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Interactions of established risk factors and a GWAS-based genetic risk score on the risk of venous thromboembolism

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Abstract

Background—Multiple genetic and environmental risk factors contribute to venous thromboembolism (VTE) risk. Understanding how genes and environmental risk factors interact may provide key insight into the pathophysiology of VTE and may identify opportunities for targeted prevention and treatment.

Objectives—To examine the main effects and the potential effect-modification between single nucleotide polymorphisms (SNPs) at established loci and lifestyle risk factors for VTE.

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Contributors

MCB was the lead author while SL and CK were the senior authors of the paper. MCB, SL and CK proposed the hypothesis and planned the analysis. MCB analyzed the data and wrote the first draft of the manuscript. MCB, SL and CK contributed to all aspects of the study design, data collection, data analysis and interpretation, and writing of the article. CK was the expert on venous thromboembolism, and SL the genetics and gene-environment interaction expert. IDV, CAC, RV, FG, MKJ, PK, and SZG contributed to data collection, data interpretation, and writing of the article. All authors read and approved the final version of the manuscript.

Conflict of Interest

The authors declare that there are no conflicts of interest: no support from any organization for the submitted work other than those described above; no financial relationships with any organizations that might have an interest in the submitted work; no other relationships or activities that could appear to have influenced the submitted work.

Patients and Methods—We performed a nested case-control study using data on 1,040 incident VTE cases and 16,936 controls from the Nurses' Health Study, Nurses' Health Study II, and Health Professionals Follow-up Study cohorts, who gave blood, were selected as participants in a previous GWAS study, and completed a biennial questionnaire at time of blood draw. We selected SNPs that were associated with VTE risk in previous GWAS studies. A genetic risk score (GRS) was constructed to evaluate the combined effect of the 16 SNPs that have reached genome-wide significance in previous GWAS of VTE. Interactions between SNPs and VTE risk factors (BMI and smoking) were also assessed.

Results and Conclusions—We found a significant association between our GRS and VTE risk. The risk of VTE among individuals in the highest GRS tertile was 2.02 times that of individuals in the lowest GRS tertile (p-trend = 9.69×10^{-19}). The OR was 1.52 ($p=1.03\times10^{-8}$) for participants in the highest GRS tertile compared to those in the medium GRS tertile. However, while BMI and smoking were associated with VTE, and their effects were additive to each other we did not observe any significant multiplicative gene-environment interactions.

Keywords

body mass index; smoking; genetic susceptibility; gene-environment interactions; venous thromboembolism

INTRODUCTION

Venous thromboembolism (VTE) describes a spectrum of disease including deep vein thromboembolism (DVT) and pulmonary embolism (PE) and is a common cause of morbidity and mortality, with complex environmental and genetic determinants. The genetic basis of VTE is strong, with a heritability estimate of about 50% (1-3). VTE inheritance follows a multifactorial, or non-Mendelian, inheritance model, with multiple genetic factors contributing to risk (1, 4-7). The risk for VTE in individuals with an affected sibling is 2.5 times that of the general population (8, 9). Carriers of a familial thrombophilic genetic risk variant have a 0.8% risk of developing VTE per year (2, 3).

In addition to genetic risk factors, numerous environmental and lifestyle risk factors contribute to VTE risk (10-13). In our previous work, we identified several common, modifiable risk factors for VTE. Body mass index (BMI) is strongly associated with idiopathic PE, with a six-fold increase in risk in the most obese individuals (14, 15). Cigarette smoking is also associated with a threefold increased PE risk among women smoking ≥35 cigarettes per day (15, 16).

Previous research suggests that VTE risk is greatest when genetic predisposition is combined with an environmental risk factor (1). Understanding how genes and environmental risk factors interact may provide key insight into the pathophysiology of VTE and may identify opportunities for targeted prevention and treatment (17-19). However, few studies have explored the effect of gene-environment interactions on VTE risk. We therefore examined the potential effect-modification between SNPs at 14 previously established VTE loci and two established lifestyle risk factors for VTE (BMI and smoking) in 1,040 VTE

cases and 16,936 controls from the Nurses' Health Study, Nurses' Health Study II and Health Professionals Follow-up Study cohorts.

METHODS

Study Populations

The Nurses' Health Study (NHS) is a prospective cohort study of 121,700 female registered nurses in 11 US states aged 30-55 years at cohort inception in 1976 (http://www.channing.harvard.edu/nhs/). Since enrollment, participants have completed biennial questionnaires to update information on demographic characteristics, lifestyle factors, and newly diagnosed diseases (20, 21). Between 1989 and 1990, 32,826 NHS cohort participants provided blood samples. Details of the blood collection and archival methods have been described previously (22, 23). The Nurses' Health Study II (NHS-II) cohort was established in 1989 to recruit a population younger than the original NHS cohort. This prospective cohort includes 116,686 female registered nurses from 15 US states, aged 25–44 years at cohort inception, who complete follow-up using methodology similar to the NHS. Between 1996-1999, 29,612 NHS-II cohort participants provided blood samples(24). The Health Professionals Follow-up Study (HPFS) is a prospective cohort study of 51,529 US men aged 40–75 years at cohort inception who enrolled in 1986 (https://www.hsph.harvard.edu/hpfs). Again, biennial follow up is similar to the NHS (25). Between 1993 and 1999, 18,159 HPFS cohort participants provided blood samples (26). The demographic and risk factor characteristics of the participants from all three cohorts who provided blood samples are very similar to those of the cohorts overall (24, 27).

Multiple genome-wide association studies (GWAS) have been conducted within the NHS, NHS-II and HPFS blood sub-cohorts to investigate genetic susceptibility to 12 complex diseases. From these studies, GWAS data are available for 20,769 individuals (28). For the current analysis, we performed a nested case-control study of VTE using data on women and men from the NHS, NHS-II, and HPFS cohorts who gave blood, were selected as participants in a previous GWAS study, and completed a biennial questionnaire at the time of their blood draw. We only included incident VTE cases since cohort inception in the present analysis. The case-control by study are: NHS (570 VTE cases, 9,723 controls), NHS-II (52 VTE cases, 868 controls), and the HPFS (418 VTE cases, 6,345 controls).

The NHS and NHS-II study protocols were approved by the Human Research Committee of Partners Healthcare, and the HPFS study protocol was approved by the Institutional Review Board of the Harvard T.H. Chan School of Public Health, with informed consent from all participants.

VTE assessment

The present study includes VTE cases, including DVT and PE diagnosed between 1976-2012 in NHS; 1989-2011 in NHS-II; 1986-2012 in HPFS. All potential VTE cases are initially identified based on the nurse's or health professional's self-reported diagnosis. Participants who report a physician-diagnosed PE on a biennial questionnaire but do not have a prior history of malignancy receive a follow-up letter requesting medical records

from the facility in which they were diagnosed with PE. PE cases are then reviewed by a physician (CK, SZG), and subjects are considered to have PE confirmed if medical records include: a positive pulmonary angiogram, a high-probability ventilation/perfusion scan, or a positive contrast-enhanced computed tomography of the chest. PE diagnoses in subjects with a history of cancer and all cases of DVT are based on subject self-report. Questionnaire-reported diagnoses and exposures have been previously validated. PE diagnosis were validated through expert medical record review, with >95% accuracy (21, 29-31). DVT self-reported diagnosis was validated in a sub-study of 101 self-reported cases of DVT for which medical records were available. We found that 95 (94%) DVT cases were confirmed, 2 (2%) cases were probable, and only 4 (4%) cases were not confirmed.

Non-genetic (lifestyle) exposures

Body Mass Index (BMI) assessment—Height and weight have been collected in all three cohorts. Height was reported at baseline. Weight is updated every 2 years. Prior validation studies have demonstrated that self-reported height and weight are highly correlated (r=0.97) with weight measured by trained technicians (29). For the purposes of the present study, we used weight at the time of DNA collection. We calculated BMI according to the standard formula (BMI = weight in kilograms/height in meters, squared). We categorized participants according to three BMI categories: <25.0, 25.0-29.9, and ≥30.0 kg/m^2.

Smoking assessment—Smoking has been assessed on every biennial questionnaire for all three cohorts since inception and can be categorized according to number of cigarettes per day or pack-years of smoking. For the purposes of the present study, we divided participants into two smoking categories: never-smokers versus ever-smokers, based on whether they reported any number of pack-years of smoking in the cycle before DNA collection.

Genetic Exposures

SNP selection—Based on the Catalog of Published Genome-Wide Association Studies (32), we identified SNPs that have been associated with VTE risk on a genome-wide significance level (5×10^{-8})(33-38). Most of those correspond to coagulation factor genes. The 16 included SNPs (and their corresponding mapped genes) are as follows: rs6025 (*F5* – also known as F5 Leiden mutation), rs1018827 (*F5*), rs6427196 (*F5*), rs1799963 (*F2* – also known as G20210A mutation of the prothrombin gene), rs3756008 (*F11*), rs4253399 (*F11*), rs7659024 (*FGA – FGG*), rs6536024 (*FGG – LRAT*), rs6087685 (PROCR), rs2519093 (*ABO*), rs495828 (*ABO – SURF6*), rs505922 (*ABO* – risk allele associated with blood type O), rs687621 (*ABO*), rs16861990 (*NME7*), rs2288904 (*SLC44A2*), rs78707713 (*TSPAN15*).

We obtained genotype information from the GWAS data biorepository for the NHS, NHS-II, and HPFS cohorts(28). Since studies used different GWAS arrays, we created three new datasets, merged by platform family (Affymetrix, Illumina HumanHap, Illumina Omniexpress), and imputed these to a common reference panel: the 1,000 Genomes phase I release. We used the 1000 Genomes Project ALL Phase I Integrated Release Version 3

Haplotypes excluding monomorphic and singleton sites (2010-11 data freeze, 2012-03-14 haplotypes) as the reference panel. SNP and indel genotypes were imputed in three steps. First, genotypes on each chromosome were split into chunks to facilitate windowed imputation in parallel using ChunkChromosome (v.2011-08-05). Then each chunk of chromosome was phased using MACH (Howie, et al., 2012; Li, et al., 2010) (v.1.0.18.c). In the final step, Minimac (v.2012-08-15) was used to impute the phased genotypes to approximately 31 million markers in the 1000 Genomes Project. The average imputation quality score across SNPs ranged from 0.75 to 1.00. Details of the methodology behind the data merging and imputation and the imputation quality statistics have been published (28).

We first assessed the main effects of previously published GWAS-significant SNPs on VTE risk in our data. Then, to maximize power to detect any potential interactions, and to test if there was an effect of the overall genetic burden in VTE risk, we constructed a GRS. The 16 SNPs that reached genome-wide significance in the original studies (32-38) were included in the GRS. For our primary analysis of the GRS, we used SNP-specific weights based on the beta coefficients reported in the original study. We also assessed an unweighted GRS where each SNP was assigned the same weight. To reduce bias, only participants without any missing data were included in our GRS analysis.

Statistical analysis

We performed logistic regression to determine the associations between BMI, smoking, individual SNPs, and VTE risk. We calculated multivariable adjusted odds ratios (OR) and corresponding 95% confidence intervals (CI). We assessed interactions between SNPs and VTE risk factors (BMI and smoking) by including an interaction term for each SNP and VTE risk factor. We also created models to assess the association between the GRS and VTE risk. All analyses were adjusted for age, cohort (NHS, NHS-II, HPFS), and platform family (Affimetrix, Illumina HumanHap, Illumina Omniexpress). Our main analyses included all cases and controls, but as sensitivity analysis, we also performed sub-analyses based on GWAS platform family.

All reported P-values are two sided, and an α level of 0.05 was used to define statistical significance. All analyses were conducted using SAS version 9.2 (SAS Institute, Cary, NC) and R package (R Foundation, Vienna, Austria)(39).

RESULTS

We included a total of 1,040 incident VTE cases and 16,637 controls from the NHS, NHS-II and HPFS cohorts. Table 1 shows the main characteristics of the study population by cohort. Our cohorts represent large and well-characterized study populations, with detailed assessment of anthropometric, socio-demographic, and lifestyle characteristics. Of those, 1,023 cases and 16,502 controls had information on BMI available, and 1,016 and 16,637 controls had complete smoking information. The genetic data are of high quality, with imputation quality scores over 0.98 for all SNPs included in the present study.

Non-genetic (lifestyle) exposures and VTE risk

Table 2 shows the main effects of BMI and smoking on VTE risk. After adjusting for age, cohort, and platform family, we observed a strong positive association between BMI and risk of VTE (OR=1.55, 95%CI = 1.33-1.79) for overweight participants (BMI 25.0-29.9), and (OR=2.22, 95%CI=1.87-2.62) for obese participants (BMI ≥30.0). We also observed an increased risk of VTE in ever smokers compared to never smokers (OR=1.16, 95%CI=1.02-1.32) (Table 2). Compared to never smokers, the association between smoking and VTE risk was stronger in current smokers (OR=1.25, 95%CI=1.03-1.51) than in former smokers (OR=1.12, 95%CI=0.98-1.27).

Genetic Susceptibility to VTE

Table 3 shows the associations between the 16 evaluated SNPs and VTE risk. The seven SNPs included in the GRS were all associated with VTE in our data (P-value ranging from 0.024 to 5.72×10^{-12}). The minor alleles in the *NME7, ABO, F11, F5* and *FGA-FGG* genes were associated with an increased risk of VTE, while the minor alleles in the *SLC44A2* and *TSPAN15* genes were associated with a decreased risk of VTE, consistent with the effect directions seen in previous studies.

Table 4 presents the association between the GRS and VTE risk. Participants in the top tertile of the weighted GRS were 2.02 (95%CI=1.73-2.34) times more likely to have VTE compared to participants in the lowest (reference) tertile (Table 4). Compared to participant to the medium tertile of the GRS (reference), participants in the top tertile had a 52% increased risk of VTE, while participants in the lowest tertile had a 25% reduction in VTE risk (results not shown). In the weighted GRS, the OR for each standard deviation was 1.23 (95%CI=1.18-1.27; $p=1.91\times10^{-26}$). Each allele in the unweighted GRS increased the risk of VTE by a factor of 1.10 (95%CI=1.08-1.11; $p=5.29\times10^{-24}$). The association between the GRS and VTE risk remained robust, even when we excluded the two SNPs with the strongest individual effects (the SNPs located in *F5* and *ABO* genes) on VTE risk from the score (Table 4). The ORs for each standard deviation increase in GRS 1.24 (95%CI=1.18-1.29; $p=7.63\times10^{-22}$), 1.26 (95%CI=1.18-1.34; $p=6.46\times10^{-15}$), and 1.38 (95%CI=1.25-1.51; $p=5.35\times10^{-11}$) for the scores excluding SNPs in *F5*, *ABO*, or both, respectively. The distribution of the number of risk alleles in cases and controls is shown in Supplementary Figure 1.

Conducting genotyping platform-specific analysis and combining the results by meta-analysis did not qualitatively alter the results (p for heterogeneity = 0.64; p for association = 1.81×10^{-27}) (Supplementary Figure 2).

Gene-environment interactions and VTE risk

We did not observe any significant multiplicative interactions between BMI or smoking and the individual SNPs (Table 5). In order to maximize the power to detect any potential interactions, and to test if there was an effect of the overall genetic burden in VTE risk, we additionally assessed the joint effects of BMI and the weighted GRS (Table 6a), and smoking and the GRS (Table 6b) on VTE risk. The effects of genetic risk factors and BMI and smoking were additive. For obese participants with a high GRS (defined as the top

tertile), the risk of VTE was twice that of patients with either obesity or a high GRS alone (Table 6a). However, we found no significant multiplicative interaction between BMI and the GRS as related to VTE risk (p for interaction = 0.75). Using non-smokers with a low GRS as reference, we found no significant association for smokers with low GRS (OR=1.19, 95%CI=0.91-1.56), while the OR for never smokers and high GRS was 2.22 (95%CI=1.74-2.86), and the OR for ever smokers and high GRS was 2.41 (95%CI=1.90-3.08) (Table 6b). Again, we did not find significant multiplicative interaction between smoking and the GRS as related to VTE risk (p for interaction = 0.60). We additionally assessed the interaction between pack-years of smoking and the GRS, and we did not observe any significant multiplicative interactions as related to VTE risk (results not shown).

DISCUSSION

In this case control study of 1,040 VTE cases and 16,637 controls, we assessed interactions between genetic susceptibility, two environmental factors previously associated with VTE risk and incident VTE. (14-16). This study is novel in several ways. This study represents the first detailed exploration of interactions between BMI, smoking, and genetic risk factors for VTE. Our GRS is also the first to demonstrate an association with VTE using SNPs associated with VTE at genome-wide significant levels. We found that VTE risk increases with the number of risk alleles, and that the risk of VTE among individuals with a high GRS is 2.02 times that of individuals with a low GRS (p for trend = 9.69×10^{-19}). The number of risk alleles was a strong predictor of VTE risk even when we excluded the SNPs located in F5 and ABO, the SNPs with strongest individual effects. We have also confirmed the strong association between BMI with VTE risk and a weaker association between smoking and VTE risk. We did not find evidence of multiplicative interaction between genetic and environmental risk factors, though we did demonstrate additive effects of both on VTE risk. These results provide insight into the relative contribution of genetic and lifestyle risk factors for VTE and add to our understanding of both the epidemiology and pathophysiology of VTE.

Our results are consistent with the few published studies evaluating a GRS for VTE risk. Soria et al performed systematic review and meta-analysis to select variants that contribute to VTE risk and created a GRS called Thrombo inCode (TiC) (40). They concluded that TiC, which includes SNPs on *F5, F2, F13, SERPINC1, SERPINA10,* and *A1* blood group genes, improved VTE risk prediction compared to using variants in *F5* and prothrombin genes alone. De Haan et al created a GRS based on 31 SNPs associated with VTE, identified through candidate gene approaches (41). In this case-control study of 2712 patients and 4634 controls, a GRS including five SNPs was highly associated with VTE risk (OR 7.48 [95% CI= 4.49-12.46]) and performed similarly to a GRS including all 31 studied SNPs. As with this score, we found that including SNPs not associated with VTE in our data, but associated in previous GWAS, did not improve but slightly attenuated our results. In contrast to the de Haan study, where the SNP representing *F5*, rs6025, was the dominant driver of their GRS, the association between our GRS and VTE did not change materially when we removed *F5* and ABO genotype from the score. This suggests that newly identified genome-wide significant SNPs add to risk prediction of VTE in the general population. While de

Haan et al did not specifically assess gene-environment interactions, their risk models did improve when they combined genetic risk factors and non-genetic risk factors, which is consistent with our findings.

Limitations

We performed our analysis based on DNA from samples collected between 1989-1990 (in NHS and NHS-II) or 1993-1995 (in HPFS). It is therefore possible that VTE cases that resulted in deaths prior DNA collection could have been missed. However, mortality rate in our cohorts prior to blood and cheek collection is very low. Potential regression dilution bias should also be considered.

Large sample sizes are required to detect gene-environment interactions, especially when ORs for the main effects are small. This may have limited our power to identify gene-environment interactions, especially for smoking, which has a relatively weak association with VTE. In order to maximize power, we assessed gene-environment interactions not only with individual SNPs but also with a GRS. The analysis of individual SNPs emphasizes likely physiologic interactions, whereas the analysis of the GRS emphasized overall genetic burden and increased power. We focused only on SNPs that reached genome-wide significance in previous studies. Although previous history of cancer or surgical procedures are considered acute provoking factors for VTE, we did not consider including those variables in our models since we do not expect them to be confounders of the association between genetic polymorphisms and VTE risk.

We are aware that gene-environment interactions may specifically be observed in patients with PE or DVT. However, we did not conduct stratified analysis by disease subtype due to the limited sample size. Additionally, the NHS, NHS-II, and HPFS populations tend to be somewhat healthier than the population at large, and the proportion of smokers is smaller, which may have limited our power. All study subjects included in the present study are of European ancestry, so our results may not generalize to other racial or ethnic groups. However, the homogeneity among NHS, NHS-II, and HPFS participants strengthens the internal validity of these findings by maximizing the quality of reported data. While this analysis was focused on BMI and smoking, we acknowledge that other interactions, such as gene × age, and gene × gene might exist. These potential interactions should be explored in future analyses. Finally, how our results or those of any GRS for VTE will be applied to clinical care is yet to be determined.

Conclusion

Using data from three large and well-characterized cohorts, we performed a large population-based study of genetic risk factors for VTE and confirmed that SNPs associated with VTE at genome-wide significant levels can be combined into a GRS with a strong, linear, positive association with VTE. Moreover, we established that genetic risk factors add to the risk of VTE imparted by high BMI and smoking but do not appear to interact to increase VTE risk. Further study of gene-environment interactions are required to improve our understanding of VTE risk so that we can target prevention and testing to the most appropriate patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

BMI	body mass index
CI	confidence interval
DVT	deep vein thrombosis
GRS	genetic risk score
GWAS	genome-wide association studies
HPFS	Health Professionals Follow-up Study
NHS	Nurses' Health Study
OR	odds ratio
PE	pulmonary embolism
SNP	single nucleotide polymorphism
VTE	venous thromboembolism

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What is known about this topic?

- The genetic basis of VTE is strong, with a heritability estimate of about 50% and with multiple genetic factors contributing to risk.
- Numerous environmental and lifestyle risk factors contribute to VTE risk. Specifically, associations have been shown between BMI and cigarette smoking and VTE risk.
- VTE risk is greatest when genetic predisposition is combined with an environmental risk factor; however, few studies have explored the effect of gene-environment interactions on VTE risk.

What does this paper add?

- This study represents the first detailed exploration of interactions between BMI, smoking, and genetic risk factors for VTE. Our results provide insight into the relative contribution of genetic and lifestyle risk factors for VTE.
- Our GRS is also the first to demonstrate an association with VTE using SNPs associated with VTE at genome-wide significant levels.

Table 1

Main characteristics of the study population by cohort.

	All cohorts (N=17,976)	NHS (N=10,293)	NHS2 (N=920)	HPFS (N=6,763)
Age (mean, SD)	59.6 (9.0)	58.1 (6.8)	44.4 (4.6)	64.1 (9.3)
BMI (mean, SD)	26.1 (4.7)	26.1 (5.1)	26.2 (6.2)	26.2 (3.6)
Smoking (% ever)	52.8%	55.0%	31.6%	52.3%
Weighted GRS (mean, SD)	3.27 (1.53)	3.27 (1.53)	3.13 (1.47)	3.31 (1.53)

Table 2

Main effect of BMI and smoking on VTE risk.

	Controls	Cases	OR[1]	95% CI	P-value[2]
BMI (kg/m^2)					
<25.0	7853	350	1.00		$<2.2\times10^{-16}$
25.0-29.9	6000	417	1.55	1.33-1.79	
≥30.0	2649	256	2.22	1.87-2.62	
Smoking					
Never	7728	435	1.00		0.017
Ever	8909	581	1.16	1.02-1.32	

[1] adjusted by age, cohort and platform

[2] P-trend

Table 3

Main effect of GWAS significant SNPson VTE risk.

SNP (mapped gene)	Controls	Cases	OR[1]	95% IC	P-value[2]
rs6025 (F5)					
CC	16,051	940	1.00		6.89×10^{-9}
CT	880	96	1.90	1.51-2.37	
TT	5	4	13.41	3.30-51.0	
rs1018827 (F5)					
AA	14,648	840	1.00		6.53×10^{-6}
AG	2,196	192	1.53	1.29-1.79	
GG	92	8	1.62	0.72-3.15	
rs6427196 (F5)					
CC	14,609	838	1.00		9.21×10^{-6}
CG	2,232	194	1.51	1.28-1.78	
GG	95	8	1.57	0.70-3.05	
rs1799963 (F2)					
GG	16,609	1,013	1.00		0.12
GA	327	27	1.37	0.90-2.01	
AA	0	0	-	-	
rs3756008 (F11)					
TT	6,127	338	1.00		1.47×10^{-2}
TA	8,091	509	1.43	0.99-1.32	
AA	2,718	193	1.30	1.08-1.56	
rs4253399 (F11)					
GG	6,475	351	1.00		1.66×10^{-3}
GT	7,980	504	1.17	1.01-1.35	
TT	2,481	185	1.39	1.16-1.68	
rs7659024 (FGA-FGG)					
AA	9,990	586	1.00		3.03×10^{-2}
AG	5,981	374	1.06	0.92-1.21	
GG	965	80	1.40	1.09-1.78	
rs6536024 (FGG-LRAT)					
TT	3,690	213	1.00		0.23
TC	8,434	505	1.05	0.89-1.24	
CC	4,812	322	1.16	0.97-1.39	
rs6087685 (PROCR)					
GG	8,320	504	1.00		0.92
GC	7,041	436	1.02	0.89-1.16	

SNP (mapped gene)	Controls	Cases	OR[1]	95% IC	P-value[2]
CC	1,575	100	1.03	0.82-1.29	
rs2519093 (ABO)					
CC	11,010	571	1.00		5.72×10^{-12}
CT	5,263	393	1.43	1.25-1.64	
TT	663	76	2.24	1.73-2.87	
rs495828 (ABO)					
GG	10,375	541	1.00		1.72×10^{-10}
GT	5,721	409	1.37	1.20-1-57	
TT	840	90	2.09	1.64-2.63	
rs505922 (ABO)					
CC	7,467	349	1.00		5.17×10^{-11}
CT	7,451	525	1.50	1.31-1.73	
TT	2,018	166	1.78	1.46-2.15	
rs687621 (ABO)					
AA	7,389	350	1.00		5.45×10^{-10}
AG	7,487	521	1.46	1.27-1.68	
GG	2,060	169	1.74	1.43-2.10	
rs16861990 (NME7)					
CC	14,849	859	1.00		2.90×10^{-7}
CA	2,032	167	1.45	1.21-1.72	
AA	55	14	4.11	2.14-7.33	
rs2288904 (SLC44A2)					
AA	10,387	656	1.00		2.42×10^{-2}
AG	5,689	348	0.97	0.85-1.11	
GG	860	36	0.63	0.44-0.89	
rs78707713 (TSPAN15)					
TT	12,862	834	1.00		6.22×10^{-3}
TC	3,800	190	0.77	0.66-0.91	
CC	274	16	0.90	0.52-1.45	

[1] adjusted by age, cohort and platform

[2] P-trend

Table 4

Association between the Genetic Risk Score (GRS) and VTE risk.

GRS	OR[1]	95% CI	P-value[2]
Unweighted GRS			
Low (≤,9.92)	1.00		3.96×10^{-16}
Medium (9.92,12.9)	1.29	1.09-1.52	
High (>12.9)	1.90	1.63-2.22	
Weighted GRS (tertiles)[3]			
Low (≤2.35)	1.00		9.69×10^{-19}
Medium (2.35,3.82)	1.33	1.12-1.57	
High (>3.82)	2.02	1.73-2.34	
Weighted GRS (no F5)[3]			
Low (≤2.20)	1.00		3.11×10^{-16}
Medium (2.20,3.57)	1.46	1.23-1.72	
High (>3.57)	1.94	1.66-2.28	
Weighted GRS (no ABO)[3]			
Low (≤ 1.60)	1.00		1.39×10^{-9}
Medium (1.60,2.26)	1.17	0.99-1.37	
High (>2.26)	1.60	1.38-1.87	
Weighted GRS (no F5, no ABO)[3]			
Low (≤1.54)	1.00		3.83×10^{-8}
Medium (1.54,2.09)	1.12	0.96-1.32	
High (>2.09)	1.53	1.32-1.78	

[1] adjusted by age, cohort and platform

[2] P-trend

[3] tertiles based on controls only

Table 5

Interaction between GWAS significant SNPs and risk factors on VTE risk.

SNP (mapped gene)	OR[1]	95% IC	P-value[2]
rs6025 (F5)			
rs6025*BMI	1.03	0.99-1.08	0.15
rs6025*smoking	1.16	0.74-1.80	0.51
rs1018827 (F5)			
rs1018827*BMI	1.02	0.99-1.05	0.31
rs1018827*smoking	1.20	0.88-1.64	0.25
rs6427196 (F5)			
rs6427196*BMI	1.02	0.99-1.05	0.29
rs6427196*smoking	1.19	0.88-1.62	0.26
rs1799963 (F2)			
rs1799963*BMI	1.01	0.94-1.08	0.76
rs1799963*smoking	1.02	0.45-2.36	0.96
rs3756008 (F11)			
rs3756008*BMI	0.99	0.98-1.01	0.44
rs3756008*smoking	0.93	0.77-1.12	0.45
rs4253399 (F11)			
rs4253399*BMI	1.00	0.98-1.01	0.66
rs4253399*smoking	0.92	0.76-1.11	0.38
rs7659024 (FGA-FGG)			
rs7659024*BMI	1.01	0.99-1.03	0.37
rs7659024*smoking	0.99	0.81-1.22	0.95
rs6536024 (FGG-LRAT)			
rs6536024*BMI	1.00	0.98-1.01	0.62
rs6536024*smoking	1.07	0.89-1.30	0.47
rs6087685 (PROCR)			
rs6087685*BMI	1.01	0.98-1.03	0.47
rs6087685*smoking	0.93	0.71-1.22	0.59
rs2519093 (ABO)			
rs2519093*BMI	0.99	0.97-1.00	0.13
rs2519093*smoking	1.11	0.90-1.37	0.35
rs495828 (ABO)			
rs495828*BMI	0.99	0.97-1.00	0.11
rs495828*smoking	1.09	0.88-1.33	0.43

SNP (mapped gene)	OR[1]	95% IC	P-value[2]
rs505922 (ABO)			
rs505922*BMI	1.00	0.98-1.01	0.56
rs505922*smoking	1.12	0.93-1.35	0.22
rs687621 (ABO)			
rs687621*BMI	0.99	0.98-1.01	0.50
rs687621*smoking	1.13	0.94-1.36	0.18
rs16861990 (NME7)			
rs16861990*BMI	1.01	0.98-1.04	0.50
rs16861990*smoking	1.21	0.88-1.66	0.24
rs2288904 (SLC44A2)			
rs2288904*BMI	1.01	0.99-1.03	0.57
rs2288904*smoking	0.86	0.68-1.08	0.20
rs78707713 (TSPAN15)			
rs78707713*BMI	1.01	0.98-1.04	0.47
rs78707713*smoking	0.94	0.70-1.27	0.70

[1] OR for the interaction term; adjusted by age, cohort and platform

[2] P-interaction

Table 6a

Joint effect of BMI and GRS on VTE risk.

	Normal (BMI < 25.0)	Overweight (BMI 25.0 - 29.9)	Obese (BMI ≥ 30.0)
Low GRS	2,539 Controls 111 Cases OR = 1.00 (REF)	2,028 Controls 126 Cases OR = 1.50 95% CI: 1.11-2.03	856 Controls 89 Cases OR = 2.39 95% CI: 1.71-3.32
Medium GRS	2,580 Controls 156 Cases OR = 1.37 95% CI: 1.03-1.84	1,977 Controls 134 Cases OR = 1.89 95% CI: 1.42-2.53	882 Controls 95 Cases OR = 3.28 95% CI: 2.42-4.46
High GRS	2,602 Controls 215 Cases OR = 2.03 95%CI: 1.56-2.67	1,958 Controls 194 Cases OR = 3.26 95%CI: 2.52-4.26	846 Controls 137 Cases OR = 4.43 95%CI: 3.32-5.94

P-interaction = 0.75

Table 6b

Joint effect of smoking and GRS on VTE risk.

	Never Smokers	Ever Smokers
Low GRS	2,549 Controls 134 Cases OR = 1.00 (REF)	2,915 Controls 189 Cases OR = 1.19 95% CI: 0.91-1.56
Medium GRS	2,488 Controls 152 Cases OR = 1.31 95% CI: 0.99-1.72	2,986 Controls 237 Cases OR = 1.65 95% CI: 1.28-2.13
High GRS	2,591 Controls 249 Cases OR = 2.22 95%CI: 1.74-2.86	2,864 Controls 306 Cases OR = 2.41 95%CI: 1.90-3.08

P-interaction = 0.60

ORs adjusted by age, cohort and platform

Weighted GRS, tertiles based on controls only