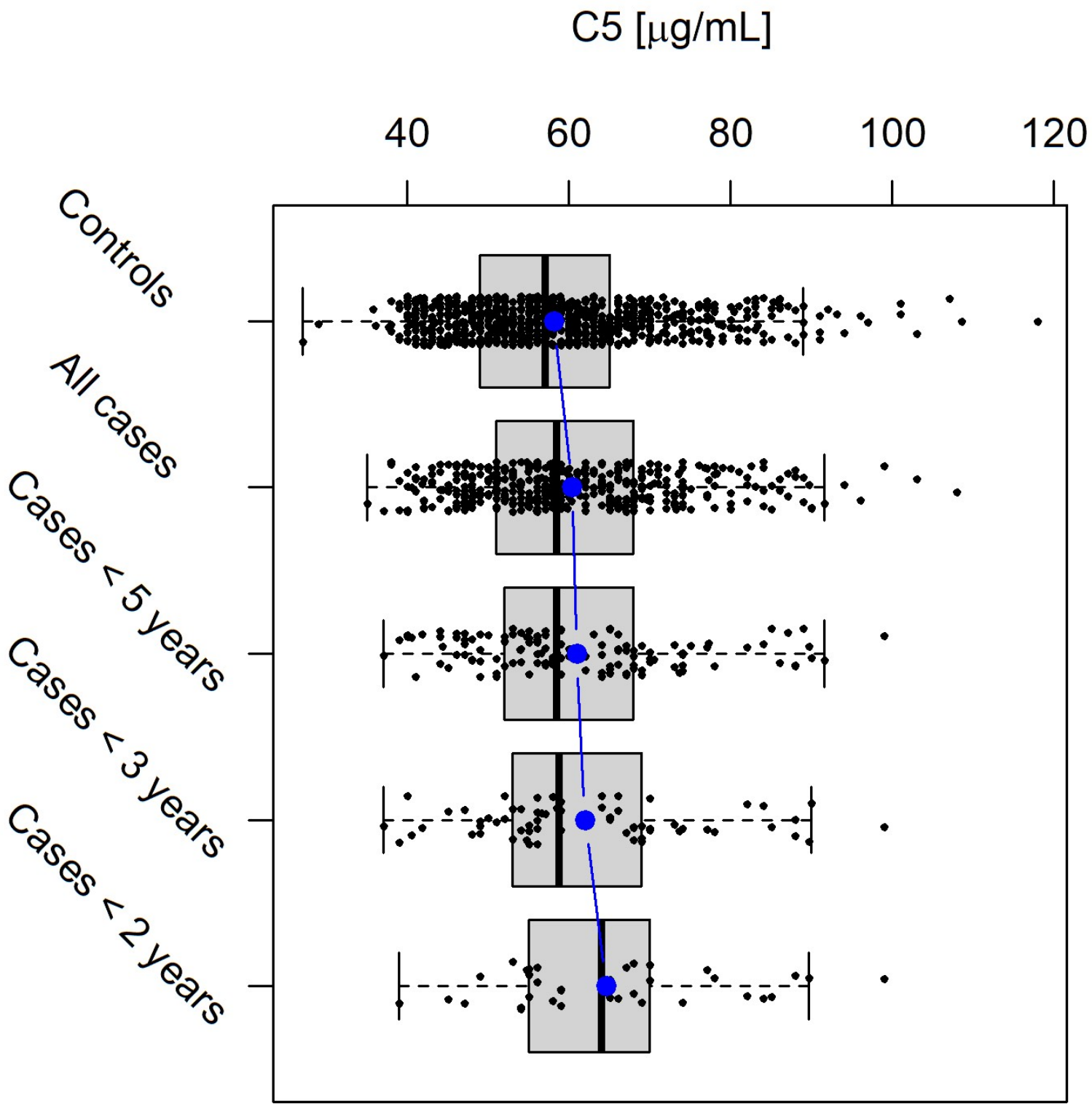

VTE OR: 1.25
10 51 91 132 172 213 254 294 335 375 415
2-year OR = 4.27
3-year OR = 2.17
5-year OR = 1.77
OR (T1 (ref) vs. T3)
Max years from Tromsø 4 to VTE


Provoked VTE OR: 1.16
10 33 56 79 102 125 149 172 195 218 240
2-year OR = 4.09
3-year OR = 2.73
5-year OR = 1.87
OR (T1 (ref) vs. T3)
Max years from Tromsø 4 to provoked VTE


Unprovoked VTE OR: 1.39
10 27 43 60 76 93 110 126 143 159 175
2-year OR = 4.71
3-year OR = 1.54
5-year OR = 1.61
OR (T1 (ref) vs. T3)
Max years from Tromsø 4 to unprovoked VTE


Model 1
Model 2
Model 3
Max 2 years to VTE
T1 (Ctrls: 301, Cases: 4)
T2 (Ctrls: 276, Cases: 13)
T3 (Ctrls: 271, Cases: 20)
1.00 (Reference)
1.00 (Reference)
1.00 (Reference)
3.38 (1.17-12.15)
3.41 (1.18-12.25)
3.27 (1.13-11.79)
4.81 (1.77-16.87)
4.63 (1.70-16.27)
4.27 (1.54-15.14)
Max 3 years to VTE
T1 (Ctrls: 301, Cases: 13)
T2 (Ctrls: 276, Cases: 21)
T3 (Ctrls: 271, Cases: 32)
1.00 (Reference)
1.00 (Reference)
1.00 (Reference)
1.68 (0.83-3.52)
1.70 (0.84-3.56)
1.63 (0.80-3.42)
2.43 (1.26-4.94)
2.31 (1.20-4.71)
2.12 (1.08-4.37)
Max 5 years to VTE
T1 (Ctrls: 301, Cases: 30)
T2 (Ctrls: 276, Cases: 37)
T3 (Ctrls: 271, Cases: 56)
1.00 (Reference)
1.00 (Reference)
1.00 (Reference)
1.29 (0.77-2.15)
1.29 (0.78-2.17)
1.27 (0.76-2.15)
1.87 (1.16-3.06)
1.80 (1.12-2.95)
1.74 (1.07-2.87)
0.5
1
2
4
8
OR (95% CI)


Model 4
VTE
T1 (Ctrls: 301, Cases: 114)
T2 (Ctrls: 276, Cases: 154)
T3 (Ctrls: 271, Cases: 147)
1.00 (Reference)
1.42 (1.02-1.98)
1.24 (0.87-1.77)
VTE provoked
T1 (Ctrls: 301, Cases: 69)
T2 (Ctrls: 276, Cases: 89)
T3 (Ctrls: 271, Cases: 82)
1.00 (Reference)
1.40 (0.94-2.09)
1.17 (0.76-1.78)
VTE unprovoked
T1 (Ctrls: 301, Cases: 45)
T2 (Ctrls: 276, Cases: 65)
T3 (Ctrls: 271, Cases: 65)
1.00 (Reference)
1.47 (0.91-2.37)
1.38 (0.84-2.27)
DVT
T1 (Ctrls: 301, Cases: 74)
T2 (Ctrls: 276, Cases: 89)
T3 (Ctrls: 271, Cases: 95)
1.00 (Reference)
1.26 (0.85-1.87)
1.25 (0.83-1.88)
DVT provoked
T1 (Ctrls: 301, Cases: 45)
T2 (Ctrls: 276, Cases: 50)
T3 (Ctrls: 271, Cases: 60)
1.00 (Reference)
1.27 (0.79-2.05)
1.35 (0.83-2.22)
DVT unprovoked
T1 (Ctrls: 301, Cases: 29)
T2 (Ctrls: 276, Cases: 39)
T3 (Ctrls: 271, Cases: 35)
1.00 (Reference)
1.27 (0.70-2.30)
1.07 (0.57-2.00)
PE
T1 (Ctrls: 301, Cases: 40)
T2 (Ctrls: 276, Cases: 65)
T3 (Ctrls: 271, Cases: 52)
1.00 (Reference)
1.76 (1.09-2.89)
1.28 (0.75-2.17)
PE provoked
T1 (Ctrls: 301, Cases: 24)
T2 (Ctrls: 276, Cases: 39)
T3 (Ctrls: 271, Cases: 22)
1.00 (Reference)
1.73 (0.94-3.28)
0.86 (0.42-1.75)
PE unprovoked
T1 (Ctrls: 301, Cases: 16)
T2 (Ctrls: 276, Cases: 26)
T3 (Ctrls: 271, Cases: 30)
1.00 (Reference)
1.86 (0.92-3.93)
2.01 (0.96-4.34)
0.5
1
2
4
OR (95% CI)


C5 [µg/mL]
40
60
80
100
120
Controls
All cases
Cases < 5 years
Cases < 3 years
Cases < 2 years