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## ORIGINAL ARTICLE

# Combined effects of five prothrombotic genotypes and cancer on the risk of a first venous thromboembolic event

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## Abstract

**Background:** The role of combined prothrombotic genotypes in cancer-related venous thromboembolism (VTE) is scarcely studied. We aimed to investigate the impact of a 5-single nucleotide polymorphism (SNP) score on the risk of VTE in patients with and without cancer using a population-based case-cohort.

**Methods:** Cases with a first VTE ( $n = 1493$ ) and a subcohort ( $n = 13\,072$ ) were derived from the Tromsø Study (1994-2012) and the Nord-Trøndelag Health Study (1995-2008). Five SNPs previously reported as a risk score were genotyped: ABO (rs8176719), F5 (rs6025), F2 (rs1799963), FGG (rs2066865), and F11 (rs2036914). Hazard ratios (HRs) for VTE were estimated according to cancer status and the number of risk alleles in the 5-SNP score (0-1, 2-3, and  $\geq 4$  alleles).

**Results:** During a median follow-up of 12.3 years, 1496 individuals were diagnosed with cancer, of whom 232 experienced VTE. The VTE risk increased with the number of risk alleles in the 5-SNP score among subjects without and with cancer. In cancer-free subjects, the HR was 2.17 (95% confidence interval [CI] 1.79-2.62) for  $\geq 4$  versus 0-1 risk alleles. In cancer patients, the corresponding HR was 1.93 (95% CI 1.28-2.91). The combination of cancer and  $\geq 4$  risk alleles yielded a 17-fold (HR 17.1, 95% CI 12.5-23.4) higher risk of VTE compared with cancer-free subjects with 0-1 risk alleles.

**Conclusion:** The risk of VTE increases with the number of prothrombotic risk alleles in subjects with and without cancer, and the combination of prothrombotic risk alleles and cancer leads to a highly elevated risk of VTE.

## KEYWORDS

5-SNP score, cancer, deep vein thrombosis, prothrombotic genotypes, pulmonary embolism, risk, venous thromboembolism

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## 1 | INTRODUCTION

Venous thromboembolism (VTE), a collective term for deep vein thrombosis (DVT) and pulmonary embolism (PE), is a severe and frequent complication of malignancy, of which the incidence is increasing.<sup>1,2</sup> Cancer is associated with a four- to seven-fold increased risk of VTE,<sup>3-5</sup> and approximately 15% of cancer patients will develop a VTE during the course of malignancy.<sup>6</sup> Cancer patients who develop VTE have shortened life expectancy compared with cancer patients without VTE.<sup>7,8</sup> The clinical consequences of VTE, such as post-thrombotic syndrome, VTE recurrence, and treatment-related bleeding, occur more often in cancer patients than in cancer-free subjects.<sup>7,9</sup> Current guidelines do not recommend routine thromboprophylaxis to all ambulatory cancer patients due to the uncertain benefit-to-harm ratio, which emphasizes the importance of identifying high-risk subjects.<sup>10-12</sup>

Simulation studies have shown that mapping genetic profiles may be useful to detect subjects at high and low risk of disease.<sup>13,14</sup> Genetic profiling may therefore help to identify subjects in need of VTE prophylaxis in high-risk situations, such as cancer, surgery, or prolonged immobilization. Several single nucleotide polymorphisms (SNPs) are associated with the risk of VTE.<sup>15-19</sup> To identify high-risk individuals, de Haan et al created a genetic score based on 31 SNPs previously reported to increase VTE risk.<sup>20</sup> In this score, the SNPs with highest odds ratios of VTE were added one-by-one to finally create a genetic risk score containing five SNPs: rs8176719 (non-O blood type) in ABO, rs6025 (factor V Leiden [FVL]) in F5, rs1799963 (prothrombin G20210A) in F2, rs2066865 in the fibrinogen gamma gene (FGG), and rs2036914 in F11. This 5-SNP score performed similarly to the score of all 31 SNPs,<sup>20</sup> and detected subjects at increased risk of both incident and recurrent VTE.<sup>20-22</sup>

Genetic alterations such as Factor V Leiden,<sup>23,24</sup> ABO rs505922 and rs8176746,<sup>25</sup> ABO rs8176719,<sup>26</sup> and prothrombin G20210A<sup>27-30</sup> have all been found to be associated with VTE risk in cancer. Moreover, the combined effect of cancer and the factor 5 SNPs rs6025 and rs4524 increased VTE risk on a supra-additive scale, indicating a biological interaction.<sup>24</sup> As prothrombotic genotypes only need to be measured once, and are not influenced by disease progression, interventions, or complications, they are attractive candidate biomarkers of VTE risk in cancer patients.

The role of genetics in cancer-related VTE is not yet fully elucidated, and to the best of our knowledge, no previous study has investigated the impact of the 5-SNP score on VTE risk in cancer patients. Therefore, we aimed to investigate the impact of increasing number of risk alleles in the 5-SNP score on the risk of VTE in subjects with and without cancer using a case-cohort recruited from the general population.

## 2 | METHODS

### 2.1 | Study population

Study participants were derived from the fourth survey of the Tromsø Study, conducted in 1994-1995,<sup>31</sup> and the second survey of the

### Essentials

- The role of prothrombotic SNPs in cancer-related venous thromboembolism (VTE) is scarcely studied.
- We investigated if a 5-SNP score was associated with VTE risk in subjects with and without cancer.
- The risk of VTE increased with increasing number of 5-SNP score risk alleles in both groups.
- The combination of prothrombotic risk alleles and cancer led to a highly elevated risk of VTE.

Nord-Trøndelag Health (HUNT) Study, conducted in 1995-1997.<sup>32</sup> The Tromsø Study (Tromsø 4) and the HUNT Study (HUNT2) are Norwegian population-based cohorts of the inhabitants of Tromsø municipality and Nord-Trøndelag County, respectively. A total of 27 158 unique individuals aged  $\geq 25$  years participated in Tromsø 4, and 66 140 individuals aged  $\geq 20$  years participated in HUNT, yielding attendance rates of 77% (Tromsø 4) and 71% (HUNT2). Detailed descriptions of the studies have been published elsewhere.<sup>31,32</sup>

Participants were followed from the date of inclusion until a verified first VTE diagnosis, migration, death, or end of follow-up (December 31, 2008 in HUNT2 and December 31, 2012 in Tromsø 4). In Tromsø 4, all VTE events were identified by searching 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 provider of diagnostic radiology and treatment of VTE in the Tromsø area. Trained personnel reviewed the medical records for each potential VTE case, and incident VTE events were included when clinical signs and symptoms of PE or DVT were combined with radiologic confirmation and treatment was initiated (unless contraindications were specified). In HUNT2, VTE events were identified by searching the hospital discharge diagnosis registry and the radiology procedure registry at the two local hospitals in the county (Levanger Hospital and Namsos Hospital) and by searching the discharge diagnosis registry of the tertiary-care center of the region, St. Olav's Hospital in Trondheim. Two physicians reviewed the medical records for each VTE event, and the validation criteria included symptomatic VTE events confirmed by radiologic procedures (ultrasound, venography, computed tomography [CT] scan, or perfusion-ventilation scan) which required treatment. The identification and adjudication process of VTEs in both studies have been previously described in detail.<sup>33,34</sup> Participants with a history of VTE before inclusion in the parent cohorts were excluded.

We created a case-cohort by including all cases with a first lifetime VTE ( $n = 1493$ ) and a randomly sampled subcohort ( $n = 13\ 072$ ) derived from the parent cohorts (Figure 1). Participants not registered as inhabitants of Tromsø or Nord-Trøndelag at study inclusion ( $n = 3$ ) and subjects with missing information on risk alleles ( $n = 170$ ) or body mass index ( $n = 80$ ) were excluded. Further, subjects with a cancer diagnosis prior to or less than 6 months after inclusion ( $n = 624$ ) were excluded. Eventually, the case-cohort consisted of

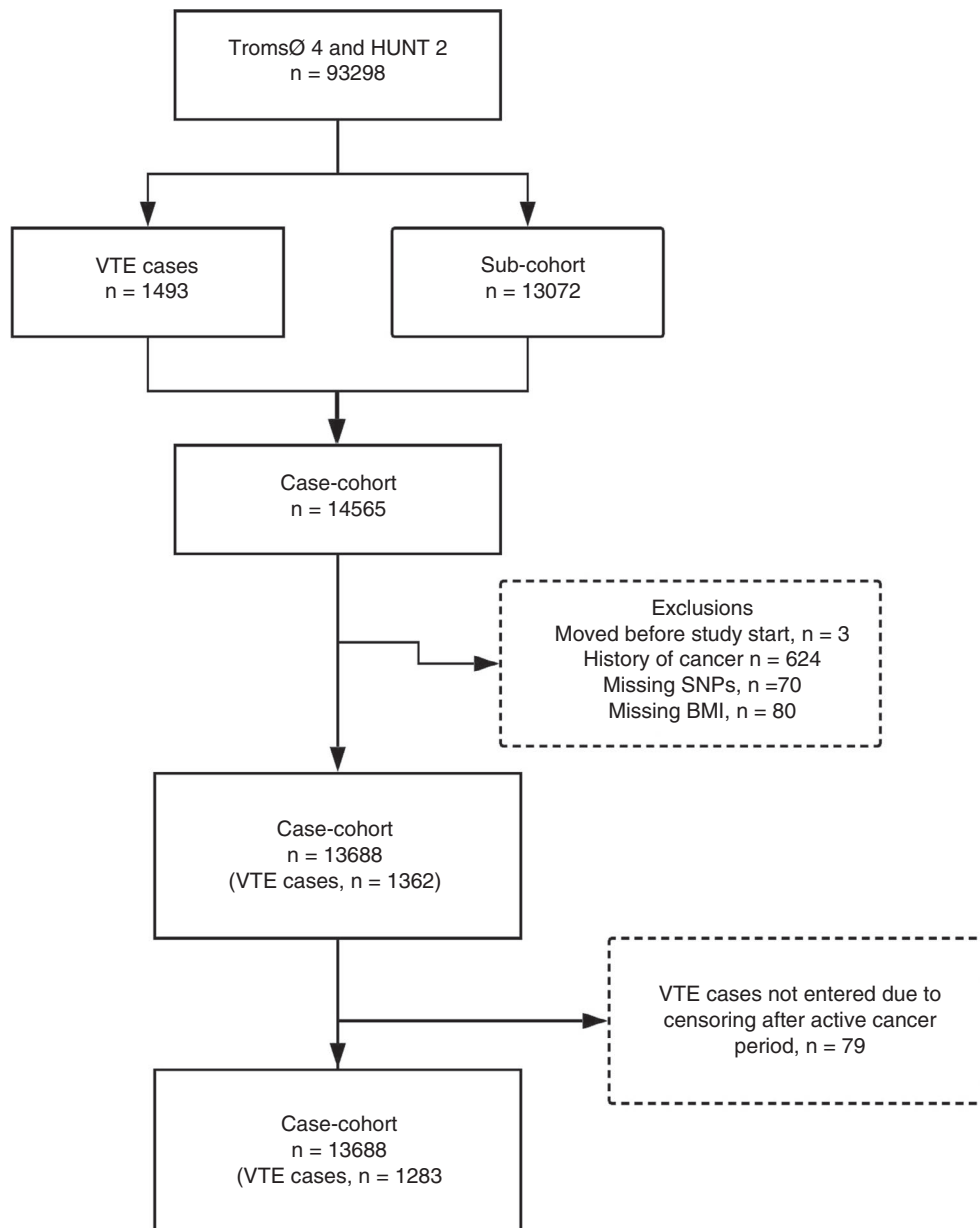
13 688 participants, of whom 1362 were VTE cases. Due to the nature of the case-cohort design, in which every person in the cohort, including the cases, has the same probability of being selected to the subcohort, 206 of the subjects randomly selected to the subcohort were also cases. All participants gave their informed written consent to participate and The Regional Committee of Medical Health Research Ethics approved the study.

## 2.2 | Baseline measurements and genotyping

Baseline information was obtained by physical examination, blood sampling, and self-administrated questionnaires in each study. Body height and weight were measured with participants wearing light

clothing and no shoes. Body mass index (BMI) was calculated by dividing the weight in kilograms (kg) by height in meters (m) squared ( $\text{kg}/\text{m}^2$ ). Information on history of cardiovascular disease (myocardial infarction, angina, or stroke), diabetes mellitus, and smoking status were obtained from the questionnaires.

DNA was isolated from blood, and the following five SNPs were genotyped: ABO rs8176719 (non-O blood type), F5 rs6025 (Factor V Leiden), F2 rs1799963 (prothrombin G20210A), FGG rs2066865, and F11 rs2036914. In Tromsø 4, the Sequenom platform was used for genotyping rs8176719 (ABO), rs6025 (F5), rs1799963 (F2), and rs2036914 (F11), while rs2066865 (FGG) was genotyped with the TaqMan platform, as previously described.<sup>35</sup> In HUNT2, genotyping was performed using the Illumina HumanCore Exome array.



**FIGURE 1** Study population. Participants were recruited from the fourth survey of the Tromsø study (1994–2012) and the second survey of the Nord-Trøndelag Health (1995–2008) study. VTE indicates venous thromboembolism; SNPs, single nucleotide polymorphisms

Subjects were considered as carriers of prothrombotic genotypes if one or two risk alleles were present. Hence, we did not differentiate between hetero- and homozygous carriers. Because the minor allele at F11 rs2036914 is associated with reduced VTE risk,<sup>36</sup> the common allele was set as risk allele in our analysis. Zero risk alleles at ABO rs8176719 was defined as O blood type, whereas one or two risk alleles were classified as non-O blood type. The 5-SNP score conceived by de Haan et al was created by summing the number of risk alleles from the five sequenced SNPs.<sup>20</sup>

### 2.3 | Cancer assessment

Information on cancer diagnosis, primary cancer site (International Classification of Diseases, Revision 7 [ICD-7] codes 140-205), and stage during follow-up, was obtained by linkage to the Cancer Registry of Norway. The Cancer Registry of Norway is considered a complete and valid registry with reported 98.8% completeness and with 94% of the cases being histologically verified.<sup>37</sup> Non-melanoma skin cancers (ICD-7 codes 191.0-191.9) were classified as non-cancer due to the pathophysiology and nature of these cancers.

As temporal proximity to cancer diagnosis is shown to be a strong predictor for VTE risk,<sup>1,24,38</sup> a VTE event was classified as related to active cancer if it occurred within 6 months prior to a cancer diagnosis until 2 years following the cancer diagnosis date. Patients who were not diagnosed with VTE and still alive at the end of the active cancer period were censored at this time, as information regarding cancer progression and remission was not available and extending the active cancer period could result in dilution of the results by including VTE events that were not related to cancer. Consequently, 79 VTE events that occurred beyond the active cancer period were never counted in the analyses. Thus, the total number of VTE events in the analyses were 1283, of which 232 occurred in the active cancer period.

### 2.4 | Statistical analysis

Statistical analyses were carried out using STATA version 15.0 (Stata Corporation). Cancer was entered as a time-varying covariate and data were split in relation to cancer diagnosis date to distinguish between non-cancer and cancer-exposed periods. Individuals who developed cancer during follow-up contributed with exposure status as cancer-free until 6 months prior to their cancer diagnosis date, and from that point onward exposure status changed to active cancer. Two years after the cancer was diagnosed, the cancer patients who were still alive and had not experienced a VTE event were censored from the analyses. Thus, individuals who developed cancer contributed to both non-exposed and cancer-exposed person-years (PY) at risk during the study period.

Cox proportional hazard regression models were used to estimate the hazard ratios (HRs) with 95% confidence intervals (CIs) for incident VTE according to the individual SNPs or categories of risk

alleles (the 5-SNP score categories, ie, 0-1, 2-3, and  $\geq 4$  risk alleles) and by cancer status (cancer free and active cancer). In analyses stratified on cancer status, subjects with 0-1 risk alleles were used as reference category. All analyses were adjusted for age, sex, and BMI. The proportional hazard assumption was tested by the use of Schoenfeld residuals and was found not to be violated.

We also investigated the combined effect of cancer and risk allele categories on VTE risk using cancer-free subjects with 0-1 risk alleles as reference category. The presence of a biological interaction between cancer and presence of SNPs was calculated using the relative excess risk attributable to interaction (RERI), the attributable proportion due to interaction (AP), and the synergy index (SI) with corresponding 95% CIs.<sup>39,40</sup> Briefly, the RERI can be interpreted as part of the total effect on outcome (eg, VTE) that is due to interaction (eg, between cancer and prothrombotic SNPs), the AP as the proportion of the combined effect that is attributable to interaction between the two exposures. A RERI and an AP  $> 0$ , and a synergy index  $> 1.0$  suggest a positive interaction, ie, the combined effect of two exposures is larger than the sum of the two separate effects.<sup>39</sup>

## 3 | RESULTS

There were 1496 subjects diagnosed with cancer during a median follow-up of 12.3 years. The baseline characteristics of cancer-free subjects and cancer patients with and without VTE are presented in Table 1. In cancer-free subjects, participants who experienced a VTE were older, had more cardiovascular disease, and higher BMI than those without VTE. In cancer patients with VTE, there was a higher proportion of women and smokers and they were of younger age than cancer patients without VTE.

The proportions of non-cancer and cancer patients across increasing number of risk alleles in the 5-SNP score are shown in Figure 2. The total number of risk alleles ranged from zero to seven in cancer-free subjects and from zero to six in cancer patients, with a median of two in both groups.

The risk estimates for VTE according to prothrombotic SNPs in subjects with and without cancer are presented in Table 2. In subjects without cancer, all five SNPs were associated with an increased risk of VTE by the presence of one or more risk allele. The highest VTE risks were found for FVL (rs6025, HR 2.50, 95% CI 2.13-2.95), prothrombin (rs1799963, HR 1.55, 95% CI 1.02-2.36), and ABO (rs8176719, HR 1.47, 95% CI 1.29-1.86). The greatest risk of a cancer-related VTE was seen in subjects with FVL (rs6025, HR 1.89, 95% CI 1.29-2.77), prothrombin (rs1799963, HR 1.39, 95% CI 0.44-4.37), and FGG (rs2066865, HR 1.34, 95% CI 1.03-1.74). Measures quantifying interaction on an additive scale (ie RERI, AP, and synergy index) suggested a positive interaction between cancer and the presence of three of the five prothrombotic SNPs; ABO (rs8176719), FVL (rs6025), and FGG (rs2066865; Table 3).

The risk of VTE increased across categories of increasing risk alleles in the 5-SNP score (0-1, 2-3,  $\geq 4$ ) in subjects with and without

**TABLE 1** Baseline characteristics of the study population with and without cancer and VTE

	No cancer		Cancer	
	Subcohort	VTE	Subcohort	VTE
Participants, n <sup>a</sup>	12 637	1051	1264	232
Age, y	50.2 ± 16.3	60.3 ± 15.1	62.0 ± 12.2	60.6 ± 13.4
Male sex	48.3 (5977)	47.8 (502)	53.2 (673)	44.4 (103)
BMI, kg/m <sup>2</sup>	26.2 ± 4.1	27.7 ± 4.5	26.8 ± 4.3	26.9 ± 4.2
Cardiovascular disease	7.7 (972)	14.1 (148)	12.3 (156)	12.1 (28)
Smoking	29.8 (3770)	25.0 (263)	32.2 (407)	34.9 (81)
rs8176719 (ABO) <sup>b</sup>	61.4 (7767)	69.9 (735)	60.6 (766)	68.1 (158)
rs6025 (F5) <sup>b</sup>	6.9 (873)	16.3 (171)	7.5 (95)	13.4 (31)
rs1799963 (F2) <sup>b</sup>	1.3(169)	2.1 (22)	1.3 (17)	1.29 (3)
rs2066865 (FGG) <sup>b</sup>	42.3 (5346)	45.9 (482)	40.8 (516)	47.8 (111)
rs2036914 (F11) <sup>b</sup>	78.2 (9879)	82.5 (867)	78.6 (994)	81.0 (188)

Note: Values are in % (n) or mean ± standard deviation.

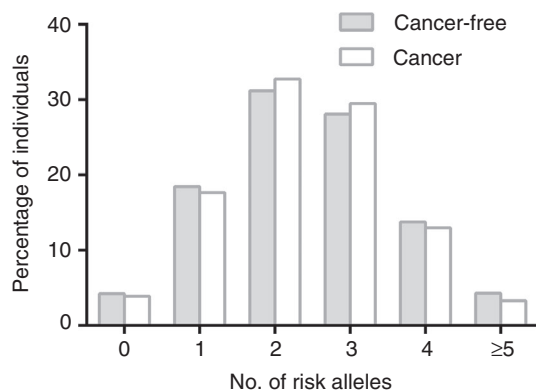
Abbreviations: BMI, body mass index; VTE, venous thromboembolism.

<sup>a</sup>Participants with cancer contributed to observation periods both in the no cancer and cancer group.

<sup>b</sup>Percentage of participants with ≥1 risk allele.

cancer. In those without cancer, the HR for ≥4 versus 0-1 risk alleles was 2.17 (95% CI 1.79-2.62). In cancer patients, the corresponding HR was 1.93 (95% CI 1.28-2.91). Similar results were found when analyzing DVT and PE separately (Table S1 in supporting information).

In subjects with 0-1 risk alleles, the risk of VTE was nine-fold higher in cancer patients than in those without cancer. Accordingly, there was a synergistic effect between the number of risk alleles and cancer on the relative risk of VTE. Subjects with cancer and ≥4 risk alleles had a 17-fold (HR 17.1, 95% CI 12.5-23.4) higher risk of VTE than cancer-free subjects with ≤1 risk allele. This combined effect was higher than expected on the basis of the individual effects of cancer and ≥4 risk alleles (RERI 6.72 95% CI 1.17-12.26). The AP revealed that 39% of the total VTE events in participants with cancer and ≥4 risk alleles were attributable to the interaction between the two exposures (ie, cancer and ≥4 risk alleles).

**FIGURE 2** Distribution (%) of individuals across number (#) of risk alleles in study participants with and without cancer

## 4 | DISCUSSION

In this case-cohort study, we investigated the risk of cancer-related VTE by the presence of prothrombotic genotypes, both as individual SNPs and as categories of the 5-SNP score (0-1, 2-3, and ≥4 risk alleles).<sup>20</sup> Moreover, we investigated whether the combination of cancer and prothrombotic genotypes had a biological interaction on VTE risk. For each prothrombotic genotype, the VTE risk increased in both cancer-free subjects and in cancer patients, and particularly for FVL, FGG, and ABO there was a more than additive effect in combination with cancer. When the 5-SNP score was applied, we found a dose-response relationship between the number of risk alleles and VTE risk in subjects with and without cancer. Likewise, the combined effect of cancer and the high-risk category of the genetic score (≥4 risk alleles) yielded a more than additive effect on VTE risk, with an HR of 17 compared with those without cancer in the low-risk category (0-1 risk alleles). The AP revealed that 39% of the VTE events occurring among cancer patients with ≥4 risk alleles could be attributed to the interaction between the risk factors.

Our findings on the role of individual prothrombotic genotypes and risk of cancer-related VTE are in line with the previously published studies. Factor V Leiden,<sup>23,24</sup> ABO rs505922 and rs8176746,<sup>25</sup> ABO rs8176719,<sup>26</sup> prothrombin G20210A,<sup>27-30</sup> and FGG rs2066865<sup>41</sup> have all been found to increase the risk of VTE in cancer patients. Moreover, SNPs in the F5 gene (FVL and rs4525) and FGG<sup>41</sup> have been shown to exert a more than additive effect on VTE risk when combined with cancer. Accordingly, we found a positive RERI for the SNPs in ABO, FVL, and FGG, which indicated a biological interaction.

Even though the 5-SNP score has been shown to predict VTE risk in the general population,<sup>20</sup> and in subjects with ischemic stroke,<sup>42</sup> the combined effect of the 5-SNP score and cancer has not been

		n	Events	HR (95% CI) <sup>a</sup>	HR (95% CI) <sup>a</sup>
Cancer	rs8176719 (ABO) <sup>b</sup>				
-	-	5210	316	Ref.	Ref.
-	+	8502	735	1.47 (1.29-1.68)	1.48 (1.29-1.69)
+	-	562	74	Ref.	8.68 (6.73-11.20)
+	+	934	158	1.31 (0.99-1.73)	11.07 (9.12-13.44)
Cancer	rs6025 (F5) <sup>b</sup>				
-	-	12 644	880	Ref.	Ref.
-	+	1044	171	2.50 (2.13-2.95)	2.50 (2.12-2.95)
+	-	1370	201	Ref.	8.16 (6.98-9.54)
+	+	126	31	1.89 (1.29-2.77)	15.95 (11.12-22.87)
Cancer	rs1799963 (F2) <sup>b</sup>				
-	-	13 497	1029	Ref.	Ref.
-	+	191	22	1.55 (1.02-2.36)	1.55 (1.02-2.37)
+	-	1476	229	Ref.	7.93 (6.85-9.18)
+	+	20	3	1.39 (0.44-4.37)	9.05 (2.91-28.17)
Cancer	rs2066865 (FGG) <sup>b</sup>				
-	-	7860	569	Ref.	Ref.
-	+	5828	482	1.14 (1.01-1.28)	1.14 (1.01-1.28)
+	-	869	121	Ref.	7.36 (6.03-8.98)
+	+	627	111	1.34 (1.03-1.74)	9.78 (7.96-12.01)
Cancer	rs2036914 (F11) <sup>b</sup>				
-	-	2942	184	Ref.	Ref.
-	+	10 746	867	1.27 (1.08-1.49)	1.27 (1.08-1.49)
+	-	314	44	Ref.	9.21 (6.62-12.81)
+	+	1182	188	1.11 (0.79-1.54)	9.63 (7.84-11.83)
Cancer	5-SNP score <sup>c</sup>				
-	0-1	3106	170	Ref.	Ref.
-	2-3	8111	589	1.33 (1.12-1.57)	1.33 (1.12-1.57)
-	≥4	2471	292	2.17 (1.79-2.62)	2.15 (1.78-2.60)
+	0-1	322	41	Ref.	9.16 (6.51-12.90)
+	2-3	931	139	1.18 (0.83-1.68)	10.3 (8.21-12.9)
+	≥4	243	52	1.93 (1.28-2.91)	17.1 (12.5-23.4)

<sup>a</sup>Adjusted for age, sex, and body mass index.

<sup>b</sup>Positive indicating subjects with one or two risk alleles.

<sup>c</sup>Number of risk alleles.

studied previously. In cancer patients, those with  $\geq 4$  risk alleles had an almost two-fold higher VTE-risk than those with 0-1 risk alleles, and the combination of cancer and  $\geq 4$  risk alleles yielded a 17-fold increased risk. As 39% of the VTEs occurring among those with cancer and the high-risk category of the genetic risk score could be attributed to the biological interaction, our findings suggest that genetics could be a useful tool for identifying cancer patients at high risk of VTE. However, the predictive performance of the 5-SNP score remains to be determined.

Risk factors for VTE in cancer can be broadly categorized into patient-related, cancer-related, and treatment-related factors. Prothrombotic genotypes are examples of patient-related factors

that determine the intrinsic thrombosis potential of a patient and are not influenced by the disease or its progression. The finding that cancer and prothrombotic genotypes have a more than additive effect on VTE risk is especially interesting, as it could indicate that prothrombotic SNPs act through pathophysiological pathways that further increases the procoagulant state of malignancy. The five SNPs are all related to functions of the coagulation system and enhance its performance. Malignant tumors release cell-free DNA, procoagulant factors such as tissue factor, and growth factors that promote the release of neutrophil extracellular traps (NETs) from neutrophils.<sup>43</sup> These are main triggers of the intrinsic and extrinsic pathways of the coagulation system that in combination with

**TABLE 2** Hazard ratios (HR) with 95% confidence intervals (CI) for venous thromboembolism (VTE) by categories of prothrombotic genotypes and cancer

**TABLE 3** Measures of interaction on an additive scale between cancer and the individual single-nucleotide polymorphisms (SNPs) or  $\geq 4$  risk alleles in the 5-SNP score

	RERI (95% CI)	AP (95% CI)	Synergy index (95% CI)
Individual SNPs (genes)			
rs8176719 (ABO)	1.91 (-0.72-4.54)	0.17 (-0.05-0.39)	1.23 (0.92-1.66)
rs6025 (F5)	6.29 (0.52-12.06)	0.39 (0.17-0.62)	1.73 (1.16-2.58)
rs1799963 (F2)	0.57 (-9.76-10.89)	0.06 (-1.01-1.13)	1.08 (0.30-3.89)
rs2066865 (FGG)	2.28 (0.03-4.53)	0.23 (0.04-0.43)	1.35 (1.01-1.81)
rs2036914 (F11)	0.16 (-2.90-3.21)	0.02 (-0.30-0.33)	1.02 (0.71-1.46)
5-SNP score ( $\geq 4$ vs $\leq 1$ )	6.72 (1.17-12.26)	0.39 (0.16-0.63)	1.71 (1.13-2.61)

Abbreviations: ABO, non-O blood type; AP, proportion attributable to interaction; CI, confidence interval; FGG, fibrinogen gamma gene; RERI, relative excess risk attributable to interaction; SNP, single nucleotide polymorphism.

prothrombotic genotypes will facilitate downstream coagulation activation with subsequent increased risk of thrombus formation. Moreover, acquired resistance to activated protein C is common in cancer patients, and may contribute to further increase the risk in patients with FVL.<sup>44</sup>

For decisions on use of thromboprophylaxis in cancer patients, the risk of VTE needs to be evaluated and weighted against the risk of bleeding. Several risk prediction models for VTE risk have been proposed, such as the Khorana score,<sup>45</sup> the Vienna CATS score,<sup>46</sup> the PROTECHT score,<sup>47</sup> and the CONKO score.<sup>48</sup> However, these risk scores focus mainly on clinical risk factors, and currently, they are not recommended in international guidelines due to unsatisfying performances in validation studies. The TiC-Onco score is the only score that includes genetics,<sup>49</sup> and in this score, eight prothrombotic SNPs were investigated together with clinical variables. The final TiC-Onco score included four SNPs (FVL rs6025, F5 rs4524, F13 rs5985, and SERPINA10 rs2232698). Of note, the TiC-Onco score performed better than the Khorana score in the derivation study,<sup>49</sup> but the performance of these two models has not been compared in a validation study. Nevertheless, the TiC-Onco results supports that prothrombotic genotypes may be promising candidates for risk prediction of VTE in cancer patients.

Major strengths of our study include the large number of genotyped subjects followed for a long period of time, the high attendance rate in the two cohorts, and the thorough outcome assessment. Further, confounding by ethnicity is limited as the study cohorts represent a general Caucasian population. Some limitations of our study need to be addressed. The number of cases was low in some subgroups, particularly for the rare genetic variants, which resulted in limited statistical power. The subgroup results must therefore be interpreted with caution. The effect of genetic variants may vary in different types of cancer, but unfortunately, we did not have power to stratify our analyses on cancer types. Unfortunately, information on cancer treatment modalities was not available, which could have provided additional insight to the relationship between genes and treatment-related risk factors for cancer.

In conclusion, cancer and a high number of prothrombotic genotypes displayed a supra-additive effect on the risk of VTE, indicating a biological interaction between the risk factors. Our findings suggest that the 5-SNP score may be useful for identifying cancer patients at increased risk of VTE.

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#### CONFLICTS OF INTEREST

There are no conflicts of interest reported by any of the authors.

#### AUTHOR CONTRIBUTIONS

Conception and design: JBH and SKB; data collection: JBH, SKB, KH, MEG, BB; data analysis and statistical support: HS, KH, SKB; draft of manuscript: HS, JBH, and SKB; revision of manuscript for intellectual content: BP, FRR, KH, KH, MEG, BB. All authors read and approved the final version of the manuscript.

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#### SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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