Research article

Genetics University of Toronto Thrombophilia Study in Women (GUTTSI): genetic and other risk factors for venous thromboembolism in women

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Abstract

Background Women may be at increased risk for venous thromboembolism (VTE) as compared with men. We studied the effects of genetic and biochemical markers of thrombophilia in women, in conjunction with other established risk factors for VTE.

Method The present retrospective case–control study was conducted in a thrombosis treatment programme at a large Toronto hospital. The cases were 129 women aged 16–79 years with objectively confirmed VTE. Age-matched control individuals were women who were free of venous thrombosis. Neither cases nor control individuals had known cardiovascular disease. Participants were interviewed regarding personal risk factors for VTE, including smoking, history of malignancy, pregnancy, and oestrogen or oral contraceptive use. Blood specimens were analyzed for common single nucleotide polymorphisms of prothrombin, factor V and methylenetetrahydrofolate reductase (MTHFR; C677T, A1298C and T1317C), and the A66G polymorphism for methionine synthase reductase (MTRR). Fasting plasma homocysteine was also analyzed.

Results Women with VTE were significantly more likely than female control individuals to carry the prothrombin polymorphism and the factor V polymorphism, or to have fasting hyperhomocysteinaemia. Homozygosity for the C677T MTHFR gene was not a significant risk factor for VTE, or were the A1298C or T1317C MTHFR homozygous variants. Also, the A66G MTRR homozygous state did not confer an increased risk for VTE.

Conclusion Prothrombin and factor V polymorphisms increased the risk for VTE in women, independent from other established risk factors. Although hyperhomocysteinaemia also heightens this risk, common polymorphisms in two genes that are responsible for homocysteine remethylation do not. These findings are consistent with previous studies that included both men and women.

Keywords case–control study, deep vein thrombosis, factor V gene, folate, genetics, homocysteine, methionine synthase reductase, methylenetetrahydrofolate reductase, prothrombin gene, pulmonary embolism, risk, thrombophilia, venous thromboembolism, women's health

CI = confidence interval; MTHFR = methylenetetrahydrofolate reductase; MTRR = methionine synthase reductase; OR = odds ratio; RH = relative hazard; tHcy = total homocysteine; VTE = venous thromboembolism.

Introduction

The development of both idiopathic and situational VTE remains multifactorial [1]. Advanced age [2], malignancy [1] and smoking [3], in addition to both genetic and biochemical abnormalities within the coagulation and endovascular systems [4], predispose to VTE. Furthermore, women are perceived to be at greater risk for VTE [2,5], principally due to the presence of both endogenous and exogenous oestrogen sources [6–9].

Published data implicate both the guanine-to-adenine nucleotide 20210 (G20210A) prothrombin and guanine-to-adenine nucleotide 1691 (G1691A) factor V gene polymorphisms in the development of VTE during pregnancy and the puerperium [10,11]. Few studies have assessed these and other genetic risk factors, as well as hyperhomocysteinaemia, among nonpregnant women, while also considering such elements as smoking [3], malignancy [1], oestrogen use [6] and recent immobilization [12].

In order to better define the role of thrombophilic defects and other risk factors in the development of VTE in women, we conducted the present study. In addition to probable genetic risk factors [10,11] and total homocysteine (tHcy) levels, we examined the relationship between VTE and four polymorphisms involved in tHcy remethylation to methionine. The first two, the adenine-to-cytosine (A1298C) and thymine-to-cytosine (T1317C) [13], which are MTHFR polymorphisms, participate in the cycling of folate coenzyme by the MTHFR enzyme, in addition to the classic MTHFR cytosine-to-thymine (C677T) polymorphism [14]. We also studied the effect of the novel adenine-to-guanine polymorphism (A66G) in MTRR, the cobalamin-dependent enzyme that is responsible for folate-dependent tHcy remethylation [14].

Method

We conducted a retrospective case–control study. Eligible cases included all women aged 16–79 years with objectively confirmed VTE seen at the University Health Network in Toronto from April 1996 to October 1999. Objective diagnostic testing comprised compression ultrasonography or venography for deep vein thrombosis of the limb, ventilation/perfusion or contrast enhanced spiral computed tomography imaging for pulmonary embolism, and magnetic resonance or computed tomography imaging for intra-abdominal or intracranial venous thrombosis. We excluded those women whose VTE was related to a central venous catheter [15], as well as those with a history of coronary artery, cerebrovascular or peripheral vascular disease [16], or a previously identified thrombophilia defect. After obtaining written informed consent, one female control individual (within 10 years of age) was matched to every case; these control individuals were derived from a cohort of healthy women recruited through the Family Medicine Unit at the Toronto University Health Network. Thirty cases (approximately 25% of the study population) had been enrolled in a previous study that examined the relationship between hyperhomocysteinaemia and VTE [17], and none of the control individuals had participated in a previous study.

At the initial visit, a clinical nurse specialist interviewed cases using a standardized data collection form. An assessment for VTE risk factors included age at first VTE, history of recurrent VTE, history of malignancy within the past 5 years, current cigarette consumption, and immobilization or surgery within 3 months before the diagnosis of VTE. Presence of an 'oestrogen exposure state' was defined as either current use of an oral contraceptive agent, hormone replacement therapy or tamoxifen, or being pregnant or within 6 weeks postpartum. Information was also collected about the anatomical site of the VTE and the method of detection. Control group data were obtained using a standard questionnaire, administered by a trained research assistant during a face-to-face interview. The same demographic information was collected for cases and control individuals, with the exception of a history of recent immobilization or surgery, which was not assessed in the control group.

All laboratory analyses were performed with the investigator blinded to each woman's clinical diagnosis. Participants were instructed to fast for at least 8 h before plasma tHcy specimens were collected. Neither cases nor control individuals were known to be taking folate or vitamin B_{12} supplements at the time of blood specimen collection. Plasma tHcy was analyzed using high-performance liquid chromatography as previously described [18], and red-cell folate was measured using a radioimmunoassay method (Quantaphase II; Bio-Rad Laboratories Incorporated, Toronto, Ontario, Canada). DNA was extracted from peripheral blood cells [19]. A single multiplex polymerase chain reaction assay was designed to identify simultaneously the prothrombin, factor V and MTHFR genotypes [20–22]. The MTRR A66G genotype was analyzed as previously described [23]. Cases (but not control individuals) were evaluated for the presence of anticardiolipin antibodies, the lupus-like anticoagulant, and deficiencies in protein C, protein S and antithrombin.

Statistical analysis

In the primary analysis, the association between the presence of VTE and each thrombophilic risk factor was evaluated using conditional logistic regression analysis. Crude and adjusted odds ratios (ORs) were estimated. The ORs were adjusted for specific covariates, which were defined *a priori*, including current cigarette smoking habits, oestrogen exposure state and recent history of cancer. Hyperhomocysteinaemia was defined as the tHcy concentration above the 95th centile value in the control group [24]. Interaction terms between the presence of hyperhomo-

cysteinaemia and the homozygous state for any MTHFR or MTRR polymorphism were also evaluated [25].

A predefined subgroup analysis was performed excluding cases and their respective control individuals with either immobilization or surgery within the past 3 months, and any case–control pairs in which either had had a malignancy within the past 5 years. The potential presence of another identifiable thrombophilia defect among the cases (eg protein S, protein C or antithrombin deficiency) was not accounted for in any of the analyses.

Genotype distributions and allele frequencies and equilibria were analyzed using Genetic Data Analysis version 1.0 (PO Lewis and D Zaykin, 2000, Sinauer Associates, Incorporated, Sunderland, MA, USA). Probabilities for allele frequencies were calculated using Fisher's exact test. For each disequilibrium (Hardy-Weinberg or pairwise linkage), the data program estimated the probability of obtaining the observed genotype distribution using 3600 permuted simulations generated by an allele-shuffling algorithm.

Baseline characteristics of cases and control individuals were compared using one-way analysis of variance for continuous variables, or the χ^2 test for categorical data. All *P* values were two-sided, and the significance level chosen was 0.05. Statistical analyses were performed using SAS Version 6.12 (SAS Institute Incorporated, Cary, NC, USA). All data were collected in an anonymous manner, and approval to conduct the study was obtained through the University of Toronto Research Ethics Committee.

Results

General findings

After initial review, 54 potential cases were excluded. Reasons for exclusion included the presence of cardiovascular disease (29 women), VTE related to central venous catheters (seven women), recurrent pregnancy loss, heparin-induced thrombocytopenia, superficial phlebitis or cellulitis (eight women), and lack of objective evidence to confirm the diagnosis of VTE (10 women). Thus, 129 cases, along with 129 age-matched control individuals, were included.

Table 1 presents the characteristics of the 129 cases with VTE and their respective control individuals. Of all case–control pairs, 120 (93%) were matched within 7 years of age, and 82 (64%) were within 5 years. The mean age of the cases and controls was 45.8 years and 40.9 years, respectively ($P = 0.03$). Cases were more likely to have a history of malignancy (24.0% versus 6.2%; $P = 0.0001$) and fewer were current smokers (13.2% versus 23.3%; $P = 0.2$).

A nonstudy thrombophilia defect was identified in nine cases (7%). Four had positive anticardiolipin antibodies, and a fifth had a positive lupus anticoagulant. Of these five women, one was homozygous for both MTHFR C677T and A1298C, and heterozygous for the factor V polymorphism; one woman was homozygous for MTHFR A1298C; one woman was homozygous for MTRR A66G; and the remaining two women had no other identifiable defects. Three other women displayed mild functional protein S deficiency, of which one demonstrated homozygosity for both the MTHFR A1298C and MTRR A66G polymorphisms; one was homozygous for the MTHFR A1298C polymorphism; and the third was homozygous for the MTRR A66G polymorphism. One other woman was found to have antithrombin deficiency, but had no other detectable abnormalities.

Gene frequency

Of the six loci examined (Table 2), the population-dependency of allele frequencies was known for the prothrombin, factor V and MTHFR C677T genes. The frequencies of these variant alleles among the control individuals (1.2, 1.9 and 27.0%, respectively) were within the range of values reported for predominantly Caucasian populations. The genotype distributions at all six loci showed no departures from those expected under the Hardy-Weinberg equilibrium. Comparison of frequency distributions for each allele pair showed significant differences for the prothrombin, factor V and MTHFR C677T polymorphisms (Fisher's exact test $P < 0.05$). As expected, the linkage disequilibrium was significant for each pairwise comparison among the three MTHFR intragenic loci (data not shown), but not for any other pair of loci (Table 2).

Venous thromboembolism risk among all women

Among the participants, women with VTE were significantly more likely than control individuals to be carriers of the prothrombin polymorphism (9.3% versus 2.3%; adjusted OR 4.3, 95% confidence interval [CI] 1.1–17.4; Table 3). One case and no control individuals were homozygous for the prothrombin gene defect. Similarly, the factor V gene polymorphism was more common among cases than control individuals (16.3% versus 3.9%; adjusted OR 3.9, 95% CI 1.4–10.8). Two cases and no control individuals were homozygous for this defect. Three cases (2.3%) were carriers of both the prothrombin and factor V polymorphisms, for which no further statistical analysis was conducted.

The MTRR A66G homozygous state was more common among cases (32.6%) than control individuals (27.1%), but not significantly (adjusted OR 1.1, 95% CI 0.6–2.2). Homozygosity for C677T MTHFR was no more frequent among cases (14.7%) than control individuals (10.1%; adjusted OR 1.4, 95% CI 0.6–3.1), and neither was the homozygous state for A1298C MTHFR (52.7% versus 53.5%; adjusted OR 1.0, 95% CI 0.6–1.7; Table 3). Only one case and one control individual (0.8% each)

Table 1

Characteristics of 129 women with VTE and age-matched control individuals

Characteristic	Women who experienced VTE (129 cases)	Women who did not experience VTE (129 controls)	Statistical comparison between cases and controls
Mean (SD) age (years)	45.8 (17.5)	40.9 (17.8)	$P = 0.03$
Number (percentage) with deep vein thrombosis of the leg	88 (68.2)	–	–
Number (percentage) with deep vein thrombosis at another site	17 (13.2)	–	–
Number (%) with pulmonary embolism*	32 (24.8)	–	–
Number (%) with recurrent VTE	33 (25.6)	–	–
Number (%) with other thrombophilia defects†	9 (7.0)	–	–
Number (%) with immobilization or surgery within 3 months	23 (17.8)	–	–
Number (%) with malignancy within 5 years	31 (24.0)	8 (6.2)	$P = 0.0001$
Number (%) with oestrogen exposure state‡	44 (34.1)	43 (33.3)	$P = 0.9$
Number (%) current cigarette smokers	17 (13.2)	30 (23.2)	$P = 0.2$
Mean (SD) fasting plasma total homocysteine (μmol/l)	11.1 (5.6)	6.5 (3.0)	$P = 0.0001$
Mean (SD) red-cell folate (nmol/l)$	1121.3 (566.1)	981.9 (452.6)	$P = 0.05$
Mean (SD) serum creatinine (μmol/l)$	82.2 (62.8)	69.2 (10.3)	$P = 0.03$

*Describes individuals who either experienced pulmonary embolism in isolation or in combination with a deep vein thrombosis. †Includes either a positive test for antiphospholipid antibodies, the lupus anticoagulant, or a deficiency in protein C, protein S or antithrombin. ‡Defined as current use of an oral contraceptive agent, hormone replacement therapy or tamoxifen, or currently pregnant or within 6 weeks postpartum. $Complete data for serum creatinine and red-cell folate concentrations were only available for 80 case–control pairs. –, Data not applicable.

Table 2

Allele frequencies of the prothrombin, factor V, MTHFR and MTRR polymorphisms among 129 women with VTE and 129 matched control individuals

	Gene polymorphism											
	Prothrombin G20210A		Factor V G1691A		MTHFR C677T		MTHFR A1298C		MTHFR T1317C		MTRR A66G	
Allele	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
–/–	117	126	108	124	49	72	68	69	122	123	25	29
+/–	11	3	19	5	61	44	49	49	6	5	62	65
+/+	1	0	2	0	19	13	12	11	1	1	42	35
Frequency	0.050	0.012	0.089	0.019	0.384	0.271	0.283	0.275	0.031	0.027	0.566	0.523
Fisher's exact test *P* value	0.02		0.0007		0.008		0.92		1.0		0.4	

–/–, Wildtype; +/–, heterozygote; +/+, homozygote.

carried the T1317C MTHFR homozygous variant (adjusted OR 1.0, 95% CI 0.1–16.3). The presence of any MTRR or MTHFR homozygous state was also not a significant risk factor for VTE (adjusted OR 1.1, 95% CI 0.6–2.0).

The mean fasting plasma tHcy was significantly higher among women with VTE (11.1 μmol/l) than control individuals (6.5 μmol/l; unpaired *t*-test $P < 0.0001$; Table 1). The 95th centile tHcy value for control individuals was 11.0 μmol/l; using this latter cut-point, hyperhomocysteinaemia was significantly more common among cases (35.6%) than control individuals (4.0%). The adjusted OR was 17.8 (95% CI 4.2–74.9) for VTE in the presence of hyperhomocysteinaemia. No significant interaction ($P > 0.50$ for each) was observed between the presence of hyperhomocysteinaemia and any MTHFR or MTRR homozygous state.

Table 3

Prevalence and adjusted ORs for thrombophilia among women with VTE

	All women				Excluding women with malignancy, recent surgery or immobilization*
Thrombophilia factor	Number (%) of cases with thrombophilia factor	Number (%) controls with thrombophilia factor	Crude OR (95% CI)	Adjusted OR† (95% CI)	Adjusted OR‡ (95% CI)
Prothrombin (++ or +/–)	12 (9.3)	3 (2.3)	4.3 (1.2–15.6)	4.3 (1.1–17.4)	7.8 (1.0–64.2)
Factor V (+/+ or +/–)	21 (16.3)	5 (3.9)	4.8 (1.8–13.2)	3.9 (1.4–10.8)	3.6 (1.2–11.4)
MTHFR C677T (+/+)	19 (14.7)	13 (10.1)	1.5 (0.7–3.3)	1.4 (0.6–3.1)	1.1 (0.4–2.9)
MTHFR 1298C (+/+)	68 (52.7)	69 (53.5)	1.0 (0.6–1.6)	1.0 (0.6–1.7)	1.2 (0.6–2.3)
MTRR A66G (+/+)	42 (32.6)	35 (27.1)	1.3 (0.8–2.2)	1.1 (0.6–2.2)	0.9 (0.4–2.2)
Any MTHFR (+/+) or A66G MTRR (+/+)	90 (69.8)	88 (68.2)	1.1 (0.6–1.8)	1.0 (0.6–2.0)	1.1 (0.5–2.3)
Fasting plasma hyperhomocysteinemia$	46 (35.6)	5 (4.0)	13.7 (5.2–36.0)	17.8 (4.2–74.9)	11.6 (2.7–50.5)

*Excludes cases with immobilization or surgery within the past 3 months, or a malignancy within the past 5 years, and their respective control individuals. †Adjusted for cigarette smoking, oestrogen exposure state and recent history of cancer. ‡Adjusted for cigarette smoking and oestrogen exposure state. $Defined as a homocysteine concentration greater than the 95th centile value in the control group. +/+, Homozygous; +/–, heterozygous.

Venous thromboembolism risk, excluding women with recent malignancy, immobilization or surgery

Upon excluding cases with secondary causes for VTE (malignancy, immobilization or surgery), and their respective control individuals, 81 case–control pairs were available for analysis (Table 3). In the presence of the prothrombin polymorphism, the adjusted OR for VTE was 7.8 (95% CI 1.0–64.2). The risk for VTE was also significant in the presence of the factor V polymorphism (adjusted OR 6.6, 95% CI 1.2–11.4). The risk for VTE among homozygotes for each MTHFR and A66G MTRR polymorphism was no higher in this subgroup, however ($P > 0.5$ for each). Finally, the adjusted OR for VTE in the presence of fasting hyperhomocysteinaemia decreased somewhat from that observed in the main analysis (adjusted OR 11.6, 95% CI 2.7–50.5; Table 3).

Discussion

We found that the presence of the prothrombin and factor V gene polymorphisms and hyperhomocysteinaemia significantly increased the risk for VTE among women. When those with recent malignancy, surgery or immobilization were excluded, the risk was even greater for carriers of the prothrombin polymorphism. Although hyperhomocysteinaemia also increased the risk for VTE, this association was not found for the MTRR A66G or any of the three MTHFR gene polymorphisms.

Of the cases studied, 7% were found to have another thrombophilia defect, such as antiphospholipid antibodies or protein S deficiency, which was not controlled for in the present analysis. It is unlikely that the presence of such defects influenced the results, however, because they were uncommon, and eight out of nine of the defects were observed among women with the MTHFR or MTRR gene polymorphisms, or both, none of which independently conferred an increased risk for VTE.

The present study might have been limited by a referral bias among the cases, potentially exaggerating our risk estimates for VTE [26]. Because 25% of cases also had recurrent VTE, these estimates might have been further inflated. In order to evaluate this, we compared the rates of thrombophilia gene defects in the cases and control individuals with those in other published case–control studies (Table 4). The prevalence of the prothrombin gene among the control population studied here (2.3%) was within the general limits of other studies (range 1.0–5.4%), and our case rate (9.3%) was similar to that of other studies (range 4.6–31.0%), albeit slightly lower than those of some studies. Similarities in the frequencies of factor V and C677T MTHFR gene polymorphisms were also observed. Thus, we believe that our case and control rates reflect those observed in other adult populations with and without VTE. Ethnic variation, not assessed herein, might explain some of the differences between studies in the prevalence of these polymorphisms, given the wide diversity of ethnicity according to geographic area [27].

Table 4

Published case–control studies of the risk for VTE in the presence of polymorphisms of the prothrombin, factor V and MTHFR C677T genes, as well as fasting hyperhomocysteinaemia

				Prevalence of thrombophilia defect (%)		
Thrombophilia defect	Reference	Population	VTE type	Cases	Controls	Risk estimate (95% CI)
Prothrombin gene (+/+ or +/–)	[41]	Men and women	Any VTE	4.6	1.0	ORc 4.8 (1.5–19.8)
	[42]	Men and women	Any VTE	14.2	4.6	ORc 3.4 (2.2–5.5)
	[43]	Men and women	Any VTE	10.2	2.8	ORa 4.0 (1.9–8.5)
	[44]	Men and women	Any VTE	18.5	5.4*	ORa 3.6 (1.8–7.3)
	[45]	Men and women	Any DVT	7.1	1.8	ORa 3.8 (1.1–13.2)
	[20]	Men and women	First DVT	6.2	2.3	ORc 2.8 (1.4–5.6)
	[46]	Men and women	First DVT	15.9	2.3	ORa 8.7 (3.8–21.4)
	[10]	Pregnant women	Any VTE	16.9	1.3	RRa 15.2 (4.2–52.6)
	[47]	Pregnant women	Any VTE	31.0	4.2	ORa 10.2 (4.0–25.9)
	Present study	Women	Any VTE	9.3	2.3	ORa 4.3 (1.1–17.4)
Factor V gene (+/+ or +/–)	[41]	Men and women	Any VTE	12.5	1.4	ORc 2.7 (1.5–4.9)
	[42]	Men and women	Any VTE	18.2	5.1	ORc 4.2 (2.7–6.4)
	[48]	Men and women	Any VTE	53.0	3.3	ORc 17.4 (11.4–93.0)
	[49]	Men and women	Any VTE	26.0	7.6	ORc 4.3 (2.3–8.1)
	[43]	Men and women	Any VTE	19.5	3.5	ORa 6.5 (3.5–12.5)
	[44]	Men and women	Any VTE	40.1	3.9†	ORa 16.3 (8.5–31.3)
	[50]	Men and women	DVT and PE	14.8	5.3	ORc 4.2 (1.5–10.3)
	[51]	Men and women	Any DVT	19.5	2.9	ORc 8.0 (4.5–14.2)
	[52]	Men and women	Any DVT	28.0	11.0	ORa 3.1 (1.7–5.5)
	[53]	Men and women	Any DVT	23.5	6.1	ORc 4.7 (1.5–15.0)
	[46]	Men and women	First DVT	21.1	3.2	ORa 7.8 (3.9–17.1)
	[10]	Pregnant women	Any VTE	43.7	7.7	RRa 9.3 (5.1–16.9)
	[47]	Pregnant women	Any VTE	23.8	1.9	ORc 16.3 (4.8–54.9)
	Present study	Women	Any VTE	16.3	3.9	ORa 3.9 (1.4–10.8)
MTHFR gene (+/+)	[54]	Men and women	Any VTE	10.0	13.0	ORc 0.7 (0.5–1.0)
	[55]	Men and women	Any VTE	28.2	17.7	ORc 1.8 (1.2–2.9)
	[49]	Men and women	Any VTE	11.5	15.0	ORc 0.7 (0.4–1.3)
	[43]	Men and women	Any VTE	12.7	12.3	ORa 1.0 (0.6–1.7)
	[44]	Men and women	Any VTE	22.8	14.3	ORa 2.1 (1.2–3.7)
	[56]	Men and women	Any VTE	12.3‡	13.0	ORc 0.9 (0.4–2.4)
	[57]	Men and women	Any VTE	8.0	6.4	ORc 1.4 (0.5–4.0)
	[58]	Men and women	Any VTE	12.3	13.1	ORc 0.9 (0.4–2.3)
	[53]	Men and women	Any DVT	7.4	10.6	ORc 0.7 (0.2–2.2)
	[59]	Men and women	Any DVT	25.6	18.1	ORc 1.7 (1.1–2.5)
	[60]	Men and women	Any DVT	10.0	9.9	ORc 1.0 (0.7–1.5)
	[46]	Men and women	First DVT	20.5	21.0	ORa 1.0 (0.7–1.2)
	[47]	Pregnant women	Any VTE	28.6	16.0	ORc 2.1 (1.0–4.5)
	Present study	Women	Any VTE	14.7	10.1	ORa 1.4 (0.6–3.1)
Fasting hyper-homocysteinaemia	[24]	Men and women	Any VTE	12.5	4.7	ORp 3.0 (2.1–4.2)$
	[55]	Men and women	Any VTE	16.0	5.0	ORc 3.6 (1.8–7.3)
	Present study	Women	Any VTE	35.6	4.0	ORa 17.8 (4.2–74.9)

*Describes the +/– genotype. †Describes the +/+ genotype. ‡Describes the prevalence of the MTHFR C677T +/+ state among individuals with factor V. $Comprises pooled data from nine observational studies. DVT, deep vein thrombosis; +/+, homozygous; +/–, heterozygous; ORa, adjusted OR; ORc, crude OR; ORp, pooled OR; RRa, adjusted relative risk.

Although the rate of fasting hyperhomocysteinaemia found among our control individuals (4.0%) was consistent with those of other studies (range 4.7–5.0%; Table 4), our case rate (35.6%) was more than twice that found by other investigators. There are several possible reasons for this. First, because plasma tHcy is both inversely related to renal function [28] and directly related to age [29], the higher tHcy levels among the cases might be partly explained by their relatively high serum creatinine concentrations and older age. Second, after omitting women with recent cancer from our subgroup analysis, the adjusted OR dropped from 17.8 to 11.6, suggesting that malignancy may play a role in tHcy metabolism, as proposed by others [30]. Third, by excluding women with a history of cardiovascular disease from the present study, we probably eliminated another potential confounder (cardiovascular disease has been association with hyperhomocysteinaemia) [16]. Finally, although we did not collect

data on serum vitamin B_{12}, it has been demonstrated that both measured vitamin B_{12} [31] and its supplementation [32] probably have little influence on plasma tHcy concentration relative to that of folate, which appeared to be higher among the cases than the control individuals in whom it was measured.

A meta-analysis of 23 observational studies [33] failed to demonstrate an association between MTHFR 677T homozygosity and arterial disease (pooled OR 1.1, 95% CI 0.9–1.4). Similarly, our data and those of others (Table 4) suggest that the MTHFR polymorphisms do not heighten the risk for VTE. Furthermore, we found no significant interaction between MTHFR homozygosity and hyperhomocysteinaemia. Thus, we recommend that clinical testing for currently identified polymorphisms in the MTHFR gene be postponed in the evaluation of VTE risk outside of the research setting. A further understanding of the interaction between the combination of hyperhomocysteinaemia and other gene polymorphisms, such as factor V [34] and prothrombin, is needed.

If hyperhomocysteinaemia is a true causative risk factor for a first [24] or subsequent [35] VTE event, then the mechanisms responsible for tHcy elevation and, hence, venous thrombosis require further elucidation. Newly isolated polymorphisms, such as MTRR A66G, may contribute to the development of VTE, but we found no such association in the group studied here. In the meantime, we expect that clinical trials of tHcy reduction [36], in addition to future work in the field of molecular and genetic epidemiology, are the next logical steps.

Others have evaluated the influence of nongenetic factors on the risk for VTE. For example, in a post-trial analysis by the Heart Estrogen Replacement Study (HERS) investigators [8], the risk for VTE was increased among women with lower-extremity fractures (adjusted relative hazard [RH] 18.1, 95% CI 5.4–60.4] or cancer (RH 3.9, 95% CI 1.6–9.4), or who had had inpatient surgery within the preceding 90 days (RH 4.9, 95% CI 2.4–9.8). Even limited periods of immobilization, such as a short illness or minor surgery or injury, appear to increase the risk for deep vein thrombosis (OR 2.9, 95% CI 1.5–5.4) [37]. We therefore conducted a subgroup analysis that excluded women with malignancy, recent surgery, or immobilization. Although we might expect that certain thrombophilia markers confer the greatest relative risk for VTE in the absence of other risk factors [4], as was the case for the prothrombin polymorphism, we cannot explain why this was not so for factor V. Future research should further explore the interplay between hereditary and acquired VTE risk factors.

Clinicians face a dilemma in deciding which women should be screened for these and other thrombophilia markers. It would seem both imprudent and costly to investigate those who develop VTE in the presence of active malignancy, because anticoagulant therapy is likely to be continued indefinitely [38]. However, among women who experience VTE either in the absence of any risk factors, while taking hormone replacement therapy [8,39] or oral contraceptives [6,7], or during pregnancy and the puerperium [10,11], a thrombophilia assessment may be a sensible option. Specifically, others have demonstrated that oestrogen use and activated protein C resistance have a synergistic effect on the risk for VTE (OR 13.3, 95% CI 4.3–41.0) [39]. It is least clear whether screening is beneficial in an otherwise healthy woman who develops VTE after surgery or a fracture of the lower extremity [5,40]. Hopefully these issues can be resolved through ongoing research and expert consensus.

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References

1. Heit JA, Silverstein MD, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ III: **Risk factors for deep vein thrombosis and pulmonary embolism: a population-based case–control study.** *Arch Intern Med* 2000, **160**:809–815.
2. Silverstein MD, Heit JA, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ III: **Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study.** *Arch Intern Med* 1998, **158**:585–593.
3. Hansson PO, Eriksson H, Welin L, Svardsudd K, Wilhelmsen L: **Smoking and abdominal obesity: risk factors for venous thromboembolism among middle-aged men: 'the study of men born in 1913'.** *Arch Intern Med* 1999, **159**:1886–1890.
4. Kearon C, Crowther M, Hirsh J: **Management of patients with hereditary hypercoagulable disorders.** *Annu Rev Med* 2000, **51**:169–185.
5. Svensson PJ, Benoni G, Fredin H, Bjorgell O, Nilsson P, Hedlund U, Nylander G, Bergqvist D, Dahlback B: **Female gender and resistance to activated protein C (FV:Q506) as potential risk factors for thrombosis after elective hip arthroplasty.** *Thromb Haemost* 1997, **78**:993–996.
6. Farmer RD, Lawrenson RA, Todd JC, Williams TJ, MacRae K: **Oral contraceptives and venous thromboembolic disease. Analyses of the UK General Practice Research Database and the UK Mediplus database.** *Hum Reprod Update* 1999, **5**:688–706.
7. Rosing J, Middeldorp S, Curvers J, Christella M, Thomassen LG, Nicolaes GA, Meijers JC, Bouma BN, Buller HR, Prins MH, Tans G: **Low-dose oral contraceptives and acquired resistance to activated protein C: a randomised cross-over study.** *Lancet* 1999, **354**:2036–2040.
8. Grady D, Wenger NK, Herrington D, Khan S, Furberg C, Hunninghake D, Vittinghoff E, Hulley S: **Postmenopausal hormone therapy increases risk for venous thromboembolic disease.** *Ann Intern Med* 2000, **132**:689–696.
9. Meier CR, Jick H: **Tamoxifen and risk of idiopathic venous thromboembolism.** *Br J Clin Pharmacol* 1998, **45**:608–611.
10. Gerhardt A, Scharf RE, Beckmann MW, Struve S, Bender HG, Pillny M, Sandmann W, Zotz RB: **Prothrombin and factor V mutations in women with a history of thrombosis during pregnancy and the puerperium.** *N Engl J Med* 2000, **342**:374–380.
11. McColl MD, Ellison, Reid F, Tait RC, Walker ID, Greer IA: **Prothrombin 20210 G→A, MTHFR C677T mutations in women with venous thromboembolism associated with pregnancy.** *Br J Obstet Gynaecol* 2000, **107**:565–569.
12. Thorogood M, Mann J, Murphy M, Vessey M: **Risk factors for fatal venous thromboembolism in young women: a case-control study.** *Int J Epidemiol* 1992, **21**:48–52.

13. Weisberg I, Tran P, Christensen B, Sibani S, Rozen R: **A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity.** *Mol Genet Metab* 1998, **64**:169–172.
14. Kang SS, Wong PW, Zhou JM, Sora J, Lessick M, Ruggie N, Grcevich G: **Thermolabile methylenetetrahydrofolate reductase in patients with coronary artery disease.** *Metabolism* 1988, **37**:611–613.
15. Martin C, Viviand X, Saux P, Gouin F: **Upper-extremity deep vein thrombosis after central venous catheterization via the axillary vein.** *Crit Care Med* 1999, **27**:2626–2629.
16. Boushey CJ, Beresford SA, Omenn GS, Motulsky AG: **A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. Probable benefits of increasing folic acid intakes.** *JAMA* 1995, **274**:1049–1057.
17. Langman LJ, Ray JG, Evrovski J, Yeo E, Cole DE: **Hyperhomocyst(e)inemia and the increased risk of venous thromboembolism: more evidence from a case–control study.** *Arch Intern Med* 2000, **160**:961–964.
18. Evrovski J, Callaghan M, Cole DEC: **Determination of homocysteine by HPLC with pulsed integrated amperometry.** *Clin Chem* 1995, **41**:757–758.
19. Lahiri DK, Bye S, Nurnberger JI Jr, Hodes ME, Crisp M: **A non-organic and non-enzymatic extraction method gives higher yields of genomic DNA from whole-blood samples than do nine other methods tested.** *J Biochem Biophys Meth* 1992, **25**: 193–205.
20. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM: **A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis.** *Blood* 1996, **88**:3698–8703.
21. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, *et al*: **A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase.** *Nature Genet* 1995, **10**:111–113.
22. Langman LJ, Wong BYL, Boggis C, Rubin LA, Cole DEC: **The prevalence and linkage disequilibrium of three methylenetetrahydrofolate reductase (MTHFR) gene polymorphisms varies in different ethnic groups [abstract].** *J Vasc Res* 1998, **35**:4S.
23. Wilson A, Platt R, Wu Q, Leclerc D, Christensen B, Yang H, Gravel RA, Rozen R: **A common variant in methionine synthase reductase combined with low cobalamin (vitamin B_{12}) increases risk for spina bifida.** *Mol Genet Metab* 1999, **67**: 317–323.
24. Ray J: **A meta-analysis of hyperhomocyst(e)inemia and the risk of venous thromboembolic disease.** *Arch Intern Med* 1998, **158**:2101–2106.
25. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R: **Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations.** *Circulation* 1996, **93**:7–9.
26. Bloemenkamp KW, Rosendaal FR, Buller HR, Helmerhorst FM, Colly LP, Vandenbroucke JP: **Risk of venous thrombosis with use of current low-dose oral contraceptives is not explained by diagnostic suspicion and referral bias.** *Arch Intern Med* 1999, **159**:65–70.
27. Rosendaal FR, Doggen CJ, Zivelin A, Arruda VR, Aiach M, Siscovick DS, Hillarp A, Watzke HH, Bernardi F, Cumming AM, Preston FE, Reitsma PH: **Geographic distribution of the 20210 G to A prothrombin variant.** *Thromb Haemost* 1998, **79**:706–708.
28. Ducloux D, Motte G, Chalopin JM: **Homocysteine in renal disease.** *Clin Lab* 2000, **46**:141–51.
29. Nygard O, Vollset SE, Refsum H, Stensvold I, Tverdal A, Nordrehaug JE, Ueland M, Kvale G: **Total plasma homocysteine and cardiovascular risk profile. The Hordaland Homocysteine Study.** *JAMA* 1995, **274**:1526–1533.
30. Kato I, Dnistrian AM, Schwartz M, Toniolo P, Koenig K, Shore RE, Akhmedkhanov A, Zeleniuch-Jacquotte A, Riboli E: **Serum folate, homocysteine and colorectal cancer risk in women: a nested case–control study.** *Br J Cancer* 1999; 79:1917–1922.
31. Ray JG, Cole DE, Boss SC: **An Ontario-wide study of vitamin B12, serum folate, and red cell folate levels in relation to plasma homocysteine: is a preventable public health issue on the rise?** *Clin Biochem* 2000, **33**:337–343.
32. Homocysteine Lowering Trialists' Collaboration: **Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials.** *Br Med J* 1998, **316**:894–898.
33. Brattstrom L, Wilcken DE, Ohrvik J, Brudin L: **Common methylenetetrahydrofolate reductase gene mutation leads to hyperhomocysteinemia but not to vascular disease: the result of a meta-analysis.** *Circulation* 1998, **98**:2520–2526.
34. Ridker PM, Hennekens CH, Selhub J, Miletich JP, Malinow MR, Stampfer MJ: **Interrelation of hyperhomocyst(e)inemia, factor V Leiden, and risk of future venous thromboembolism.** *Circulation* 1997, **95**:1777–1782.
35. Eichinger S, Stumpflen A, Hirschl M, Bialonczyk C, Herkner K, Stain M, Schneider B, Pabinger I, Lechner K, Kyrle PA: **Hyperhomocysteinemia is a risk factor of recurrent venous thromboembolism.** *Thromb Haemost* 1998, **80**:566–569.
36. Willems HP, den Heijer M, Bos GM: **Homocysteine and venous thrombosis: outline of a vitamin intervention trial.** *Semin Thromb Hemost* 2000, **26**:297–304.
37. Eekhoff EM, Rosendaal FR, Vandenbroucke JP: **Minor events and the risk of deep venous thrombosis.** *Thromb Haemost* 2000, **83**:408–411.
38. Levine M, Rickles FR: **Treatment of venous thromboembolism in cancer patients.** *Haemostasis* 1998, **28(suppl 3)**: S66–S70.
39. Lowe G, Woodward M, Vessey M, Rumley A, Gough P, Daly E: **Thrombotic variables and risk of idiopathic venous thromboembolism in women aged 45–64 years. Relationships to hormone replacement therapy.** *Thromb Haemost* 2000, **83**: 530–535.
40. Ryan DH, Crowther MA, Ginsberg JS, Francis CW: **Relation of factor V Leiden genotype to risk for acute deep venous thrombosis after joint replacement surgery.** *Ann Intern Med* 1998, **128**:270–276.
41. Leroyer C, Mercier B, Oger E, Chenu E, Abgrall JF, Ferec C, Mottier C: **Prevalence of 20210 A allele of the prothrombin gene in venous thromboembolism patients.** *Thromb Haemost* 1998, **80**:49–51.
42. Margaglione M, Brancaccio V, Giuliani N, D'Andrea G, Cappucci G, Iannaccone L, Vecchione G, Grandone E, Di Minno G: **Increased risk for venous thrombosis in carriers of the prothrombin G→A20210 gene variant.** *Ann Intern Med* 1998, **129**: 89–93.
43. Alhenc-Gelas M, Arnaud E, Nicaud V, Aubry ML, Fiessinger JN, Aiach M, Emmerich J: **Venous thromboembolic disease and the prothrombin, methylene tetrahydrofolate reductase and factor V genes.** *Thromb Haemost* 1999, **81**:506–510.
44. Salomon O, Steinberg DM, Zivelin A, Gitel S, Dardik R, Rosenberg N, Berliner S, Inbal A, Many A, Lubetsky A, Varon D, Martinowitz U, Seligsohn U: **Single and combined prothrombotic factors in patients with idiopathic venous thromboembolism: prevalence and risk assessment.** *Arterioscler Thromb Vasc Biol* 1999, **19**:511-518.
45. Hillarp A, Zoller B, Svensson PJ, Dahlback B: **The 20210 A allele of the prothrombin gene is a common risk factor among Swedish outpatients with verified deep venous thrombosis.** *Thromb Haemost* 1997, **78**:990–992.
46. Cattaneo M, Chantarangkul V, Taioli E, Santos JH, Tagliabue L: **The G20210A mutation of the prothrombin gene in patients with previous first episodes of deep-vein thrombosis: prevalence and association with factor V G1691A, methylenetetrahydrofolate reductase C677T and plasma prothrombin levels.** *Thromb Res* 1999, **93**:1–8.
47. Grandone E, Margaglione M, Colaizzo D, D'Andrea G, Cappucci G, Brancaccio V, Di Minno G: **Genetic susceptibility to pregnancy-related venous thromboembolism: roles of factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations.** *Am J Obstet Gynecol* 1998, **179**: 1324–1328.
48. Garcia-Gala JM, Alvarez V, Pinto CR, Soto I, Urgelles MF, Menendez MJ, Carracedo C, Lopez-Larrea C, Coto E: **Factor V Leiden (R506Q) and risk of venous thromboembolism: a case-control study based on the Spanish population.** *Clin Genet* 1997, **52**:206–210.
49. Ocal IT, Sadeghi A, Press RD: **Risk of venous thrombosis in carriers of a common mutation in the homocysteine regulatory enzyme methylenetetrahydrofolate reductase.** *Mol Diagn* 1997, **2**:61–68.

50. de Moerloose P, Reber G, Perrier A, Perneger T, Bounameaux H: **Prevalence of factor V Leiden and prothrombin G20210A mutations in unselected patients with venous thromboembolism.** *Br J Haematol* 2000, **110**:125–129.
51. Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH: **High risk of thrombosis in patients homozygous for factor V Leiden (activated protein C resistance).** *Blood* 1995, **85**:1504–1508.
52. Svensson PJ, Zoller B, Mattiasson I, Dahlback B: **The factor VR506Q mutation causing APC resistance is highly prevalent amongst unselected outpatients with clinically suspected deep venous thrombosis.** *J Intern Med* 1997, **241**:379–378.
53. Akar N, Akar E, Akcay R, Avcu F, Yalcin A, Cin S: **Effect of methylenetetrahydrofolate reductase 677 C-T, 1298 A-C, and 1317 T-C on factor V 1691 mutation in Turkish deep vein thrombosis patients.** *Thromb Res* 2000, **97**:163–167.
54. Brown K, Luddington R, Baglin T: **Effect of the MTHFRC677T variant on risk of venous thromboembolism: interaction with factor V Leiden and prothrombin (F2G20210A) mutations.** *Br J Haematol* 1998, **103**:42–44.
55. Gemmati D, Previati M, Serino ML, Moratelli S, Guerra S, Capitani S, Forini E, Ballerini G, Scapoli GL: **Low folate levels and thermolabile methylenetetrahydrofolate reductase as primary determinant of mild hyperhomocystinemia in normal and thromboembolic subjects.** *Arterioscler Thromb Vasc Biol* 1999, **19**:1761–1767.
56. Rintelen C, Mannhalter C, Lechner K, Eichinger S, Kyrle PA, Papagiannopoulos M, Schneider B, Pabinger I: **No evidence for an increased risk of venous thrombosis in patients with factor V Leiden by the homozygous 677 C to T mutation in the methylenetetrahydrofolate-reductase gene.** *Blood Coagul Fibrinolysis* 1999, **10**:101–105.
57. Lin JS, Shen MC, Tsai W, Lin B: **The prevalence of C677T mutation in the methylenetetrahydrofolate reductase gene and its association with venous thrombophilia in Taiwanese Chinese.** *Thromb Res* 2000, **97**:89–94.
58. Tosetto A, Missiaglia E, Frezzato M, Rodeghiero F: **The VITA project: C677T mutation in the methylene-tetrahydrofolate reductase gene and risk of venous thromboembolism.** *Br J Haematol* 1997, **97**:804–806.
59. Margaglione M, D'Andrea G, d'Addedda M, Giuliani N, Cappucci G, Iannaccone L, Vecchione G, Grandone E, Brancaccio V, Di Minno G: **The methylenetetrahydrofolate reductase TT677 genotype is associated with venous thrombosis independently of the coexistence of the FV Leiden and the prothrombin A20210 mutation.** *Thromb Haemost* 1998, **79**:907–911.
60. Kluijtmans LA, den Heijer M, Reitsma PH, Heil SG, Blom HJ, Rosendaal FR: **Thermolabile methylenetetrahydrofolate reductase and factor V Leiden in the risk of deep-vein thrombosis.** *Thromb Haemost* 1998, **79**:254–258.