Interaction of Hereditary Thrombophilia and Traditional Cardiovascular Risk Factors on the Risk of Arterial Thromboembolism

Pooled Analysis of Four Family Cohort Studies

Bakhtawar K. Mahmoodi, MD, PhD, MPH; Nic J.G.M. Veeger, MSc, PhD; Saskia Middeldorp, MD, PhD; Willem M. Lijfering, MD, PhD; Jan-Leendert P. Brouwer, MD, PhD; Jur ten Berg, MD, PhD; Karly Hamulyák, MD, PhD; Karina Meijer, MD, PhD

Background—Hereditary thrombophilia is associated with a slightly increased risk of arterial thromboembolism (ATE). Whether hereditary thrombophilia interacts with traditional cardiovascular risk factors on the risk of ATE has yet to be established.

Methods and Results—A total of 1891 individuals belonging to 4 family cohorts from the Netherlands were included in the analyses. Five hereditary thrombophilic defects, including factor V Leiden, prothrombin G20210A defect, and deficiencies of the natural anticoagulants (ie, antithrombin, protein C, and protein S), were assessed, and data on risk factors and previous ATE were collected. Thrombophilia was associated with elevated risk of ATE (hazard ratio =1.74, 95% confidence interval, 1.18–2.58; \( P =0.005 \)). Overall, the association of thrombophilia with ATE tended to be stronger in the presence of traditional cardiovascular risk factors, especially the synergistic effect of thrombophilia with diabetes mellitus was striking (hazard ratio of thrombophilia–ATE association was 1.41 in nondiabetics versus 8.11 in diabetics). Moreover, the association of thrombophilia with ATE tended to be stronger in females and before the age of 55 years as compared with males and individuals >55 years of age, respectively.

Conclusions—Thrombophilia is associated with ATE. This association may be stronger in the presence of traditional cardiovascular risk factors in particular in individuals with diabetes mellitus. Future studies on thrombophilia–ATE risk should focus on high-risk populations with high prevalence of traditional cardiovascular risk factors. (Circ Cardiovasc Genet. 2016;9:79-85. DOI: 10.1161/CIRCGENETICS.115.001211.)

Key Words: arterial thrombosis ◼ cardiovascular disease ◼ epidemiology ◼ factor V Leiden ◼ hereditary thrombophilia ◼ protein C deficiency ◼ protein S deficiency ◼ prothrombin G20210A

Factor V Leiden, prothrombin G20210A polymorphism, and hereditary deficiencies of the anticoagulant proteins (ie, antithrombin, protein C, and protein S) are well-recognized risk factors for venous thromboembolism (VTE).1-3 In contrast, the association of these hereditary thrombophilic defects with arterial thromboembolism (ATE) is relatively less well-established and is believed to be weak.4-5 Most previous studies on the association of thrombophilic defects with ATE are case–control studies often conducted in young individuals with low risk profile.

Clinical Perspective on P 85

Endothelial damage and subsequent atherosclerosis are considered the main etiologic factors for ATE, whereas coagulation disturbance and stasis of the blood are major risk factors for VTE.4 This is also consistent with the much higher relative risk of VTE as compared with ATE in individuals with hereditary thrombophilia.2-5 Although the process of atherosclerosis is driven by traditional cardiovascular risk factors, such as hypertension, diabetes mellitus, smoking, hyperlipidemia, and obesity, the occurrence of ATE (ie, transient ischemic attack, ischemic stroke, or myocardial infarction) is typically caused by rupture of atherosclerotic plugs with subsequent thrombus formation.6 Therefore, synergistic interaction of hereditary
thrombophilia with traditional cardiovascular risk on ATE risk seems likely. Moreover, cerebral thromboembolic events because of cardiac embolization, such as in patients with atrial fibrillation, may also be promoted by thrombophilia given the stasis of blood in the left atrium during atrial fibrillation. However, previous studies on interaction of traditional cardiovascular risk factors with factor V Leiden or prothrombin G20210A polymorphism are limited and report conflicting results.\textsuperscript{2-11} The conflicting results may be at least partially because of selection bias and residual confounding, which is generally better tackled in family cohort studies as compared with case–control and cohort studies of nonrelated individuals.\textsuperscript{12} Moreover, lack of power in individual studies may have contributed to risk differences across studies.

To assess potential interaction of the hereditary thrombophilic defects (ie, factor V Leiden, prothrombin G20210A polymorphism, and deficiencies of antithrombin, proteins C, and protein S) with traditional cardiovascular risk factors on the risk of ATE, we pooled 4 retrospective family cohort studies with similar ascertainment protocols.

Methods

Participants
Details of the study protocols have been published elsewhere.\textsuperscript{2,5,13-15} In brief, individual-level data from 4 large retrospective family cohort studies with various thrombophilic index defects were pooled. The major difference between these cohorts was the index defect of the proband. Additionally, in the cohort with the index defect of the anticoagulant proteins deficiency, the individual with the index deficiency (probond) had to have VTE in the presence of antithrombin, protein C, or protein S deficiency.\textsuperscript{3} In the other 3 cohorts, probands had to have either VTE or premature ATE (any ATE before the age of 50 years) in the presence of prothrombin G20210A polymorphism, elevated factor VIII (>150 IU/L), or hyperhomocysteinemia.\textsuperscript{2,13-15}

To avoid selection bias, probands of the later 3 cohorts were dropped from the analysis because these were also selected on the basis of ATE. Moreover, the cohort with the index defect of the anticoagulant protein deficiency was a single-center study conducted at the University Medical Center (UMC) Groningen, whereas the other 3 studies were conducted at UMC Maastricht, Amsterdam Medical Center, and UMC Groningen.\textsuperscript{2} All studies were approved by the institutional review boards of the participating hospitals.

First-degree relatives of the probands, >15 years of age were identified by pedigree analysis. As previously reported,\textsuperscript{4} for antithrombin deficiency also second-degree relatives were included. For living relatives >15 years of age, the response rate ranged between 68% and 92% per cohort. Individuals were enrolled after informed consent was obtained. Detailed information on previous episodes of VTE and ATE, risk factors for atherosclerosis, and anticoagulant treatment were collected, using a standardized questionnaire and reviewing medical records. Blood samples were taken after clinical data had been collected. Probands and relatives were tested for other thrombophilic defects in addition to their index deficiencies, including factor V Leiden, prothrombin G20210A polymorphism, and deficiencies of antithrombin, protein C and protein S. Traditional cardiovascular risk factors included hypertension, defined by self-reported diagnosis of hypertension made by a physician or use of antihypertensive drugs; diabetes mellitus, defined by self-reported diagnosis of diabetes mellitus made by a physician or use of glucose lowering drugs; hyperlipidemia, defined by self-reported diagnosis of hyperlipidemia made by a physician or the use of lipid-lowering drugs; and cigarette smoking, including both previous and current smokers. History of these traditional cardiovascular risk factors was ascertained at the time of enrolment. The body mass index (BMI) was calculated as bodyweight in kilograms divided by height in meters squared.

Diagnosis of ATE
Myocardial infarction and strokes were self-reported during an interview with a medical professional and identified by screening of the medical charts. The outcomes were considered confirmed if reported in the medical charts of the diagnosing hospital, using the following criteria: myocardial infarction was confirmed by typical ECG features, elevated levels of cardiac enzymes, radionuclide imaging techniques, or coronary angiography. Ischemic stroke was documented by computed tomographic scanning or magnetic resonance imaging. Transient ischemic attack required typical neurological symptoms and signs lasting <24 hours.

Laboratory Studies
Thrombophilic defects were identified using the same assays in all 4 studies. Factor V Leiden and the prothrombin G20210A mutation were demonstrated by polymerase chain reactions. Protein S and protein C antigen levels were measured by Enzyme Linked Immuno Sorbent Assay (reagents obtained from DAKO, Glostrup, Denmark), activity of protein C (Berichrom Protein C; Dade Behring, Liederbach, Germany), and antithrombin (Coastest; Chromogenix, Mölndal, Sweden) by chromogenic substrate assays. Normal ranges (means±SD) were determined in healthy blood donors who had no (family) history of thrombembolism, were not pregnant, and had not used oral contraceptives for at least 3 months. Protein S deficiency type I was defined by lowered total protein S antigen levels (<68 IU/dL). Protein C deficiency type I and type II were defined by reduced levels of either protein C antigen (<63 IU/dL) and activity (<64 IU/dL), and antithrombin deficiency was defined by decreased levels of antithrombin activity (<74 IU/dL). Deficiencies were considered inherited if they were confirmed by measuring a second sample collected later and were found in at least one family member in addition to the proband, whereas acquired conditions were excluded. If there was a discrepancy between the results of the 2 tests, a third sample was tested. A deficiency of protein S was considered acquired through use of oral contraceptives or pregnancy, unless it was confirmed at least 3 months after withdrawal of oral contraceptives or delivery, respectively. Individuals who were on treatment with vitamin K antagonist, blood samples for protein C and S were taken after treatment had been interrupted for at least 2 weeks, while low molecular weight heparin was given subcutaneously. Factor VIII:C was measured by 1-stage clotting assay and was considered increased at levels >150 IU/dL. Levels of homocysteine were measured by high-performance liquid chromatography after overnight fasting. Hyperhomocysteinemia was defined as a fasting homocysteine level >18.0 μM corresponding with the 95th percentile of normal in the Dutch population.

Statistical Analysis
Because all probands in the cohort of the anticoagulant deficiencies were selected on the basis of VTE, and not ATE, they were included in the current analysis for ATE. In the remaining 3 cohorts, probands were excluded from the analysis to avoid selection bias because probands were also selected on the presence of premature ATE. Individuals who had at least one of the 5 thrombophilic defects (ie, antithrombin-, protein C-, or protein S deficiency, factor V Leiden of prothrombin G20210A polymorphism) were classified as having thrombophilia. Individuals who tested negative for all 5 thrombophilic defects were classified as not having thrombophilia. Individuals who tested negative for the thrombophilic defects of interest but had incomplete data on at least one of the 5 thrombophilic defects were dropped from the analysis. Elevated factor VIII and hyperhomocysteinemia were not considered as part of thrombophilia in the current analysis because these correlate with traditional cardiovascular risk factors and each other.\textsuperscript{13,16,17} Missing values of other variables were allowed, and if the variable served as an adjustment covariate in a model, its missing values were substituted by its mean in the overall sample to retain maximum numbers of events. Given the low prevalence of missing values in the data set, this simple adjustment for missing values should not influence the estimates, and its effect was evaluated in a complete-case analysis.
To adjust for differences in baseline risk across studies and clustering within families, study-stratified Cox regression models with adjustment for clustering within families were applied and robust variances were estimated. Moreover, given the lower prevalence of traditional cardiovascular risk factors in thrombophilic individuals as compared with nonthrombophilic individuals, in a sensitivity analysis, the estimates were also adjusted for potential selection bias using inverse probability weighting. Observation time was defined as the period from 15 years of age until the first episode of ATE or the end of the study. Because the observation period is a proxy of age, estimates were routinely adjusted for age; however, to account for differences in calendar time, year of birth was included in the model. Cox regression model with time-varying option was used to assess the age effect on the association of thrombophilia with ATE. Both estimates of main effects and interaction terms were derived from fully adjusted models. Fully adjusted model included thrombophilia, date of birth, sex, hypertension, hyperlipidemia, diabetes mellitus, smoking, and BMI. Influence of elevated factor VIII and hyperhomocysteinemia on estimates for thrombophilia was evaluated in a sensitivity analysis. Results were expressed as hazard ratios (HRs) with 95% confidence intervals (CIs) and P values.

In addition to multiplicative interaction, for potential public health purposes, the interaction of thrombophilia with the modifiable risk factors was also quantified on additive scale by calculating the attributable proportion (AP) due to interaction. To calculate AP due to interaction of thrombophilia with a traditional cardiovascular risk factor, relative excess risk due to interaction was divided by the HR in the doubly exposed group. APs were calculated using the nclom command in STATA after fully adjusted model that included the interaction term. Delta method was applied to assess the standard errors of the APs. AP measures the proportion of the combined effect that is caused by interaction in the doubly exposed group. AP value can go from -1 to 1; AP value >0 means positive additive interaction, AP value <0 means negative additive interaction, and AP value of 0 means no additive interaction. Differences between groups of categorical data were evaluated by Chi-square test and of continuous data by 1-way ANOVA. Statistical significance was considered as a 2-tailed probability <0.05. Statistical analyses were performed using STATA software, version 12.1 (StataCorp LP, College Station, TX).

**Results**

Characteristics of the study participants are summarized in Table. After exclusion of duplicate records (n=23) and individuals with incomplete data (n=109) on at least one of the 5 thrombophilic defects (ie, antithrombin, protein C, or protein S deficiency, factor V Leiden, or prothrombin G20210A polymorphism), a total of 1891 individuals belonging to 495 probands were included in the analyses. The sample sizes of the individual cohorts varied from 398 to 526 participants. In the first cohort in which probands were selected on the basis of antithrombin, protein C, or protein S deficiency, a total of 68% of participants had at least one of the 5 thrombophilic defects. In the second cohort in which probands were selected on the basis of prothrombin G20210A polymorphism, 60% had at least one of the 5 defects. Because in the third and fourth cohort the index defects (ie, elevated factor VIII and hyperhomocysteinemia) were not part of the 5 thrombophilic defects of interest, the prevalence of thrombophilia was only 19% and 17%, respectively. In addition to thrombophilia, also the prevalence of traditional cardiovascular risk factors and male sex varied somewhat across the studies (P<0.039). Mean age was similar (P=0.09) across studies. Overall, missing values for covariates were <1%, except for smoking (2.2% missing) and BMI (3.5% missing). In the pooled data set, the prevalence of traditional cardiovascular risk factors was somewhat higher in nonthrombophilic individuals as compared with individuals with thrombophilia (P≤0.03; Figure I in the Data Supplement).

As compared with individuals without thrombophilia, individuals with thrombophilia had a 74% (HR 1.74; 95% CI, 1.18–2.55; P=0.005) higher risk of ATE (Figure 1) after adjustment for age, sex, and traditional cardiovascular risk factors. This adjusted risk was higher in individuals with ≥2 thrombophilic defects (HR 1.91) as compared with individuals with only one thrombophilic defect (HR 1.46), and it was somewhat higher in individuals with the natural anticoagulants deficiency (HR 1.69) as compared with individuals with either factor V Leiden or prothrombin G20210A polymorphism (HR 1.50). Except hyperlipidemia, the adjusted HRs conferred by traditional cardiovascular risk factors were in magnitude comparable to the HR conferred by thrombophilia. The association of diabetes mellitus and BMI ≥30 with ATE did not reach statistical significance in the fully adjusted model.

The association of thrombophilia across age categories (age ≥55 years versus <55 years), sex, and traditional cardiovascular risk factors is shown in Figure 2. The association of thrombophilia with ATE is significantly stronger before the age of 55 years as compared with after the age of 55 years. The association of thrombophilia with ATE tended to be stronger in females as compared with males. Moreover, the association of thrombophilia with ATE tended to be somewhat stronger in the presence of modifiable traditional cardiovascular risk factors, in particular in the presence of diabetes mellitus and continuous BMI modeled with spline terms with knots

| Table. Baseline Characteristics |
|-------------------------------|
| Variables | ACS Deficiency | Elevated FVIII | PT G20210A | Hyperhomo |
| Participants, n | 525* | 526 | 398 | 442 |
| Thrombophilia | 359 (68%) | 101 (19%) | 237 (60%) | 77 (17%) |
| ACS deficiency | 308 (59%) | 10 (2%) | 6 (2%) | 10 (2%) |
| PT G20210A | 38 (8%) | 14 (3%) | 213 (54%) | 13 (3%) |
| Factor V Leiden | 81 (16%) | 80 (15%) | 52 (13%) | 54 (12%) |
| Male sex | 252 (48%) | 205 (39%) | 180 (45%) | 202 (46%) |
| Age, mean (SD) | 45.9 (17.4) | 45.7 (16.8) | 46.5 (17.5) | 46.5 (15.7) |
| Hypertension | 68 (13%) | 104 (20%) | 78 (20%) | 96 (22%) |
| Diabetes mellitus | 18 (3%) | 32 (6%) | 10 (3%) | 24 (5%) |
| Hyperlipidemia | 55 (10%) | 74 (14%) | 46 (12%) | 72 (16%) |
| Smoking | 210 (40%) | 281 (54%) | 212 (59%) | 280 (63%) |
| BMI, mean (SD) | 24.8 (4.2) | 26.1 (4.5) | 25.4 (4.3) | 26.2 (5.1) |
| Arterial thromboembolism | 42 (8%) | 24 (5%) | 20 (5%) | 33 (7%) |
| Venous thromboembolism | 174 (33%) | 26 (5%) | 28 (7%) | 15 (3%) |
| Long-term VKA use | 100 (19%) | 8 (2%) | 9 (2%) | 9 (2%) |

ACS deficiency denotes antithrombin, protein C, or protein S deficiency; BMI, body mass index; elevated FVIII, factor VIII levels above 150 IU; Hyperhomo, hyperhomocysteinemia; long-term VKA use, vitamin K antagonists use for >12 mo; and PT G20210A, prothrombin G20210A.

*Of 416 tested individuals, 2 were positive for lupus anticoagulant.
at BMI of 25 and 30 kg/m² (Figure II in the Data Supplement). Interaction of modifiable risk factors with thrombophilia was also quantified on additive scale in Figure 3. The results were in agreement with the multiplicative interaction presented in Figure 2 because there was some evidence for synergistic effect for all modifiable risk factors.

In sensitivity analyses, the association of thrombophilia with ATE did not alter if the fully adjusted model was additionally adjusted for elevated factor VIII and hyperhomocysteinemia (HR 1.73; 95% CI, 1.19–2.54). Similarly, the association of thrombophilia with ATE (HR 1.67, 95% CI, 1.12–2.49) did not change when missing values for an adjustment variable were not substituted by the variable’s mean, but cases with missing values were dropped from the analysis. Moreover, the association of thrombophilia with myocardial infarction (HR 1.70; 95% CI, 1.02–2.84) was similar to the association of thrombophilia with stroke or transient ischemic attack (HR 1.70; 95% CI, 0.99–2.99). Finally, the interaction between cardiovascular risk factors and the anticoagulant protein deficiencies was comparable to the interaction between cardiovascular risk factors and factor V Leiden and prothrombin G20210A polymorphism (Figure III in the Data Supplement), except that for factor V Leiden and prothrombin G20210A polymorphism, the interaction with hyperlipidemia was also significant. Adjustment for potential selection bias by using the inverse-probability weighting method had no significant effect on the interaction of thrombophilia with cardiovascular risk factors (Figure IV in the Data Supplement). Finally, while contrasting individual thrombophilic defects, a higher risk was observed for protein C and S deficiencies as compared with antithrombin deficiency (Figure V in the Data Supplement).

### Discussion

Hereditary thrombophilia (antithrombin-, protein C-, or protein S deficiency, factor V Leiden, or prothrombin G20210A polymorphism) is associated with elevated risk of ATE in this pooled analysis of 4 large family cohort studies. The association of thrombophilia with ATE was somewhat stronger in females and in the presence of traditional cardiovascular risk factors, in particular diabetes mellitus. Moreover, the association of thrombophilia with ATE was diminishing with older age with significantly higher risk before the age of 55 years as compared with after the age of 55 years. Results were similar for myocardial infarction versus stroke and transient ischemic attack. Also no major differences were observed when the interaction of traditional cardiovascular risk factors with the anticoagulant protein deficiencies was contrasted to factor V Leiden and prothrombin G20210A polymorphisms.

Although the association of factor V Leiden and prothrombin G20210A with ATE has been extensively evaluated, the association of the hereditary anticoagulant deficiencies with

| Variable       | Number of participants | Hazard ratio (95% CI) | P-value |
|----------------|------------------------|-----------------------|---------|
| Thrombophilia  | 774/1891               | 1.74 (1.18, 2.55)     | 0.005   |
| • 1 defect     | 599/1826               | 1.46 (0.94, 2.24)     | 0.09    |
| • ≥2 defects   | 110/1826               | 1.91 (0.84, 4.34)     | 0.12    |
| • ACS deficiency | 334/1877         | 1.69 (1.00, 2.85)     | 0.05    |
| • FII or FVL   | 497/1854               | 1.50 (0.97, 2.31)     | 0.07    |

**Tables:**

- **Table 1.** Adjusted association of thrombophilia and traditional cardiovascular risk factors with arterial thromboembolism. The hazard ratios and the corresponding confidence intervals and P values for overall thrombophilia, sex, hypertension, diabetes mellitus, hyperlipidemia, and smoking were derived from the main study-stratified Cox model that included the mentioned variables, BMI (continuous), year of birth (continuous variable), and adjustment for family clustering. Estimates for BMI ≥30 vs <30 kg/m² were obtained by substituting the continuous BMI variable in the main model by a dichotomized BMI variable. Estimates for 1 and ≥2 thrombophilic defects were obtained by substituting the overall thrombophilia variable in the main model by 2 dichotomous variables: one representing 1 thrombophilic defect and the other representing ≥2 thrombophilic defects. Similarly, estimates for anticoagulant proteins and presence of factor V Leiden or prothrombin G20210A were obtained by substituting the overall thrombophilia variable in the main model by 2 dichotomous variables: one for the presence of anticoagulant proteins and the other for the presence of either factor V Leiden or prothrombin G20210A. Homozygosity for factor V Leiden or prothrombin G20210A were considered as 2 defects. ACS deficiency denotes antithrombin, protein C, or S deficiency; BMI, body mass index; and FII or FVL, prothrombin G20210A or factor V Leiden.

**Figure 1.** Adjusted association of thrombophilia and traditional cardiovascular risk factors with arterial thromboembolism. The hazard ratios and the corresponding confidence intervals and P values for overall thrombophilia, sex, hypertension, diabetes mellitus, hyperlipidemia, and smoking were derived from the main study-stratified Cox model that included the mentioned variables, BMI (continuous), year of birth (continuous variable), and adjustment for family clustering. Estimates for BMI ≥30 vs <30 kg/m² were obtained by substituting the continuous BMI variable in the main model by a dichotomized BMI variable. Estimates for 1 and ≥2 thrombophilic defects were obtained by substituting the overall thrombophilia variable in the main model by 2 dichotomous variables: one representing 1 thrombophilic defect and the other representing ≥2 thrombophilic defects. Similarly, estimates for anticoagulant proteins and presence of factor V Leiden or prothrombin G20210A were obtained by substituting the overall thrombophilia variable in the main model by 2 dichotomized variables: one for the presence of anticoagulant proteins and the other for the presence of either factor V Leiden or prothrombin G20210A. Homozygosity for factor V Leiden or prothrombin G20210A were considered as 2 defects. ACS deficiency denotes antithrombin, protein C, or S deficiency; BMI, body mass index; and FII or FVL, prothrombin G20210A or factor V Leiden.
ATE is based only on a few cohort studies. Compared with factor V Leiden and prothrombin G20210A polymorphisms, the hereditary anticoagulant protein deficiencies generally predispose to higher prothrombotic state and are associated with higher risk of VTE. For ATE, similar comparison has not been evaluated in previous studies, and in the current study, the association of anticoagulant protein deficiencies with ATE was only slightly more pronounced as compared with traditional cardiovascular risk factors.7–10 Though results of prior studies with ATE tended to be stronger in the presence of traditional cardiovascular risk factors.7–10 Moreover, in our study, there was also a signal that the association of factor V Leiden and prothrombin with smoking, hyperlipidemia, and hypertension were stronger than in our study. Additional adjustment for oral contraceptives use (ever versus never), which was available in 3 of the 4 cohorts, had no influence on the reported risk association of thrombophilia with ATE (HR=1.73). Finally, to reduce the chance of phenocopies for the natural anticoagulant deficiencies, we changed the cut-off value of antithrombin, protein C, or protein S deficiency to ≤60 IU/dL, which had no influence on the thrombophilia–ATE risk association (HR=1.75).

Although previous data on potential interaction of the anticoagulant proteins deficiencies and traditional cardiovascular risk factors on ATE risk is lacking, the interaction of factor V Leiden and prothrombin G20210A polymorphism with cardiovascular risk factors has been investigated in a few previous studies.7–10 Overall, the association of these defects with ATE tended to be stronger in the presence of traditional cardiovascular risk factors.7–10 Though results of prior studies are somewhat inconsistent,11 the reported synergistic interaction of factor V Leiden and prothrombin with smoking, hyperlipidemia, and hypertension were stronger than in our study. Moreover, in our study, there was also a signal that the association of thrombophilia with ATE was stronger in females as compared with males. Previous studies on this issue are not informative because these were limited to a single sex7–10; however, similarly higher relative risk of coronary heart disease conferred by traditional cardiovascular factors has been reported in females as compared with males.20 Finally, the association of thrombophilia was stronger before the age of

| Variable | Strata | Number of ATE participants | Number of participants | Hazard ratio (95% CI) | P–value for interaction |
|----------|--------|-----------------------------|------------------------|----------------------|------------------------|
| Age      | <55    | 56                          | 1891                   | 2.74 (1.63, 4.60)    | 0.01                   |
|          | ≥55    | 63                          | 548                    | 1.09 (0.63, 1.88)    |                        |
| Sex      | Female | 51                          | 1052                   | 2.60 (1.42, 4.73)    | 0.07                   |
|          | Male   | 68                          | 839                    | 1.30 (0.81, 2.08)    |                        |
| Hypertension | No | 58                          | 1544                   | 1.53 (0.93, 2.49)    | 0.51                   |
|           | Yes    | 61                          | 346                    | 1.96 (1.10, 3.48)    |                        |
| Hyperlipidemia | No | 61                          | 1640                   | 1.60 (0.97, 2.65)    | 0.68                   |
|           | Yes    | 57                          | 247                    | 1.88 (1.06, 3.36)    |                        |
| Diabetes | No     | 103                         | 1806                   | 1.41 (0.94, 2.10)    | 0.003                  |
|           | Yes    | 16                          | 84                     | 8.11 (2.79, 23.55)   |                        |
| Smoking  | No     | 36                          | 867                    | 1.66 (0.87, 3.17)    | 0.89                   |
|           | Yes    | 82                          | 983                    | 1.78 (1.10, 2.81)    |                        |
| BMI      | <30    | 83                          | 1554                   | 1.50 (0.98, 2.31)    | 0.53                   |
|           | ≥30    | 29                          | 270                    | 2.04 (0.84, 4.94)    |                        |

Figure 2. Adjusted association of thrombophilia with arterial thromboembolism (ATE) across various traditional cardiovascular risk factors, sex, and age. The hazard ratios and the corresponding confidence intervals represent the association of thrombophilia with ATE stratified by age, sex, and various traditional cardiovascular risk factors. Because age is a proxy of the follow-up time (ie, age minus 15 years), the age-stratified estimates were derived from a time-varying study-stratified Cox model with adjustment for family clustering, date of birth, and the remaining cardiovascular risk factors. For sex and each cardiovascular risk factor, separate study-stratified Cox models were fit with a single interaction term between the stratifying variable and thrombophilia with additional adjustment for family clustering, date of birth, and the remaining variables (except age) presented in the figure. Interaction terms between confounding variables and thrombophilia were not included to avoid model overfitting. In models in which body mass index (BMI) served as an adjustment variable, BMI was entered as continuous variable.
55 years, even though the absolute risks are lower, which may be because of variation in disease penetrance or that at older age other much stronger risk factors overrules the risk of ATE conferred by thrombophilia. Risk decay at older age has also been reported for some traditional cardiovascular risk factors. Moreover, at older age, thrombophilic individuals were often using oral anticoagulants, which could have diminished the risk of ATE conferred by thrombophilia. However, excluding the individuals on long-term (ie, >12 months) oral anticoagulants did not change the age interaction (data not shown).

This study has several limitations that warrant addressing. The prevalence of traditional cardiovascular risk factors was self-reported at enrollment and was retrospectively ascertained. However, differential recall or ascertainment bias seems unlikely as clinical information was collected before testing for thrombophilia. Moreover, traditional cardiovascular risk factors in patients with ATE may not be in all patients diagnosed before the ATE because we did not have the exact date of the diagnosis for traditional cardiovascular risk factors. If this has occurred, then the true effect of thrombophilia may have been underestimated; however, the prevalence of the traditional cardiovascular risk factors was lower in individuals with thrombophilia as compared with individuals without, which argues against relevant reverse association between traditional cardiovascular risk factors and ATE. The lower prevalence of traditional cardiovascular risk factors in thrombophilies may raise concern for potential selection bias, which could bias the main effect estimates, but the estimates of multiplicative interaction would be still valid. Moreover, the interaction estimates did not change when we attempted to adjust for the lower prevalence of traditional cardiovascular risk factors in thrombophilies, using the inverse probability weighting method. This study may be underpowered for detection of significant interaction effects because interaction tests are known for low power; therefore, instead of focusing on interaction P values, we think that the magnitude of the interaction effects and the corresponding confidence intervals warrant more emphasize.

Finally, in observational studies, residual confounding cannot be ruled out, which could bias both the main effect and the interaction estimates. In addition, ignoring inclusion of interaction terms between confounders and thrombophilia because of models overfitting may have increased the chance of residual confounding. However, a family-cohort design of related individuals with adjustment for family-clustering would likely account for important residual or unmeasured confounding.

Despite these limitations, this is the first study addressing interaction between anticoagulant protein deficiencies and traditional cardiovascular risk factors on ATE risk. Furthermore, previous studies addressing interaction of factor V Leiden and prothrombin G20210A polymorphisms with cardiovascular risk factors are either case–control or cohort studies with non-related individuals with their inherent methodological limitations, including residual confounding.

In conclusion, thrombophilic defects are associated with ATE. Thrombophilic defects and traditional cardiovascular risk factors, in particular diabetes mellitus, showed synergistic interaction on ATE risk. Although studies addressing the

| Presence of CV risk-factors | Presence of thrombophilia | Interaction attributable proportion (95% CI)* | P-value |
|----------------------------|---------------------------|---------------------------------------------|---------|
| Hypertension               |                           | 1.0                                        |         |
| −                          | −                         | 1.7                                        |         |
| −                          | +                         | 3.5                                        | 0.33 (−0.06 − 0.71) | 0.10   |
| +                          | +                         | 5.5                                        |         |
| Hyperlipidemia             |                           | 1.0                                        |         |
| −                          | −                         | 1.7                                        | 0.36 (0.01 − 0.72) | 0.05   |
| −                          | +                         | 4.7                                        |         |
| +                          | −                         | 7.4                                        |         |
| Diabetes                   |                           | 1.6                                        |         |
| −                          | −                         | 2.3                                        | 0.74 (0.47 − 1.00) | <0.001 |
| −                          | +                         | 2.3                                        |         |
| +                          | −                         | 9.7                                        |         |
| Smoking                    |                           | 1.3                                        |         |
| −                          | −                         | 1.7                                        | 0.22 (−0.19 − 0.64) | 0.29   |
| −                          | +                         | 2.0                                        |         |
| +                          | +                         | 3.4                                        |         |
| BMI ≥30                    |                           | 1.5                                        |         |
| −                          | −                         | 2.2                                        | 0.32 (−0.22 − 0.66) | 0.24   |
| −                          | +                         | 2.6                                        |         |
| +                          | −                         | 4.2                                        |         |
| +                          | +                         | 0.10                                       |         |

Figure 3. Crude annual incidence of arterial thromboembolism and covariate-adjusted additive interaction of thrombophilia with modifiable traditional cardiovascular risk factors on arterial thromboembolism risk. Interaction attributable proportion due to interaction is the proportion of the combined effect that is due to interaction in the doubly exposed group. Attributable proportions due to interaction were calculated in 2 steps. First step: for each cardiovascular risk factor, separate study-stratified Cox models were fit with a single interaction term between the variable of interest and thrombophilia with additional adjustment for family clustering, sex, year of birth, and the remaining variables presented in the figure. Interaction terms between confounding variables and thrombophilia were not included to avoid model overfitting. In models in which BMI served as an adjustment variable, BMI was entered as a continuous variable. Second step: from the adjusted Cox models fitted in the first step, the attributable proportion as a result of interaction were calculated as described in the statistical analysis section. BMI indicates body mass index; CI, confidence interval; and CV, cardiovascular. Error bars show crude annual incidence and the corresponding 95% confidence intervals. Plus and minus signs on the figure denote presence and absence, respectively, of the traditional cardiovascular risk factors and thrombophilia.
association of thrombophilia with ATE are thus far focusing on individuals with low-risk profile, future studies should focus on individuals with traditional cardiovascular risk factors to assess potential synergistic interaction with thrombophilic defects.

Sources of Funding
No external funding for current analysis. The original studies were supported by grant 28 to 2783 from the Prevention Fund/ZonMW (The Hague, The Netherlands) and grant 99.187 from the Dutch Heart Foundation (The Hague, The Netherlands).

Disclosures
None.

References
1. Mahmoodi BK, Brouwer JL, Ten Kate MK, Lijfering WM, Veeger NJ, Nijmeijer HM, et al. A prospective cohort study on the absolute risks of venous thromboembolism and predictive value of screening asymptomatic relatives of patients with hereditary deficiencies of protein S, protein C or antithrombin. J Thromb Haemost. 2010;8:1193–1200. doi: 10.1111/j.1538-7836.2010.03840.x.
2. Lijfering WM, Brouwer JL, Veeger NJ, Bank I, Coppens M, Middeldorp S, et al. Selective testing for thrombophilia in patients with first venous thrombosis: results from a retrospective family cohort study on absolute thrombotic risk for currently known thrombophilic defects in 2497 relatives. Blood. 2009;113:5314–5322. doi: 10.1182/blood-2008-10-184879.
3. Brouwer JL, Veeger NJ, Klain-Nelemans HC, van der Meer J. The pathogenesis of venous thromboembolism: evidence for multiple interrelated causes. Ann Intern Med. 2006;145:307–815.
4. Ye Z, Liu EH, Higgins JP, Keaveny BD, Lowe GD, Collins R, et al. Seven haemostatic gene polymorphisms in coronary disease: meta-analysis of 66,155 cases and 91,307 controls. Lancet. 2006;367:651–658. doi: 10.1016/S0140-6736(06)68263-9.
5. Mahmoodi BK, Brouwer JL, Veeger NJ, van der Meer J. Hereditary deficiency of protein C or protein S confers increased risk of arterial thromboembolic events at a young age: results from a large family cohort study. Circulation. 2008;118:1659–1667. doi: 10.1161/CIRCULATIONAHA.108.780759.
6. Mackman N. Triggers, targets and treatments for thrombosis. Nature. 2008;451:914–918. doi: 10.1038/nature06797.
7. Rosendaal FR, Siscovick DS, Schwartz SM, Psaty BM, Raghuunathan TE, Vos HL. A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. Blood. 1997;90:1747–1750.
8. Rosendaal FR, Siscovick DS, Schwartz SM, Beverly RK, Psaty BM, Longstreth WT Jr, et al. Factor V Leiden (resistance to activated protein C) increases the risk of myocardial infarction in young women. Blood. 1997;89:2817–2821.
9. Inbal A, Freimark D, Modan B, Chetrit A, Matezky S, Rosenberg N, et al. Synergistic effects of prothrombotic polymorphisms and atherogenic factors on the risk of myocardial infarction in young males. Blood. 1999;93:2186–2190.
10. Doggen CJ, Caius JM, Bertina RM, Rosendaal FR. Interaction of coagulation defects and cardiovascular risk factors: increased risk of myocardial infarction associated with factor V Leiden or prothrombin 20210A. Circulation. 1998;97:1037–1041.
11. Ridker PM, Hennekens CH, Miletich JP. G20210A mutation in prothrombin gene and risk of myocardial infarction, stroke, and venous thrombosis in a large cohort of US men. Circulation. 1999;99:939–944.
12. Borecki IB, Province MA. Genetic and genomic discovery using family studies. Circulation. 2008;118:1057–1063. doi: 10.1161/CIRCULATIONAHA.107.714592.
13. Lijfering WM, Veeger NJ, Brouwer JL, van der Meer J. The risk of venous and arterial thrombosis in hyperhomocysteinemic subjects may be a result of elevated factor VIII levels. Haematologica. 2007;92:1703–1706. doi: 10.3324/haematol.11611.
14. Bank I, van de Poel MH, Coppens M, Hamulyák K, Prins MH, van der Meer J, et al. Absolute annual incidences of first events of venous thromboembolism and arterial vascular events in individuals with elevated FVIIIc. A prospective family cohort study. Thromb Haemost. 2007;98:1040–1044.
15. Bank I, Libourel EJ, Middeldorp S, Van Pampus EC, Koopman MM, Hamulyák K, et al. Prothrombin 20210A mutation: a mild risk factor for venous thromboembolism but not for arterial thrombotic disease and pregnancy-related complications in a family study. Arch Intern Med. 2004;164:1912–1918. doi: 10.1001/archinte.164.17.19132.
16. Catena C, Colussi G, Nair F, Capobianco F, Sechi LA. Elevated homocysteine levels are associated with the metabolic syndrome and cardiovascular events in hypertensive patients. Am J Hypertens. 2015;28:943–950. doi: 10.1093/ajh/hpu248.
17. Wannamethee SG, Lowe GD, Shaper AG, Rumley A, Lennon L, Whincup PH. The metabolic syndrome and insulin resistance: relationship to haemostatic and inflammatory markers in older non-diabetic men. Atherosclerosis. 2005;181:101–108. doi: 10.1016/j.atherosclerosis.2004.12.031.
18. Knol MJ, VanderWeele TJ, Groenwold RH, Klungel OG, Rovers MM, Grobbee DE. Estimating measures of interaction on an additive scale for preventive exposures. Eur J Epidemiol. 2011;26:433–438. doi: 10.1007/s10654-011-9554-9.
19. Roshani S, Lijfering WM, Coppens M, Hamulyák K, Prins MH, Bülter HR, et al. Risk factors of arterial cardiovascular complications in patients with prior venous thromboembolism. Neth J Med. 2011;69:27–30.
20. Maas AH, Appelman YE. Gender differences in coronary heart disease. Neth Heart J. 2010;18:598–602.
21. Hainer V, Aldhoom-Hainerová I. Obesity paradox does exist. Diabetes Care. 2013;36(suppl 2):S276–S281. doi: 10.2337/dc13-2023.
22. Ko DT, Mamdani M, Alter DA. Lipid-lowering therapy with statins in high-risk elderly patients: the treatment-risk paradox. JAMA. 2004;291:1864–1870. doi: 10.1001/jama.291.15.1864.
23. Morimoto LM, White E, Newcomb PA. Selection bias in the assessment of gene-environment interaction in case-control studies. Am J Epidemiol. 2003;158:259–263.

CLINICAL PERSPECTIVE
Hereditary thrombophilia is associated with a hypercoagulable state; however, the risk of arterial thromboembolism (ATE) conferred by hereditary thrombophilia is believed to be only slightly elevated. Whether hereditary thrombophilia interacts with traditional cardiovascular risk factors (ie, hypertension, diabetes mellitus, hyperlipidemia, obesity, and smoking) on the risk of ATE has yet to be established. Given that ATE is generally a process of atherosclerotic plaque rupture, which is driven by traditional cardiovascular risk factors, followed by platelet aggregation and coagulation activation, synergistic interaction of traditional cardiovascular risk with hereditary thrombophilia on ATE risk seems likely. In the current analysis of 4 family-cohort studies with a total of 1891 participants, the association of thrombophilia with ATE tended to be stronger in the presence as compared with the absence of traditional cardiovascular risk factors, which was most evident in diabetics as compared with nondiabetics. Moreover, the association of thrombophilia with ATE was stronger in participants aged <55 as compared with ≥55 years of age and females as compared with males. Future studies are warranted to confirm these findings. If confirmed in future research, given the high prevalence of particularly factor V Leiden (≈5%) and prothrombin G20210A (≈2%–3%) in the general population, these 2 may be useful for primary prevention purposes because tighter control of traditional cardiovascular risk factors in these individuals may be necessary.