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Study conception and design: SKB and JBH, data collection: ENS, KAF, KH, MEG, SKB, JBH, statistical analysis: BP, OVG, SKB, drafting of manuscript: BP, SKB, JBH, Critical revision of manuscript: ENS, KAF, KH, MEG, SKB, JBH, HS, FRR. All authors read and approved the final version of the finalized manuscript.

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Abstract

Venous thromboembolism is a frequent complication in patients with cancer. Homozygous carriers of the fibrinogen gamma gene (FGG) rs2066865 have a moderately increased risk of venous thromboembolism, but the effect of the FGG variant in cancer is unknown. We aimed to investigate the effect of the FGG variant and active cancer on the risk of venous thromboembolism. Cases with incident venous thromboembolism (n= 640) and a randomly selected age-weighted sub-cohort (n=3734) were derived from a population-based cohort (the Tromsø study). Cox-regression was used to estimate hazard ratios with 95% confidence intervals for VTE according to categories of cancer and FGG. In those without cancer, homozygosity at the FGG variant was associated with a 70% (HR 1.7 95% CI 1.2-2.3) increased risk of venous thromboembolism compared to non-carriers. Cancer patients homozygous for the FGG variant had a 2-fold (HR 2.0 95% CI 1.1-3.6) higher risk of venous thromboembolism than cancer patients without the variant. Moreover, the 6-month cumulative incidence of venous thromboembolism among cancer patients was 6.4% (95% CI, 3.5%-11.6%) in homozygous carriers of FGG and 3.1% (95% CI, 2.3%-4.7%) in those without risk alleles. A synergistic effect was observed between rs2066865 and active cancer on the risk of VTE (Synergy index: 1.81, 95% CI: 1.02-3.21, Attributable proportion: 0.43, 95% CI: 0.11-0.74). In conclusion, homozygosity at the FGG variant and active cancer yielded synergistic effect on the risk of venous thromboembolism.

Introduction

Venous thromboembolism (VTE), a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common disease associated with substantial short- and long-term morbidity and mortality [1, 2]. The incidence of VTE is 1-2 per 1000 person-years, and it increases steeply with age [3]. Malignant disease is associated with a four- to seven-fold increased risk of VTE, and 20-25% of all first lifetime VTE-events are cancer-related [4, 5]. VTE, particularly in cancer, leads to prolonged and more frequent hospitalizations, and has a substantial impact on quality of life [6, 7]. Complications of a VTE, such as recurrence, post-thrombotic syndrome and treatment-related bleeding, occur more frequently in cancer patients [6, 8, 9], and the risk of death is higher in cancer patients with than without VTE [10, 11].

Family and twin studies suggest that VTE is highly heritable, and likely results from an interplay between inherited and environmental factors [12, 13]. Fibrinogen, the precursor of fibrin, is an essential component in the final stage of the coagulation cascade. The fibrinogen molecule has three subunits called Aα, Bβ and γ, which occur in pairs for a total number of six subunits. The γ chain, transcribed from the fibrinogen gamma gene (FGG) located on chromosome 4, has two isoforms, γA and γ'. In the Leiden Thrombophilia Study, the fibrinogen gamma gene (FGG) rs2066865 single nucleotide polymorphism (SNP) was first proposed as a risk factor for VTE by reducing fibrinogen γ' levels [14]. Several later genotyping [15, 16] and genome-wide association studies (GWAS) [17, 18] confirmed an association between rs2066865 and VTE risk, whereas two cohort studies found no significant association [19, 20]. In a recent meta-analysis including seven studies, rs2066865 was associated with an increased risk of VTE (OR 1.61, 95% CI 1.34-1.93) [21].

The majority of the genetic studies have excluded individuals with cancer-related thrombosis. However, as prothrombotic genotypes are fixed, and not influenced by disease, interventions and complications, they may be attractive candidates as biomarkers of VTE risk in cancer patients. Recent studies have suggested that interactions between cancer and other prothrombotic genotypes (FV

variants rs6025 and rs4524 and prothrombin G20210A) have synergistic effects on the risk of VTE [22-25]. To the best of our knowledge, no study has investigated the impact of rs2066865 on the risk of VTE in cancer patients. Therefore, we aimed to investigate the joint effect of rs2066865 and active cancer on the absolute and relative risks of VTE in a population-based case-cohort.

Methods

Study population

The Tromsø Study is a single-center population-based cohort, following residents of the municipality of Tromsø, Norway, with repeated health surveys. The case-cohort was derived from the fourth survey (Tromsø 4), which included 27 158 participants aged 25-97 years. A detailed cohort profile of the Tromsø study has been published previously [26]. The study was approved by the Regional Committee for Medical and Health Research Ethics in Northern Norway, and all participants provided informed written consent to participation. From enrolment in Tromsø 4 (1994/95), subjects were followed until December 31, 2012. Detailed information regarding identification and validation of VTE-events are described in the method supplementary of appendix.

In total, 710 participants developed VTE during follow-up. Of these, 26 did not have blood samples available or of sufficient quality for DNA analyses. The remaining 684 subjects were included as the cases in our study. A subcohort (n=3 931) was composed by randomly sampling individuals from Tromsø 4 weighted for the age distribution of the cases in 5-year age-groups. Due to the nature of the case-cohort design, where each participant has the same probability of sampling, 72 of the cases were also in the subcohort. Subjects with a history of cancer prior to inclusion (n=232) and subjects with missing information on rs2066865 (n=9) were excluded from the analysis. The final case-cohort consisted of 4 374 subjects, with 640 cases and 3 734 in the subcohort. A flow chart of the case-cohort is displayed in figure 1.

Baseline measurements and genotyping

Baseline measurements and genotyping methods are described in the Online supplementary methods.

Cancer exposure

Cancer assessment is described in the Online supplementary methods. Previous studies have shown a strong temporal relation between cancer diagnosis and incident VTE, and up to 50 % of cancer-related VTE events presents within a 2.5 year interval (from 6 months preceding the cancer diagnosis until 2 years following the cancer diagnosis) [27, 28]. Therefore, a VTE was defined as related to active cancer if it occurred within this time period.

Subjects who survived the active cancer period without a VTE were censored at the end of the active cancer period (i.e. 2 years after cancer was diagnosed). The censoring was performed because information regarding remission and relapse of cancer was unavailable, and extension of the observation period of cancer could result in dilution of the estimates due to inclusion of VTEs not necessarily caused by cancer. This approach resulted in censoring of 14 VTE cases that occurred after the active cancer period. Thus, 626 VTE cases were included in the final analyses.

Statistical Analysis

Statistical analyses were performed using STATA version 15.0 (Stata Corporation LP, College Station, TX, USA). Cox proportional hazards regression models were used to obtain age- and sex- adjusted hazard ratios (HR) with 95% confidence intervals (CIs) for VTE across categories of cancer status (no cancer/active cancer) and FGG risk alleles. Cancer was assessed as a time-dependent covariate in the model. Subjects who developed cancer contributed person-time as unexposed from the inclusion date until six months prior a cancer diagnosis, and thereafter contributed person-time in the active

cancer group as exposed. Absolute incidence rates (IR) were calculated based on person-time from the original cohort (n=27 128). To calculate joint effects conferred by active cancer and FGG risk alleles, subjects with no cancer and no risk alleles were used as the reference group in the Cox model. Based on the total active cancer person-time at risk derived from the source cohort, 1-Kaplan-Meier curves were used to estimate the cumulative incidence of VTE in subjects with active cancer according to the presence of FGG risk alleles. Methods for assessing synergism between FGG and active cancer on the risk of VTE are described in detail in the Online supplementary methods.

Results

The mean follow-up of the case-cohort was 12.6 years. In total, 854 subjects had active cancer, of which 167 experienced an incident VTE. The baseline characteristics of the entire case-cohort and in those with active cancer during follow-up are presented in Table 1. Subjects who developed active cancer were slightly older (61 ± 10 years vs. 58 ± 13 years) and reported a higher frequency of daily smoking (46% vs. 35%) compared to the entire case-cohort. The minor allele frequency of rs2066865 was 0.26, which is comparable to reference populations [14, 29]. The homozygous variant of the FGG was present in 289 (6.6%) subjects, the heterozygous variant in 1 723 (39.4%) subjects, while 2 362 (54.0%) subjects were non-carriers of the FGG variant. The allele frequency was essentially similar in subjects who developed cancer. Expected versus observed proportions of hetero- and homozygous individuals in the subcohort according to the Hardy-Weinberg equilibrium are presented in Supplementary table 1.

The clinical characteristics of the VTE events stratified by the presence of active cancer are shown in table 2. Compared to the non-cancer-related VTEs, cancer-related VTEs were more often a DVT (59.2% vs. 55.5%) than a PE (40.7% vs. 44.4%). The prevalence of provoking factors such as acute medical conditions, immobilization and surgery were essentially similar between the two groups, as were the total proportion of VTEs with one or more concurrent provoking factors (44.3% vs. 44.7%). Non-cancer related VTEs were more likely to be associated with traumas (9.6% vs. 2.4%) while other provoking factors (i.e. venous catheters) were more frequent in cancer-related VTE (8.4% vs. 3.7%).

In participants without cancer, the IR of VTE increased from 1.2 (95% CI 1.1-1.4) per 1000 person-years among non-carriers of FGG rs2066865 to 2.0 (95% CI 1.5-2.7) per 1000 person-years among those with two risk alleles. Accordingly, the risk of VTE was 70% (HR 1.7, 95% CI 1.2-2.3) higher in those with two risk alleles at FGG compared to non-carriers (Table 3). In subjects with active cancer, the risk was 12-fold higher (HR 11.9, 95% CI 9.3-15.2) in those with no FGG risk alleles, and 22-fold higher (HR 22.2, 95% CI 12.9-38.1) in those with two FGG risk alleles, compared to cancer-

free subject without risk alleles. Cancer patients with two risk alleles at FGG had a 2-fold higher (HR 2.0, 95% CI 1.1-3.6) risk of VTE compared to cancer patients without risk alleles. In sub-analyses, the effect of active cancer and homozygosity at FGG yielded higher risk estimates for PE (HR 2.9, 95% CI 1.3-6.6) than for DVT (HR 1.6, 95% CI 0.7-3.5).

The cumulative incidence of VTE during the active cancer period is shown in Figure 2. The cumulative incidence of VTE increased particularly during the first six months following a cancer diagnosis, where we found a substantially steeper incline in the incidence curve for subjects with two risk alleles at FGG rs2066865. The cumulative incidence of VTE among homozygous carriers was 5.0% (95% CI, 2.4%- 9.6%), 6.4% (95% CI, 3.5%-11.6%), and 8.0% (95% CI, 4.6%-13.9%) at 3 months, 6 months and 24 months after cancer diagnosis, respectively. The corresponding figures for cancer patients who were non-carriers were 2.1% (95% CI, 1.5%-3.0%), 3.1% (95% CI, 2.3%-4.7%), and 4.8% (95% CI, 3.8%-6.2%), respectively.

A supra-additive effect on the risk of VTE was observed for the combination of homozygosity at the FGG variant and active cancer (table 4). The RERI was 9.61 (95% CI -2.38-21.61) and the RSI was 1.81 (95% CI 1.02-3.21). The proportion attributable to interaction (AP) was 0.43 (95% CI 0.11-0.74). In sub-group analysis, the estimates of biological interaction were stronger for PE (RSI = 2.37, 95% CI 1.05-5.39) than for DVT (RSI = 1.46, 95% CI 0.65-3.27).

Discussion

In the present study, we aimed to investigate the joint effect of the rs2066865 SNP at FGG and active cancer on the risk of VTE in a case-cohort recruited from the general population. Homozygosity at rs2066865, occurring in 6.6% of the study population, was associated with increased risk of VTE. The combination of an rs2066865 homozygous risk genotype and active cancer showed a synergistic effect on VTE risk (on an additive scale). The effect was particularly strong for PE. The cumulative incidence of VTE increased substantially during the first six months following a cancer diagnosis, especially among patients with two risk alleles at FGG rs2066865. Our findings suggest that homozygosity at FGG rs2066865 may aid to differentiate patients at high and low risk of cancer-related VTE.

Several observational studies have reported an association between homozygous genotype of rs2066865 and increased risk of VTE in Caucasians [14-16, 21]. In a recent meta-analysis including seven observational studies, the odds ratio of VTE was 1.61 for homozygosity at rs2066865 [21]. Accordingly, in cancer-free subjects, we found that those with two rs2066865 risk alleles had a 1.7-fold higher VTE risk than those with 0 risk alleles. The risk estimates for DVT and PE were essentially similar in cancer-free subjects.

Even though the role of prothrombotic genotypes in cancer-related VTE have been scarcely studied, previous studies have found that some prothrombotic genotypes (e.g. Factor V Leiden and prothrombin G20210A) are associated with increased risk of cancer-related VTE [22, 23, 30, 31]. Further, the combined effect of cancer and Factor V variants (Factor V Leiden and rs4524) exceeded the sum of the individual effects, implicating a biological interaction on VTE risk [22, 24]. Accordingly, we found that the combination of FGG and active cancer yielded a synergistic effect on VTE risk.

In cancer patients, the cumulative incidence curve of VTE was substantially steeper in individuals homozygous for FGG during the first six months following the cancer diagnosis. According to the thrombosis potential model [32], several risk factors need to be present concurrently to exceed

the thrombosis potential and facilitate development of a VTE. In the period following a cancer diagnosis, treatment with surgery and/or chemotherapy is typically initiated, and treatment-related complications such as acute infection and immobilization frequently occur. Thus, the accumulation of several treatment-related risk factors, which adds to the background risk in patients with cancer and risk alleles at FGG, may partly explain the substantial increase in VTE incidence the first half year following a cancer diagnosis.

In contrast to cancer-free subjects, we found that the effect of rs2066865 was stronger for PE than for DVT in cancer patients. This suggests that the FGG variant may play a more essential role in the pathogenesis of PE than DVT in cancer patients. The underlying mechanism(s) for the latter observation is unknown, but may imply that rs2066865 is associated with fragile thrombi, which are prone to embolization and manifest clinically as PE rather than DVT in cancer patients.

The mechanism by which the rs2066865 affects susceptibility to VTE is not fully elucidated. However, the current hypothesis is that it acts through a phenotype with altered fibrinogen composition and formation. The rs2066865 SNP tags the FGG-H2 haplotype. Previous studies have shown that homozygous carriers of the FGG-H2 haplotype had lower levels of γ′ fibrinogen and γ′ fibrinogen/total fibrinogen concentration [14] without alterations in the total fibrinogen level [33]. The suggested mechanism is that the FGG variant favors formation of the abundant γ-chain isoform (Aγ) above the minor γ-chain (γ′) through alternative splicing of the mRNA of the FGG-gene [14, 33]. Fibrinogen γ′ exhibits an inhibitory activity towards thrombin, due to a high affinity binding site on the γ′ chain for thrombin exosite II [34], which inhibits thrombin-mediated activation of FVIII [35], FV [36] and platelets [37]. Moreover, fibrinogen γ′ has been shown to increase the activated protein C (APC) sensitivity [38]. However, studies on the association between low plasma levels of fibrinogen γ′ and VTE risk have shown somewhat inconsistent results [14, 20]

Current anticoagulant prophylaxis regimens efficiently prevent first VTE in cancer patients, but at the expense of a substantial risk of major and life-threatening bleedings [39]. Therefore, current

international guidelines do not recommend prophylactic anticoagulation to all ambulatory cancer patients [40, 41]. Thus, it is vital to recognize patients that are at high risk of cancer associated VTE, in order to identify those who would benefit most from thromboprophylaxis. Prothrombotic genotypes are attractive biomarker candidates, which could be used to distinguish between high and low risk of VTE in cancer patients, since they are fixed and not affected by the clinical status or treatment-related factors. In the present study, 6.4% of cancer patients with two risk alleles at FGG rs2066865 developed VTE during the first 6 months after cancer diagnosis compared to 3.1% of cancer patients without risk alleles. Our findings suggest that FGG may be an attractive gene candidate to pursue in future research on prediction models of VTE risk in cancer patients. We and others have previously reported similar discriminative power of two variants in the F5 gene (rs6025 and rs4524) [23, 24], and a genetic model including 9 SNPs reported promising predictive capacity on VTE risk in breast cancer [42]. Recently, a new risk prediction model for cancer-related VTE, including clinical characteristics and genetic variants, reported a strong predictive capacity with AUC of 0.73 and performed better that the Khorana score (AUC 0.58) [43].

The main strengths of present study are the prospective design, high participation rate and long-term follow-up, making it possible to capture a large quantity of both incident cancer- and VTE-events in the study population. Since all participants live within a single hospital catchment area, the probability of missing outcomes is low. Moreover, both incident VTE-events and cancer diagnoses were systematically validated and objectively confirmed. The study was limited by the lack of statistical power in sub-group analysis (i.e. DVT/PE), illustrated by wide confidence intervals for our risk estimates. In addition, we did not have access to information on treatment regimens or medical complications among cancer patients. Although there is no reason to believe that the type or intensity of treatment would be influenced by the genetic makeup, such data could have provided further insights into the possible interplay between genes and treatment-related risk factors.

In conclusion, we found that homozygosity at rs2066865 was associated with an increased risk of VTE, and yielded a synergistic effect on the VTE risk in combination with active cancer, particularly on the risk of PE.

Acknowledgements

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TABLE 1. Baseline characteristics in the entire case-cohort and in the active cancer group

	Entire case-cohort	Active cancer
Subjects (n)	4374	854
Age (years)	58 ± 13	62 ± 10
Sex (Males)	47.0 (2048)	53.0 (456)
BMI (kg/m^2)	26.0 ± 4	26.0 ± 4
Daily smoking	34.5 (1464)	43.5 (364)
WBC count (10^9/L)	7.1 ± 1.8	7.2 ± 1.8
Platelet count (10^9/L)	251 ± 60	250 ± 58
rs2066865*	0.26	0.26
1 risk alleles	1723	334
2 risk alleles	289	51

Values are numbers or percentages with numbers in parenthesis or means ±SD.

Active cancer: period from six months before a cancer diagnosis until two years after.

BMI indicates body mass index; Daily smoking indicates smoking at the time of enrollment. *Allele frequency

TABLE 2. Characteristics of subjects with cancer-related and non-cancer-related first venous thromboembolism (VTE)

	Cancer-related VTE	
	Yes (167)	No (459)
Age at VTE diagnosis (years)	69 ±11	68±14
Sex (Males)	44.9 (75)	47.3(217)
VTE type		
Deep vein thrombosis	59.2 (99)	55.5 (255)
Proximal upper limb	5.1 (5)	2.0 (5)
Distal upper limb	1.0 (1)	0 (0)
Proximal lower limb	62.6 (62)	65.9 (168)
Distal lower limb	12.1 (12)	28.2 (72)
Other localizations	19.1 (19)	3.9 (10)
Pulmonary embolism	40.7 (68)	44.4 (204)
Unprovoked event	NA	54.9 (252)
Provoking factors		
Surgery [a]	12.6 (21)	15.3 (70)
Trauma [a]	2.4 (4)	9.6 (44)
Acute medical condition [b]	15.0 (25)	14.2 (65)
Immobilization [c]	20.4 (34)	20.0 (92)
Other provoking factor [d]	8.4 (14)	3.7 (17)
Total provoked [e]	44.3 (74)	44.7 (205)

Values are numbers or percentages with numbers in parenthesis or means ±SD; NA, Not applicable; [a] Within 8 weeks before the VTE-event; [b] Myocardial infarction, ischemic stroke of major infectious disease; [c] Bedrest>3 days, wheelchair, long haul travel>4 h in the past 14 days; [d] presence of other provoking factors noted by the physician (e.g. intravenous catheters); [e] one or more provoking factor above

TABLE 3: Age and sex adjusted hazard ratios for venous thromboembolism (VTE) according to categories of fibrinogen gamma gene (FGG) risk alleles and cancer status.

	Risk Alleles	VTE			PE			DVT		
		Events	HR (95% CI)	HR (95% CI)	Events	HR (95% CI)	HR (95% CI)	Events	HR (95% CI)	HR (95% CI)
No cancer	0	242	Ref.	-	112	Ref.	-	130	Ref.	-
	1	170	1.0 (0.8-1.2)	-	70	0.9 (0.6-1.2)	-	100	1.1 (0.8-1.4)	-
	2	47	1.7 (1.2-2.3)	-	22	1.7 (1.1-2.7)	-	25	1.6 (1.1-2.5)	-
Active cancer	0	89	11.9 (9.3-15.2)	Ref.	32	8.3 (5.6-12.5)	Ref.	57	15.3 (11.2-21.1)	Ref.
	1	64	12.2 (9.2-16.1)	1.1 (0.8-1.5)	29	10.6 (7.1-16.3)	1.3 (0.8-2.2)	35	13.4 (9.2-19.6)	1.0 (0.6-1.5)
	2	14	22.2 (12.9-38.1)	2.0 (1.1-3.6)	7	22.8 (10.6-49.1)	2.9 (1.3-6.6)	7	21.6 (10.0-46.4)	1.6 (0.7-3.5)

Active cancer: period from six months before a cancer diagnosis until two years after; CI: Confidence interval; DVT: Deep vein thrombosis; HR: Hazard ratio; PE: Pulmonary embolism; VTE: Venous thromboembolism

TABLE 4. Measures of interaction between the homozygous FGG variant and active cancer on VTE risk
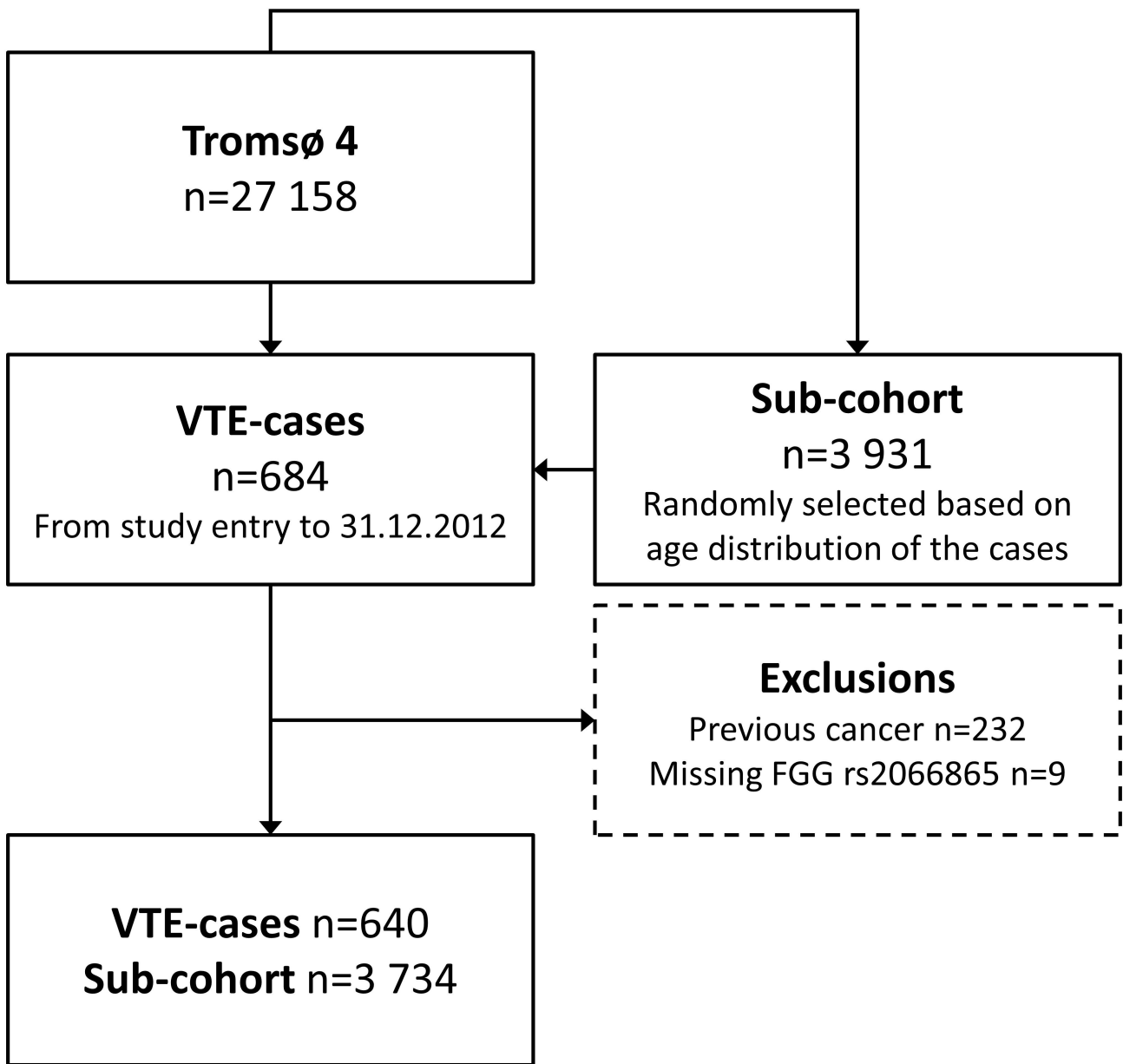
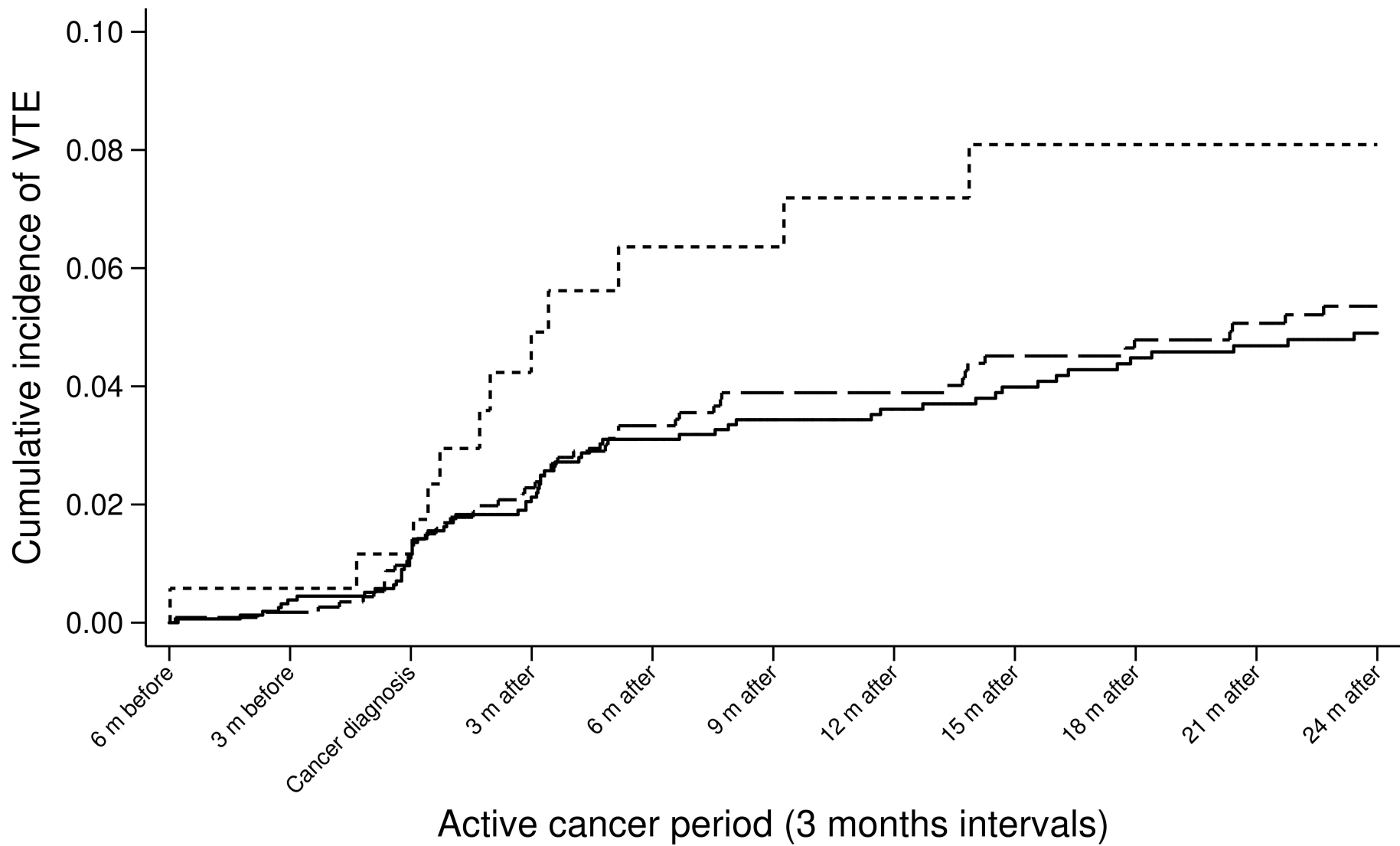|	Rothmans synergy index (RSI) (95% CI)	Relative excess risk by interaction (RERI) (95% CI)	Proportion due to interaction (AP) (95% CI)
FGG rs2066865			
VTE	1.81 (1.02 to 3.21)	9.6 (-2.4 to 21.6)	0.43 (0.11 to 0.74)
PE	2.37 (1.05 to 5.39)	13.4 (-4.8 to 31.7)	0.56 (0.21 to 0.90)
DVT	1.46 (0.65 to 3.27)	6.3 (-9.6 to 22.1)	0.30 (-0.24 to 0.83)

Rothmans synergy index (RSI) >1 indicates a positive interaction or more than additivity; Relative excess risk by interaction (RERI) >0 indicates a positive interaction or more than additivity; Proportion due to interaction (AP) >0 indicates a positive interaction or more than additivity

Figure legend:

FIGURE 1. Flow chart for the case-cohort

FIGURE 2. Cumulative incidence of VTE in the presence of FGG rs2066865 risk alleles during the active cancer period

Cumulative incidence of VTE

Active cancer period (3 months intervals)

(x-axis labels): 6 m before, 3 m before, Cancer diagnosis, 3 m after, 6 m after, 9 m after, 12 m after, 15 m after, 18 m after, 21 m after, 24 m after

Supplementary appendix for the manuscript:

Fibrinogen gamma gene rs2066865 and risk of cancer-related venous thromboembolism

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Methods

VTE identification and validation

All first lifetime events of VTE occurring among the participants in this period were identified using the hospital discharge diagnosis registry, the autopsy registry and the radiology procedure registry from University Hospital of North Norway (UNN), which is the sole provider of diagnostic radiology and treatment of VTE in the Tromsø area. Trained personnel adjudicated and recorded each VTE by extensive medical records review. The adjudication criteria for VTE were presence of signs and symptoms of PE or DVT combined with objective confirmation by radiological procedures, which resulted in treatment initiation (unless contraindications were specified). A VTE-event was classified as either a DVT or a PE. When a PE and DVT occurred concurrently, the event was classified as a PE. The identification and adjudication process of VTEs has been previously described in detail [1].

Baseline measurements

Baseline information regarding lifestyle and vital parameters was obtained through physical examination, blood samples and self-administered questionnaires. Body height and weight were measured with participants wearing light clothes and no shoes. Body mass index (BMI) was calculated by dividing body weight by height squared (kg/m^2). Information on medical history (e.g. cardiovascular diseases and diabetes mellitus), life style factors (e.g. smoking status, amount of physical activity) and level of education was derived from the questionnaires.

Cancer exposure

Registration of cancer is mandatory by law in Norway; and The Cancer Registry of Norway (CRN) performs surveillance of cancer diagnoses on a national basis. Information on date of diagnosis, malignancy location (International Classification of Disease, Revision 7 (ICD-7) codes 140-205), morphology and histology, and initial treatment was obtained by linking The Tromsø Study to the CRN using the unique national civil registration numbers. Diagnoses of non-melanoma skin cancer (ICD 190.0-191.9) was regarded as non-cancer. The cancer registry of Norway is of high quality when it comes to completeness and validation. An evaluation of the CRN performed by Larsen et al, estimated 98.8% completeness of the cancer diagnoses, with a histological verification of 94% [2].

Genotyping and quality control

We genotyped one SNP at the fibrinogen gamma gene (rs2066865), which has previously been implicated as a candidate marker of VTE [3], using the Taqman platform and an initial input of 100 ng DNA. Samples were genotyped with the Applied Biosystems 7900HT (Foster City, CA, USA) according to the recommended protocol and processed using SDS 2.4 (Thermo Fisher, Foster City, CA, USA). Genotypes passing a quality value threshold of 95 were used.

Statistics

Synergism refers to the phenomenon where the presence of two or more elements produces a stronger effect than the sum of the individual components. Rothmans synergy index (RSI) [4], relative excess risk caused by interaction (RERI) and attributable portion due to interaction (AP) were calculated to adjudicate whether the combined effect of risk alleles and active cancer on VTE risk exceeded the sum of the risks that each factor yielded alone. RSI measures departure from additive risks, and a value of 1 suggests that there is no interaction between the exposures or perfect additivity, whereas RSI>1 suggests positive interaction or more than additivity. RERI and AP measures of 0 indicates no biological interaction, while measures >0 suggest positive interaction (i.e. more than additivity).

Supplementary Table 1:

FGG risk alleles	Subcohort-members	
	Observed	Expected
0	2133 (54.3%)	2157.6 (55.0%)
1	1552 (39.6%)	1502.7 (38.3%)
2	237 (6.0%)	261.7 (6.7%)

Distribution of observed and expected allele-combination of FGG rs2066865 among individuals in the sub-cohort.

References

1.   Braekkan SK, Mathiesen EB, Njolstad I, Wilsgaard T, Stormer J, Hansen JB. Family history of myocardial infarction is an independent risk factor for venous thromboembolism: the Tromso study. J Thromb Haemost. 2008;6(11):1851-1857.
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