SUPPLEMENTARY APPENDIX

Joint effects of cancer and variants in the *factor 5* gene on the risk of venousthromboembolism

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Supplementary Appendix for the manuscript:

Joint effects of cancer and variants in the Factor 5 gene on the risk of venous thromboembolism

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Methods

Study Population

VTE validation

Identification of incident VTE events was attained by searching the hospital discharge diagnosis registry, the radiological procedure registry and the autopsy registry at the University Hospital of North Norway, as previously described by Brækkan et al.[1] The University Hospital of North Norway is the sole hospital providing diagnostic verification and management of VTE in the Tromsø municipality. The medical records of each potential VTE case were reviewed by trained personnel and a VTE event was confirmed when all of the following diagnostic criteria were met: (1) objective confirmation by diagnostic procedures (spiral computed tomography, compression ultrasonography, venography, ventilation-perfusion scanning, pulmonary angiography or autopsy); (2) recorded diagnosis of DVT or PE in the patient's medical notes by a physician; (3) signs and symptoms consistent with a VTE diagnosis; and (4) appropriate treatment initiated (anticoagulants, thrombolytics or vascular surgery).

A VTE event was classified as either a DVT or PE. When these events occurred concurrently, the event was classified as a PE. VTE cases from the autopsy registry were recorded when the death certificate stated VTE as the cause of death or a condition contributing to the cause of death.

Baseline Measures

Physical examination, blood samples and self-administered questionnaires were performed to obtain baseline information. Blood samples were collected from an antecubital vein and analyzed at the Department of Clinical Chemistry at the University Hospital of North Norway. DNA was isolated

from whole blood and stored at -70°C at the national CONOR biobank, located at the HUNT Biobank in Levanger, Norway.

Body weight and height were measured in subjects wearing light clothing and no shoes. Body mass index (BMI) was calculated by the weight in kilograms (kg) divided by height in meters (m) squared (kg/m²). Information regarding history of cardiovascular disease (myocardial infarction, angina or stroke), diabetes mellitus, smoking status (never/former/current and duration in years), physical activity and level of education was obtained by using self-reported questionnaires. The level of education was categorized into basic schooling (7-10 years), upper secondary education (high school/vocational school), and tertiary education (college/university). Detailed information regarding the baseline variables is described elsewhere.[2]

Sequenom Genotyping and Quality Control

We genotyped two SNPs in the F5 gene (rs6025, rs4524), that have been previously implicated as candidate markers for VTE,[3] using the Sequenom platform, which uses single-base extension followed by mass spectrometry to measure the molecular mass of the extended primers. Samples were genotyped using the Sequenom iPlex Gold Assay according to the recommended protocol, using an initial input of 10-20 ng DNA, and were analyzed using the MassARRAY Analyzer 4. Only genotypes with a high quality score of "A. Conservative" or "B. Moderate" were used. When multiple attempts were made to genotype an individual, one of the highest quality genotypes across all attempts was chosen for each SNP.

Cancer Assessment

Information regarding cancer exposure was obtained by linking the Tromsø Study with The Cancer Registry of Norway (CRN). The CRN performs surveillance of cancer diagnoses in the Norwegian population. The CRN provides information regarding the cancer diagnosis date, primary site of the malignancy (International Classification of Disease, Revision 7 (ICD-7) codes), tumor morphology and initial treatment. In a recent evaluation of the data quality, the completeness of the CRN was estimated at 98.8% with 94% of the cases being histologically verified.[4] Subjects with non-melanoma skin cancers (ICD 191.0–191.9) were classified as cancer-free.

Statistical Analysis

Synergism refers to the interaction of two of more elements that when combined produce a total effect greater than the sum of the individual components. Interaction between F5 variants and active cancer on VTE risk were assessed by calculating the additive (expressed by the relative excess risk caused by interaction or RERI) interaction. RERI was calculated as $HR_{11} - HR_{10} - HR_{01} + 1$, where HR_{11} is the hazard ratio for both risk factors present, HR_{10} for the first risk factor present (i.e. FVL or rs4525)

and HR_{01} the hazard ratio for the second risk factor (active cancer). RERI values <0 signify a negative interaction, values equaling 0 indicate exact additivity, and values >0 indicate a synergistic interaction. Confidence intervals for RERI were estimated based on 10 000 bootstrap samples. The sampling process produced some obvious outliers as a result of very few cases in some categories. These outliers were removed prior to finding bias-corrected confidence intervals.[5] The attributable proportion due to interaction (AP) was calculated as AP=RERI/HR_{11}. The AP is interpreted as the proportion of cases in the combined group that is due to interaction between the two exposures. An AP value <0 indicates negative interaction or less than additivity, and an AP value >0 indicates a positive additive interaction. We investigated the possible interaction between the F5 variants and active cancer on a multiplicative scale by fitting the statistical interaction terms into our Cox regression model adjusted for age and sex and was considered statistically significant if the two-sided p value was <0.05.

Supplementary Table 1.

Cancer Site	**Number (%) TOTAL 461**
Colorectal	77 (16.7)
Upper GI	31 (6.7)
Pancreatic	15 (3.3)
Lung	82 (17.8)
Gynecological	29 (6.3)
Breast	41 (8.9)
Prostate	57 (12.4)
Hematological/Lymphoma	32 (6.9)
Central nervous system	14 (3.0)
Remaining sites*	39 (8.5)
Missing cancer site	44 (9.5)

*ear, noses and throat, melanomas, endocrine, sarcomas, unknown site

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