Common C677T Polymorphism of the Methylenetetrahydrofolate Reductase Gene and the Risk of Venous Thromboembolism: Meta-Analysis of 31 Studies

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Key Words

Venous thromboembolism · Deep vein thrombosis · Pulmonary embolism · Homocysteine · Methylenetetrahydrofolate reductase · Thrombophilia · Genetics · Meta-analysis

Abstract

Background: Although the common 677 C → T polymorphism of the methylenetetrahydrofolate reductase (MTHFR) gene is implicated in the development of hyperhomocysteinemia, its correlation with venous thromboembolism (VTE) remains controversial. We conducted a meta-analysis of previously published studies to clarify the role of the MTHFR 677 TT homozygous genotype in association with VTE. **Methods and Results:** Relevant articles were retrieved from a systematic search of Medline and Embase from 1990 to September 2001. Two independent reviewers abstracted data on the characteristics of the cases with VTE and controls without VTE. We pooled the rates of the MTHFR 677 TT genotype in both groups, as well as the odds ratio (OR) of VTE in the presence of the TT versus CC or CT genotypes. In 31 published studies, comprising 4,901 cases and 7,886 controls, the pooled prevalence of the MTHFR 677 TT genotype was slightly higher among cases (14.3%) than controls (11.7%), conferring a borderline degree of heightened risk [pooled OR (OR_p) 1.2, 95% confidence interval

(CI) 1.1–1.4]. After excluding cases with a classic thrombophilia factor, the pooled prevalence rates of the MTHFR 677 TT genotype among 11 studies were 17.7 and 12.3%, respectively (OR_p 1.5, 95% CI 1.2–1.9). **Conclusions:** The classic MTHFR C677T gene polymorphism is weakly associated with an increased risk of VTE. It is unlikely that the purported relationship between hyperhomocysteinemia and VTE is mediated by this gene defect to a substantial degree, although other undiscovered gene polymorphisms may explain this association. Until more compelling data are made available, we do not recommend that testing for the MTHFR C677T polymorphism be routinely included as part of any clinical thrombophilia assessment.

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Introduction

Elevated plasma homocysteine (tHcy) has been associated with an increased risk of venous thromboembolism (VTE) [1]. Hyperhomocysteinemia may result from inadequate folic acid or cobalamin stores, renal insufficiency or one of several gene-based mechanisms, including the common thermolabile variant of 5,10-methylenetetrahydrofolate reductase (MTHFR), an enzyme that provides the folate derivative for remethylation of tHcy to methionine [2, 3]. The enzyme defect often results from a mis-

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sense mutation at base pair 677 C → T of MTHFR, which, in its homozygous (TT) state, leads to the phenotypic expression of the enzyme defect [3].

Although hyperhomocysteinemia may be an independent risk factor for cardiovascular disease [4, 5], a meta-analysis of 23 case-control studies failed to show a direct association between MTHFR C677T homozygosity and cardiovascular disease [odds ratio (OR) 1.1, 95% confidence interval (CI) 0.9–1.4] [6]. Similarly, there have been conflicting opinions about the importance of the MTHFR C677T polymorphism as a risk factor for VTE [7, 8]. To clarify the thrombophilic role of MTHFR C677T, we conducted a meta-analysis of published studies examining the association between the MTHFR C677T homozygous genotype and VTE.

Methods

Literature Search

Two authors (D.S. and J.G.R.) searched both PubMed Medline and Embase from 1990 to September 2001, merging the following textword and MeSH headings: ('MTHFR' or 'methylenetetrahydrofolate reductase') and ('deep vein thrombosis', 'venous thromboembolism', 'venous thrombosis', 'pulmonary embolism' or 'thrombophlebitis'). We also examined the references of all studies for other potential citations, but did not search for unpublished studies. English-language reports that met all of the following criteria were included: (1) use of a cohort or case-control design; (2) cases were aged 16 years and older; (3) at least 20 cases were included, and (4) the presence of the MTHFR C677 gene polymorphism was assessed both among subjects with (i.e. cases) and without (i.e. controls) VTE. In those situations in which it was unclear whether cases had been used in more than one published study, we contacted the principal author by E-mail to ask for clarification.

Table 1. Characteristics of study participants

Study	Cases with VTE							Controls without VTE		
	source and definition	excluded those cancer or situational VTE?	excluded those with a 'classic' thrombophilia marker? ^{†1}	number of males	mean/median age years	VTE type	objective assessment of VTE?	source and definition	number of males	mean/median age years
Arruda et al. 1997 [7]	Brazilian outpatients with VTE	yes	yes	51 (40)	35	DVT or PE	yes	consecutive newborns delivered at same hospital	NA	new-born
Cattaneo et al. 1997 [12]	Italian patients with DVT	yes	no	35 (45)	39	DVT or PE	yes	healthy adults without a history of VTE or arterial occlusive disease	70 (45)	43
Ocal et al. 1997 [13]	US patients with VTE and available blood samples	no	no	145 (44)	44	DVT or PE	NA	healthy blood donors	84 (50)	46
Salden et al. 1997 [14]	UK patients with VTE	yes	yes	NA	43	DVT or PE	yes	healthy plasma donors	NA	34
Tosetto et al. 1997 [15]	Italian patients with VTE	no	no	21 (32)	52	DVT or PE	yes	individuals randomly selected from the population without a history of VTE	42 (32)	51
Brown et al. 1998 [16]	UK patients with VTE	no	no	NA	45	VTE	yes	blood donors from same geographic area as cases	NA	38
Dilley et al. 1998 [17]	African-American outpatients with VTE	no	no	39 (42)	55	DVT or PE	yes	African-American outpatients undergoing routine laboratory tests without a history of VTE or CVD	91 (49)	56
Grandone et al. 1998 [18]	Italian women with VTE during pregnancy or puerperium	no	no	0 (0)	36	DVT or PE	yes	parous women without a history of VTE or arterial thrombosis	0 (0)	34
Kluijtmans et al. 1998 [19]	Dutch anticoagulant clinic patients with first DVT	no	no	205 (44)	44	DVT	yes	individuals without a history of VTE	206 (44)	44
Margaglione et al. 1998 [20]	Italian patients with first VTE	yes	yes	98 (44)	38	DVT or PE	yes	healthy individuals without a history of VTE	244 (56)	36
Rintelen et al. 1999 [21]	Austrian patients with VTE	yes	yes	27 (3)	41	DVT or PE	yes	hospital staff without a history of VTE or arterial thrombosis	NA	36
Alhenc-Gelas et al. 1999 [22]	French patients aged ≤60 years with ≥1 VTE events	no	no	92 (45)	42	DVT or PE	yes	healthy individuals for routine checkup without a history of VTE	199 (50)	43

Table 1 (continued)

Study	Cases with VTE							Controls without VTE		
	source and definition	excluded those cancer or situational VTE?	excluded those with a 'classic' thrombophilia marker? ¹	number of males	mean/median age years	VTE type	objective assessment of VTE?	source and definition	number of males	mean/median age years
Cattaneo et al. 1999 [23]	Italian patients with first DVT	yes	no	45 (38)	39	DVT	yes	healthy individuals without a history of VTE	164 (39)	43
Ho 2000 [24]	Taiwanese Chinese with VTE	no	no	28 (57)	58	NA	yes	individuals presenting for a routine checkup or outpatient blood work, without a history of VTE	830 (70)	48
Franco et al. 1999 [25]	Brazilian patients aged ≤65 years with DVT	yes	no	72 (38)	41	DVT	yes	healthy blood donors without a personal or family history of VTE	72 (38)	41
Gemmati et al. 1999 [26]	Italian patients aged ≤75 years with ≥1 VTE event	yes	yes	124 (56)	54	DVT or PE	yes	blood donors without a personal or family history of VTE	135 (61)	46
Gemmati et al. 1999 [27]	Italian patients aged ≤65 years with ≥1 VTE event	yes	yes	89 (49)	50	DVT or PE	yes	blood donors without a personal or family history of VTE	100 (50)	49
Salomon et al. 1999 [28]	Israeli patients with ≥1 VTE event	yes	yes	67 (41)	42	DVT or PE	yes	patients consecutively admitted to hospital without a history of VTE	153 (46)	71
Lin et al. 2000 [29]	Chinese Taiwanese patients with VTE	yes	yes	56 (50)	48	DVT or PE	yes	healthy individuals without a history of VTE	67 (54)	46
Akar et al. 2000 [30]	Turkish patients with DVT and no atherothrombosis	no	no	NA	NA	DVT	yes	individuals without a personal or family history of VTE or atherothrombosis	NA	NA
Angchaisuksiri et al. 2000 [31]	Thai patients with VTE	no	no	18 (45)	38	DVT or PE	yes	healthy adults	NA	NA
Couturaud et al. 2000 [32]	French patients with VTE	yes	no	171 (47)	65	DVT or PE	yes	volunteer blood donors	NA	NA
De Franchis et al. 2000 [33]	Italian patients aged <45 years with VTE	yes	yes	90 (47)	45	DVT or PE	yes	healthy individuals aged <45 years without a history of VTE	101 (50)	41
Fujimara et al. 2000 [34]	Japanese outpatients with VTE	no	yes	28 (39)	56	DVT or PE	yes	outpatients attending a venous clinic for management of varicose veins, without sonographic evidence of DVT	32 (38)	53
Gerhardt et al. 2000 [35]	German women with VTE during pregnancy or puerperium	yes	no	0 (0)	34	DVT or PE	yes	female blood donors, previously pregnant, without a history of VTE	0 (0)	34
Toydemir et al. 2000 [36]	Turkish patients with VTE	no	no	20 (67)	51	DVT	yes	healthy individuals without a history of VTE or arterial thrombosis	37 (37)	32
Zheng et al. 2000 [37]	Chinese patients with DVT	no	yes	33 (62)	44	DVT	yes	hospital blood donors and staff members	64 (52)	52
Guedon et al. 2001 [38]	French patients with VTE and no inflammatory bowel disease	no	no	32 (29)	49	NA	NA	individuals without a personal or family history of thrombosis or inflammatory bowel disease	34 (41)	36
Hanson et al. 2001 [39]	US patients with DVT	no	no	66 (48)	44	DVT	NA	employees of a university medical center without a history of VTE or CHD	205 (62)	49
Hsu et al. 2001 [40]	Chinese Taiwanese patients with VTE	no	no	82 (77)	61	DVT or PE	yes	patients without a history of VTE or CHD	82 (77)	61
Ray et al. 2001 [41]	Canadian women with VTE and no CVD	no	no	0 (0)	46	DVT or PE	yes	healthy women without a history of VTE or CVD	0 (0)	41

Figures in parentheses represent percentages. NA = Not available; DVT = deep vein thrombosis; PE = pulmonary embolism; CHD = coronary heart disease; CVD = cardiovascular disease.

¹ Defined as a deficiency of either protein C, protein S or antithrombin or the presence of antiphospholipid antibodies.

Data Abstraction

Results were independently abstracted by two authors (W.S.C. and J.G.R.), as listed in tables 1–3. We collected data on the absolute number of individuals with and without VTE who had the MTHFR C677T wild-type (CC), heterozygous (CT) or homozygous (TT) genotypes (tables 2, 3). Again, authors were contacted by E-mail if the latter figures were incomplete. We also abstracted information about the methods used to recruit the cases and controls, and whether there was exclusion of those with VTE related to surgery, trauma or cancer or a known ‘classic’ thrombophilia factor (i.e. a deficiency of either protein C, protein S or antithrombin or the presence of antiphospholipid antibodies). Finally, we assessed whether cases were diagnosed with VTE using objective diagnostic methods, such as compression ultrasonography for deep vein thrombosis of the limb and ventilation-perfusion scanning for pulmonary embolism.

Statistical Analysis

In the primary analysis, the rate of the MTHFR C677T TT genotype was calculated among cases and controls and compared to the presence of either the CT or CC genotype. An unadjusted OR and its 95% CI was calculated for each study, and then pooled (OR_p) using a random effects model, which considers both between-study and within-study variability [9]. The prevalence rates of the TT genotype among cases and controls were pooled using the same random effects method. A second analysis excluded those studies that did not explicitly describe the use of objective testing methods to diagnose VTE.

A third and fourth analysis comprised only those studies that systematically excluded cases with malignancy or VTE in the presence of surgery or trauma (i.e. situational VTE), as well as a ‘classic’ thrombophilia factor. Heterogeneity across studies was assessed using the Breslow and Day [10] test, with statistical significance set at $p < 0.10$. The applied statistical software program used was Meta-Analyst^{0.988} [11].

Table 2. Number and rate (in parentheses) of the C677T MTHFR genotypes and the risk of VTE from all 31 studies

Study	Cases with VTE				Controls without VTE			
	CC	CT	TT	total	CC	CT	TT	total
Arruda et al., 1997 [7]	61 (48)	52 (41)	14 (11)	127 (100)	170 (58)	114 (38)	12 (4.0)	296 (100)
Cattaneo et al., 1997 [12]	25 (32)	36 (47)	16 (21)	77 (100)	45 (29)	74 (48)	35 (23)	154 (100)
Ocal et al., 1997 [13]	139 (42)	154 (47)	38 (11)	331 (100)	62 (37)	79 (47)	26 (16)	167 (100)
Salden et al., 1997 [14]	88 (41)	102 (47)	26 (12)	216 (100)	71 (43)	75 (46)	18 (11)	164 (100)
Tosetto et al., 1997 [15]	21 (32)	36 (55)	8 (12)	65 (100)	42 (32)	71 (55)	17 (13)	130 (100)
Brown et al., 1998 [16]	261 (47)	242 (43)	55 (10)	558 (100)	224 (45)	209 (42)	67 (13)	500 (100)
Dilley et al., 1998 [17]	77 (83)	15 (16)	1 (1)	93 (100)	152 (82)	29 (16)	4 (2)	185 (100)
Grandone et al., 1998 [18]	12 (29)	18 (43)	12 (29)	42 (100)	69 (32)	110 (52)	34 (16)	213 (100)
Kluijtmans et al., 1998 [19]	211 (45)	213 (45)	47 (10)	471 (100)	224 (47)	203 (43)	47 (10)	474 (100)
Margaglione et al., 1998 [20]	67 (30)	95 (43)	60 (27)	222 (100)	212 (49)	141 (33)	78 (18)	431 (100)
Rintelen et al., 1999 [21]	31 (38)	40 (49)	10 (12)	81 (100)	35 (45)	32 (42)	10 (13)	77 (100)
Alhenc-Gelas et al., 1999 [22]	91 (44)	88 (43)	26 (13)	205 (100)	156 (39)	193 (49)	49 (12)	398 (100)
Cattaneo et al., 1999 [23]	33 (29)	56 (50)	23 (21)	112 (100)	138 (34)	181 (45)	84 (21)	403 (100)
Ho, 2000 [24]	24 (49)	17 (35)	8 (16)	49 (100)	583 (49)	472 (40)	125 (11)	1,180 (100)
Franco et al., 1999 [25]	71 (37)	82 (43)	37 (20)	190 (100)	81 (43)	78 (41)	31 (16)	190 (100)
Gemmati et al., 1999 [26]	51 (23)	107 (49)	62 (28)	220 (100)	65 (30)	116 (53)	39 (18)	220 (100)
Gemmati et al., 1999 [27]	53 (29)	86 (48)	41 (23)	180 (100)	68 (34)	102 (51)	30 (15)	200 (100)
Salomon et al., 1999 [28]	56 (34)	69 (43)	37 (23)	162 (100)	136 (40)	152 (45)	48 (14)	336 (100)
Lin 2000 [29]	53 (47)	50 (45)	9 (8)	112 (100)	76 (61)	41 (33)	8 (6)	125 (100)
Akar et al., 2000 [30]	30 (44)	33 (48)	5 (8)	68 (100)	40 (61)	19 (29)	7 (10)	66 (100)
Angchaisuksiri et al., 2000 [31]	34 (85)	6 (15)	0 (0)	40 (100)	365 (73)	128 (26)	7 (1)	500 (100)
Couturaud et al., 2000 [32]	233 (64)	102 (28)	31 (8)	366 (100)	59 (56)	36 (34)	10 (10)	105 (100)
De Franchis et al., 2000 [33]	88 (46)	58 (30)	46 (24)	192 (100)	112 (46)	50 (24)	42 (20)	204 (100)
Fujimura et al., 2000 [34]	32 (44)	30 (42)	10 (14)	72 (100)	36 (42)	43 (51)	6 (7)	85 (100)
Gerhardt et al., 2000 [35]	50 (44)	53 (46)	11 (10)	114 (100)	101 (48)	91 (43)	20 (9)	212 (100)
Toydemir et al., 2000 [36]	15 (50)	12 (40)	3 (10)	30 (100)	39 (39)	51 (51)	10 (10)	100 (100)
Zhang et al., 2000 [37]	12 (23)	31 (58)	10 (19)	53 (100)	62 (51)	45 (37)	15 (12)	122 (100)
Guedon et al., 2001 [38]	45 (41)	52 (47)	13 (12)	110 (100)	32 (38)	43 (51)	9 (11)	84 (100)
Hanson et al., 2001 [30]	58 (42)	63 (46)	16 (12)	137 (100)	130 (40)	158 (48)	41 (12)	329 (100)
Hsu et al., 2001 [40]	60 (57)	40 (37)	7 (6)	107 (100)	55 (51)	44 (41)	8 (8)	107 (100)
Ray et al., 2001 [41]	49 (38)	61 (47)	19 (15)	129 (100)	72 (56)	44 (34)	13 (10)	129 (100)
Total number of individuals studied	4,901				7,886			
Pooled prevalence of TT genotype, %	14.3 (95% CI 12.0–16.9)				11.7 (95% CI 10.0–13.5)			
OR_p for VTE in the presence of the TT versus CC or CT genotype					1.2 (95% CI 1.1–1.4)			
Presence of heterogeneity for the OR_p ($p < 0.10$)?					yes			

Results

Study and Participant Characteristics

A total of 73 citations were retrieved on PubMed Medline and 12 through Embase. Thirty-one original studies from 16 countries met the inclusion criteria (tables 1, 2). The demographic characteristics of the cases and controls are listed in table 1. Most studies had an equal representation of men and women, but 3 studies included only women [18, 35, 41]. The mean age of both cases and controls was 45 years; the majority were aged 40–50 years.

MTHFR C677T Homozygosity and VTE Risk among All Study Participants

Only 4 of the 31 studies independently demonstrated a significant association between the MTHFR 677 homozygous state and VTE [7, 18, 20, 26] (fig. 1). The pooled prevalence of the MTHFR 677 TT genotype was higher among the 4,901 cases (14.3%, 95% CI 12.0–16.9%) than the 7,886 unaffected controls (11.7%, 95% CI 10.0–13.5%) (OR_p 1.2, 95% CI 1.1–1.4) (table 2; fig. 1). There was, however, evidence of statistical heterogeneity for this pooled estimate ($p < 0.10$).

Secondary Analyses of MTHFR C677T Homozygosity and VTE Risk

After excluding 3 studies that did not report on the use of objective VTE testing [13, 38, 39], the associated risk for VTE in the presence of MTHFR 677 TT was not altered substantially from that observed in the main analysis (OR_p 1.3, 95% CI 1.1–1.6). An analysis of the 14 studies that excluded individuals with VTE in the presence of malignancy or situational risk factors (table 1) also showed no difference in the risk for VTE (OR_p 1.4, 95% CI 1.2–1.6). Finally, 11 studies formally excluded cases identified as having a ‘classic’ thrombophilia factor (i.e. protein C, protein S or antithrombin deficiency or the presence of antiphospholipid antibodies) (tables 1, 3). For these studies, the pooled prevalence of the MTHFR 677 TT genotype was 17.7 (95% CI 13.7–22.5%) among 1,171 cases and 12.3% (95% CI 9.5–15.7%) among 2,231 controls (OR_p 1.5, 95% CI 1.2–1.9). There was no evidence of statistical heterogeneity for any of these secondary analyses ($p > 0.10$).

Table 3. Number and rate (in parentheses) of the C677T MTHFR genotypes and the risk of VTE after the exclusion of cases with ‘classic’ thrombophilia factors

Study	Cases with VTE				Controls without VTE			
	CC	CT	TT	total	CC	CT	TT	total
Arruda et al., 1997 [7]	49 (49)	41 (41)	10 (10)	100 (100)	170 (58)	114 (38)	12 (4)	296 (100)
Salden et al., 1997 [14]	63 (41)	72 (47)	17 (12)	216 (100)	71 (43)	75 (46)	18 (11)	164 (100)
Margaglione et al., 1998 [20]	NA	NA	28 (24)	119 (100)	NA	NA	78 (18)	431 (100)
Rintelen et al., 1999 [21]	31 (38)	40 (49)	10 (12)	81 (100)	35 (45)	32 (42)	10 (13)	77 (100)
Gemmati et al., 1999 [26]	51 (23)	107 (49)	62 (28)	220 (100)	65 (30)	116 (53)	39 (18)	220 (100)
Gemmati et al., 1999 [27]	11 (22)	24 (48)	15 (30)	50 (100)	62 (33)	100 (53)	27 (14)	189 (100)
Salomon et al. 1999 [28]	NA	NA	10 (12)	80 (100)	NA	NA	46 (14)	336 (100)
Lin et al., 2000 [29]	19 (36)	26 (50)	7 (14)	52 (100)	64 (60)	37 (35)	6 (6)	107 (100)
De Franchis et al., 2000 [33]	88 (46)	58 (30)	46 (24)	192 (100)	112 (46)	50 (24)	42 (20)	204 (100)
Fujimara et al., 2000 [34]	19 (40)	25 (52)	4 (8)	48 (100)	36 (42)	43 (51)	6 (7)	85 (100)
Zheng et al., 2000 [37]	12 (23)	31 (58)	10 (19)	53 (100)	62 (51)	45 (37)	15 (12)	122 (100)
Total number of individuals studied	1,171				2,231			
Pooled prevalence of TT genotype, %	17.7 (95% CI 13.7–22.5)				12.3 (95% CI 9.5–15.7)			
OR _p for VTE in the presence of the TT versus CC or CT genotype	1.5 (95% CI 1.2–1.9)							
Presence of heterogeneity for the OR _p (p < 0.10)?	no							
'Classic' thrombophilia factors were defined as a deficiency of either protein C, protein S or antithrombin or the presence of antiphospholipid antibodies. NA = Data not available.

Fig. 1. MTHFR 677 TT versus CC or CT genotypes and the risk of VTE.

Discussion

Principal Findings

We examined 31 published studies, comprising nearly 13,000 individuals, and demonstrated a weak association between the MTHFR 677 TT genotype and VTE. This relationship was only slightly more impressive after excluding individuals with malignancy or situational VTE or a classic thrombophilia factor.

Strengths and Weaknesses

A major strength of this review was the large number of individuals and participating countries included herein. Most studies contained at least 50 cases with objectively confirmed deep vein thrombosis or pulmonary embolism,

the majority being middle aged and without malignancy or situational risk factors for VTE.

Such features make these data applicable to many patients with idiopathic VTE in whom a thrombophilia workup might be considered. Had publication bias been a major factor herein, then it is likely that the observed effect size would have been deflated further had unpublished studies been included as well [42].

Sources of Heterogeneity

The presence of statistical heterogeneity detected in the primary analysis of all 31 studies might be explained, in part, by the different methods used to recruit cases and controls. For example, some cases were enlisted at the time of their initial hospitalization for VTE, while others

were referred to a specialized clinic at a later date. Similarly, study controls were a heterogeneous group of individuals, ranging from newborn infants [7] to hospitalized adults [28]. The variety of countries included herein is another likely source of heterogeneity, since ethnogeographic differences in the prevalence of the MTHFR C677T polymorphism have been demonstrated [43, 44].

Clinical Considerations

Given the weak direct relationship between the MTHFR C677T polymorphism and VTE, we do not believe that this test should be routinely included in any clinical thrombophilia workup until more compelling data are made available. Our data parallel those of Brattstrom et al. [6], whose meta-analysis failed to demonstrate an association between MTHFR C677T homozygosity and arterial disease (OR_p 1.1, 95% CI 0.9–1.4). Since we did not assess the influence of the MTHFR C677T polymorphism in conjunction with either folate or cobalamin

deficiency or in the presence of other genetic thrombophilia factors [45], we cannot exclude a possible role therein [44]. However, we recently observed that none of three MTHFR polymorphisms (C677T, A1298C and T1317C), nor the A66G polymorphism in methionine synthase reductase, were demonstrable risk factors for VTE, either alone or in conjunction with fasting plasma hyperhomocysteinemia [41].

Future Research

The purported positive relationship between elevated tHcy and VTE [1] requires further explication beyond the MTHFR C677T polymorphism. The current challenge is to understand which other gene(s) play a role not only in the elevation of tHcy, but also in the development of idiopathic VTE. A large prospective population-based cohort study [46] is one appropriate way to evaluate both the environmental and multigenic factors that predispose to VTE [47].

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