(*Arteriosclerosis, Thrombosis, and Vascular Biology.* 1997;17:1662-1666.)

A Common Mutation in the Methylenetetrahydrofolate Reductase Gene (C677T) Increases the Risk for Deep-Vein Thrombosis in Patients With Mutant Factor V (Factor V:Q^{506})

Marco Cattaneo; Michael Y. Tsai; Paolo Bucciarelli; Emanuela Taioli; Maddalena L. Zighetti; Michelle Bignell; ; Pier Mannuccio Mannucci

From the A. Bianchi Bonomi Hemophilia and Thrombosis Center, Institute of Internal Medicine (M.C., P.B., M.L.Z., P.M.M.), and the Epidemiology Unit (E.T.), IRCCS Maggiore Hosptital, University of Milano, Italy; the Department of Environmental Medicine, Epidemiology Program, New York University Medical Center (E.T.); and the Clinical Chemistry Section, University of Minnesota Hospital and Clinic, Department of Laboratory Medicine and Pathology, Minneapolis (M.Y.T., M.B.).

Abstract

Abstract Hyperhomocysteinemia is a frequent risk factor for deep-vein thrombosis. A common mutation (C677T) in the gene encoding for methylenetetrahydrofolate reductase (MTHFR) is responsible, in the homozygous state, for decreased enzyme activity and mild hyperhomocysteinemia and is associated with increased risk for cardiovascular disease. We studied the prevalence of C677T MTHFR in 77 patients with deep-vein thrombosis and in 154 age- and sex-matched healthy control subjects. In the same individuals, we also evaluated the frequency of the coexistence of C677T MTHFR with mutant factor V:Q^{506}, a common risk factor for deep-vein thrombosis. Sixteen patients (20.8%) and 35 control subjects (22.7%) were homozygous for the C677T MTHFR mutation (odds ratio [OR]=0.8, 95% confidence interval [CI]=0.4-2.0). Sixteen patients (20.8%) and 4 control subjects (2.6%) had factor V:Q^{506}; of them, 10 patients and 3 control subjects had isolated factor V:Q^{506} (adjusted OR=6.3, 95% CI=1.6-25.3) and 6 patients and 1 control subject also had C677T MTHFR (adjusted OR=17.3, 95% CI=2.0-152.9). The OR for the coexistence of the two mutations was 65% to 75% higher than the expected joint effect calculated by either an additive (OR=6.0) or multiplicative(OR=4.4) model . The homozygous C677T mutation of MTHFR per se is not a risk factor for deep-vein thrombosis but increases the risk associated with factor V:Q^{506}. Due to the high prevalence of C677T MTHFR, it is likely that previous studies, which did not look for this mutation, overestimated the relative risk of thrombosis associated with factor V:Q^{506} alone.

Key Words: hyperhomocysteinemia • deep-vein thrombosis • methylenetetrahydrofolate reductase • factor V:Q^{506} (or factor V Leiden) • homocysteine

Introduction

Thrombophilia is a tendency to venous thromboembolism that can be acquired or determined genetically.[1] In the last few years, the previously held concept that inherited thrombophilia is due to alterations of a single gene has been challenged. It is now accepted that in thrombophilia multiple gene defects often coexist, with the clinical penetrance of the syndrome being a function of the number of defects present in an individual. This has been shown for patients with inherited deficiencies of antithrombin, protein C, or protein S,[2] [3] [4] whose risk of developing thrombotic manifestations is enhanced when there is coexistence of mutant factor V:Q^{506}. Mutant factor V:Q^{506} is responsible for resistance to activated protein C,[5] [6] is highly prevalent in populations of European descent (up to about 15%), and is the most common cause of inherited thrombophilia.[7]

We previously showed that hyperhomocysteinemia is associated with an increased risk for venous thrombosis in the young,[8] which was subsequently confirmed.[9] Later, it was demonstrated in patients who had suffered either recurrent[10] or first episodes of venous thrombosis.[11] Hyperhomocysteinemia may be caused by genetic abnormalities in either the transsulfuration pathway or the remethylation of homocysteine to methionine.[12] [13] In addition, acquired conditions such as vitamin B_{12} or folate deficiency can also lead to hyperhomocysteinemia.[12] [13] Although we showed that in about half of the patients the hyperhomocysteinemia was inherited,[8] we still have no direct demonstration of the association between gene defects causing hyperhomocysteinemia and venous thrombosis. In addition, it is unknown whether the association between hyperhomocysteinemia and other genetic risk factors for venous thrombosis increases the thrombotic risk.

A relatively common thermolabile variant of the enzyme MTHFR has been reported.[14] MTHFR synthesizes 5-methyltetrahydrofolate, the methyl donor in the conversion of homocysteine to methionine. The molecular basis of thermolability of MTHFR has recently been elucidated and is due to the C-to-T substitution at nucleotide 677 (C677T), which converts the codon for alanine to valine.[15] The thermolabile MTHFR has approximately 50% of the normal enzyme activity, and homozygosity for this mutation is associated with mild hyperhomocysteinemia and increased risk for cardiovascular disease.[16] In this study, the prevalence of the C677T mutation of MTHFR in patients with DVT was evaluated. We also examined whether the thrombotic risk that is associated with the presence of factor V:Q^{506} is modified by the coexistence of mutant MTHFR.

Methods

Seventy-seven consecutive patients, referred to our center between April 1995 and January 1996 to be screened for thrombophilic states because of previous episodes of DVT of the lower limb were enrolled in the study (Table 1✦). All diagnoses of DVT had been confirmed by phlebography or compression ultrasonography. Twenty-five patients also had pulmonary emboli, which were diagnosed by perfusion/ventilation scintigraphy or pulmonary angiography. One hundred fifty-four control subjects (M/F 70/84; median age 43 years, range 16 to 74 years) were chosen from the same geographical area and with the same cultural background as the study population. Previous episodes of venous thromboembolism and arterial occlusive disease were

excluded for all control subjects by a structured questionnaire validated for retrospective diagnosis of thrombosis.[17] No subject had abnormal liver or renal function or overt autoimmune or neoplastic diseases. All subjects gave their informed consent to the study.

Number [total (M/F)]	**77 (35/42)**
Age, y [median (range)]	**39 (19-69)**
Age at first episode of DVT, y [median (range)]	**30 (15-64)**
Time elapsed since last episode, mo [median (range)]	**12 (3-552)**
Type of episode	
DVT, n	**52**
DVT+pulmonary embolism, n	**25**
Number of thrombotic episodes	
One DVT only	**50**
Two or more	**27**
DVT only	**12**
Venous thrombosis of other districts	**15**
With circumstantial risk factors at first episode	**45**
Recent surgery	**12**
Trauma and immobilization	**10**
Pregancy/puerperium	**7**
Oral contraceptives	**16**

Table 1. Characteristics of Patients with Deep-Vein Thrombosis

After an overnight fast, venous blood samples were drawn at about 9 am into vacuum tubes containing 3.8% trisodium citrate (for coagulation measurements), EDTA (for measurement of homocysteine and extractions of leukocyte DNA), or no anticoagulant (for measurements of anticardiolipin antibodies, folates, and vitamin B_{12}). l-Methionine (3.8 g/m^2 body surface area) was then administered orally in about 200 mL of fruit juice. Four hours later, a second blood sample was drawn for plasma homocysteine assay.[8]

To detect the C677T mutation in the MTHFR gene, the polymerase chain reaction was performed as described by Frosst et al[15] on DNA extracted from peripheral leukocytes, followed by *Hin*fI restriction enzyme analysis. DNA analysis for G1691A substitution in coagulation factor V gene, responsible for factor V:Q^{506}, was carried out as described by de Ronde and Bertina.[18]

Total homocysteine levels were measured by high-performance liquid chromatography and fluorometric detection.[19] Hyperhomocysteinemia was diagnosed when fasting plasma homocysteine or post–methionine-load absolute increments above fasting levels exceeded the 95th percentile of distribution values in control subjects. Serum folates and vitamin B_{12} were measured by radioimmunoassay (Becton Dickinson).

Resistance to activated protein C, antithrombin, protein C, and total and free protein S were measured as described.[20]

Antiphospholipid antibodies were diagnosed when lupus anticoagulant and/or anticardiolipin antibodies were present. The lupus anticoagulant was diagnosed according to the criteria issued by the Scientific and Standardisation Committee (SSC) of the International Society on Thrombosis and Haemostasis,[21] and the presence of anticardiolipin antibodies was defined as IgG titers above 10 U, confirmed at least once after 8 weeks.[22]

Statistical Analysis

Continuous variables are presented as mean±SD. In univariate analyses, the Student *t* test was used to compare means; log transformation of the data was performed if necessary to obtain normalization. Pearson correlation coefficients were calculated to determine the association between folate and vitamin B_{12} levels, both basal and after an oral methionine load. Odds ratios (OR) and 95% CIs were calculated as a measure of the association between the DVT and the MTHFR and factor V:Q^{506} genotypes. Logistic regression was used to adjust the data for possible confounding factors.

Results

The mean (±SD) levels of fasting plasma homocysteine were 12.3±9.2 µmol/L for patients and 10.9±6.3 µmol/L for control subjects (not significant [NS]). The absolute increments of plasma homocysteine levels after the methionine load were 16.2±7.1 µmol/L for patients and 14.9±7.1 µmol/L for control subjects (NS). The 95th percentiles of fasting homocysteine levels and postload increments in control subjects were 19.5 µmol/L and 30.2 µmol/L. Of the 77 patients, 7 (9.1%) had fasting homocysteine levels and 4 (5.2%) had postload increments above the 95th percentile compared with 7 (4.5%) and 6 (3.9%) of 154 control subjects. Of these, 2 patients (2.6%) and 2 control subjects (1.3%) had both fasting levels and postload increments of homocysteine above the 95th percentile. The mean (±SD) serum concentration of folates and vitamin B_{12} was 5.4±2.7 ng/mL and 429±187 pg/mL for patients versus 6.1±2.7 ng/mL and 412±168 pg/mL for control subjects (NS).

The prevalence of the homozygous C677T mutation in the MTHFR gene (+/+ genotype) was similar for patients (n=16; 20.8%) and control subjects (n=35; 22.7%) (Table 2). Individuals with the +/+ genotype had higher plasma homocysteine levels and postload increments than individuals with the +/- or -/- genotypes (Table 3); there was no statistically significant difference in fasting homocysteine levels or postload increments between individuals with the +/- and -/- genotypes (Table 3). For the above reasons, the +/- and -/- genotypes were combined in the subsequent analyses. The fasting homocysteine levels were inversely correlated with folate concentration for control subjects (r=-.25, P=.0026) and cases (r=-.31, P=.0079). Postload homocysteine increments were inversely correlated with folate concentration in cases (r=-.28, P=.018), but not in control subjects (r=-.11, P=.17). Vitamin B_{12} concentration was inversely correlated with fasting homocysteine levels in the cases only (r=-.28, P=.018).

Genotype	DVT Patients (n=77)		Control Subjects (n=154)		OR (95% CI)
	%	n	%	n	
-/-	32.5	25	29.2	45	1.0
+/-	46.8	36	48.1	74	0.9 (0.5-1.7)
+/+	20.8	16	22.7	35	0.8 (0.4-1.9)

Table 2. Prevalence of MTHFR Genotypes Among Patients With DVT and Control Subjects

	Genotype		
	-/-	+/-	+/+
Control Subjects (n=154)			
Fasting homocysteine levels	9.7±5.3	10.6±5.9	13.2±7.6[1]
Postload absolute increments	14.1±6.9	14.1±6.3	17.7±8.3[2]
Patients (n=77)			
Fasting homocysteine levels	9.7±2.6	11.8±7.0	17.5±16.1[3]
Postload absolute increments	14.9±6.3	15.2±5.4	20.5±9.9[4]
All (n=231)			
Fasting homocysteine levels	9.7±4.5	10.9±6.2	14.6±11.0[5]
Postload absolute increments	14.4±6.7	14.5±6.1	18.6 ±8.8[6]

Table 3. Total Plasma Fasting Homocysteine Levels and Post–Methionine-Load Absolute Increments in DVT Patients and Control Subjects, by MTHFR Genotype

Values (in μmol/L) are given as mean±SD. Differences in fasting homocysteine levels and postload increments between the -/- and +/- genotypes were not statistically significant for any category of subjects.

[1] *P*=.04,

[2] *P*=.009,

[3] *P*=.1,

[4] *P*=.05

[5] *P*=.01

[6] *P*=.006 vs -/- and +/- combined.

Of the 77 patients, 4 (5.2%) had antithrombin deficiency, 2 (2.6%) had protein C deficiency, 1 (1.3%) had protein S deficiency, and 16 (20.8%) had factor V:Q^{506}. In the control group, 1 (0.6%) had antithrombin deficiency, none had protein C deficiency, 2 (1.3%) had protein S deficiency, and 4 (2.6%) had the factor V:Q^{506} mutation. Due to the high frequencies of homozygous mutant MTHFR (which is responsible for mild hyperhomocysteinemia) and mutant factor V, we calculated prevalences and ORs for thrombosis in individuals with both mutations or either one in relation to individuals with neither. Table 4 shows that the presence of the factor V mutation alone was associated with an OR of 7.6 and that the OR for the coexistence of the two mutations was 13.7. A similar relationship (OR of 6.3 versus 17.3) persisted after adjustment for quartiles of folate and vitamin B_{12} serum concentrations (Table 4). The OR for the coexistence of the two mutations was 65% to 75% higher than the expected joint effect calculated by either an additive (OR=6.0) or multiplicative (OR=4.4) model. The interaction between factor V:Q^{506} and mutant MTHFR was particularly evident in patients who developed spontaneous episodes of DVT, ie, those with no obvious association with circumstantial risk factors such as surgery, trauma/immobilization, pregnancy/puerperium, and oral contraceptive intake (Table 5).

Factor V:Q^{506}	MTHFR[1]	Cases, n	Control Subjects, n	OR (95% CI)	Adjusted OR[2] (95% CI)
-	-	51	116	1.0 (reference)	1.0 (reference)
-	+	10	34	0.7 (0.3-1.5)	0.7 (0.3-1.6)
+	-	10	3	7.6 (2.0-28.7)	6.3 (1.6- 25.3)
+	+	6	1	13.7 (1.6-116.3)	17.3 (2.0-152.9)

Table 4. Effect Modification of the Association Between Factor V:Q^{506} and Mutant MTHFR

[1] Genotypes -/- and +/- combined.
[2] Adjusted for quartiles of folate (0 to 4.2, 4.3 to 5.3, 5.4 to 7.9, and >7.9 ng/mL) and quartiles of vitamin B_{12} (0 to 301, 302 to 383, 384 to 499, and >499 pg/mL) serum levels.

FV:Q[506]	MTHFR[1]	Control Subjects, n	Cases					
			No Risk Factor		Surgery/Trauma		OC/Pregnancy, Puerperium	
			N	OR (95% CI)	n	OR (95% CI)	n	OR (95% CI)
-	-	116	21	1.0 (reference)	15	1.0 (reference)	15	1.0 (reference)
-	+	34	3	0.5 (0.1-1.7)	6	1.4 (0.5-3.8)	1	0.3 (0.1-1.8)
+	-	3	4	7.4 (1.5-35.3)	1	2.6 (0.3-26.4)	5	12.9 (2.8-59.5)
+	+	1	4	22.1 (2.4-207.6)	0	...	2	15.5 (1.3-181.0)

Table 5. Effect Modification of the Association Between Factor V:Q[506] and Mutant MTHFR, as a Function of the Presence and Type of Circumstantial Risk Factors for DVT

OC indicates oral contraceptives.

[1] Genotypes -/- and +/- combined.

Discussion

In 77 patients with DVT, we found a high percentage of patients who were homozygous for the C677T mutation in the MTHFR gene, which is responsible for the thermolabile variant of the enzyme and is associated with mild hyperhomocysteinemia. However, the same high prevalence of about 20% was also found in 154 healthy control subjects. Since the prevalence in control subjects is higher than that previously found in French Canadian (12%),[15] Dutch (5%),[16] and Australian[23] healthy individuals, we considered the possibility that our control group had been inappropriately selected. However, three lines of evidence are against this possibility: (1) control subjects that were matched with cases for sex, age, and cultural background were chosen from the same geographical area in Northern Italy as the study population; (2) we carefully ruled out that they had had previous episodes not only of venous thrombosis but also of arterial occlusive disease, for which the C677T MTHFR mutation is a risk factor[16] ; and (3), the prevalence of factor V:Q[506], a common risk factor for DVT, was of the same magnitude as that previously found in healthy Italian individuals.[24] Therefore, our data indicate that the homozygous C677T mutation in the MTHFR gene is very common in Northern Italy and that the mutation cannot be considered per se a risk factor for DVT.

The mean homocysteine levels in plasma from individuals who were homozygous for the C677T mutation in the MTHFR gene were significantly higher than in heterozygotes or subjects with the normal genotype. In contrast with a recent study,[25] we found that both fasting homocysteine levels and postload increments were high in

homozygotes for MTHFR mutation, indicating that the methionine loading test explores not only the transsulfuration pathway but also the remethylation pathway of homocysteine metabolism.[26]

Since homozygosity for the C677T mutation of MTHFR is associated with hyperhomocysteinemia, which is a risk factor for venous thrombosis, its lack of association with DVT is surprising and hard to interpret. Perhaps the very mild increase in homocysteine levels associated with homozygosity for C677T MTHFR is not sufficient per se to predispose to DVT. Indeed, it has been demonstrated that the risk of DVT does not change with homocysteine levels up to 22 μmol/L, above which it sharply increases, indicating a threshold effect of homocysteine rather than a continuous dose-response relation.[11] On the other hand, our finding that homozygosity for the C677T mutation of MTHFR increases the thrombotic risk for patients with factor V:Q^{506} suggests that lower levels of plasma homocysteine may be sufficient to synergize with other congenital or acquired risk factors for thrombosis.

This study evaluates the effects of the combination of homozygous C677T MTHFR and factor V:Q^{506}. Previously, the combined effects of hyperhomocysteinemia and factor V:Q^{506} were evaluated. A study of patients who had had a first episode of venous thrombosis was inconclusive, since the small number of subjects with both defects made the results statistically unstable and sensitive to the cutoff chosen for hyperhomocysteinemia.[11] On the other hand, the study by Mandel et al[27] showed that patients with the coexistence of severe hyperhomocysteinemia due to homozygous homocystinuria and factor V:Q^{506} have a higher risk of thrombosis than patients with homozygous homocystinuria alone. Our results extend the observation by these authors, indicating that patients with factor V:Q^{506} combined with the very mild hyperhomocysteinemia that is associated with the common C677T mutation of MTHFR are at higher risk of DVT than patients with either defect alone. The synergistic effect of factor V:Q^{506} and mutant MTHFR was particularly evident in patients who had spontaneous DVT; in contrast, factor V:Q^{506}, either alone or in combination with mutant MTHFR, did not increase the risk for DVT secondary to trauma or surgery. Due to the high frequency of the C677T mutation of MTHFR in the population (5% to 22%), it is likely that previous epidemiological studies, which did not look for this mutation, overestimated the relative risk of thrombosis associated with factor V:Q^{506}; the extent of this overestimation would be inversely correlated with the prevalence of the MTHFR mutation in the general population.

In conclusion, our study further supports the hypothesis that inherited thrombophilia is a multigene disorder, in which the likelihood to develop a thrombotic episode increases with the number of genetic risk factors present in a subject. Since the C677T mutation of MTHFR increases the risk of DVT for patients with factor V:Q^{506} and is very common, it should be searched for in patients with other genetic risk factors for thrombosis. The possibility of decreasing the thrombotic risk in affected patients by supplementation with folate is possible and should be tested in properly designed clinical trials.

Selected Abbreviations and Acronyms

CI	= confidence interval
DVT	= deep-vein thrombosis
MTHFR	= 5,10-methylenetetrahydrofolate reductase
OR	= odds ratio

Footnotes

Reprint requests to M. Cattaneo, MD, Hemophilia and Thrombosis Center, Via Pace 9, 20122 Milano, Italy.

Received January 17, 1997; accepted March 12, 1997.

References

1. De Stefano V, Finazzi G, Mannucci PM. Inherited thrombophilia: pathogenesis, clinical syndromes, and management. *Blood.* 1996;87:3531-3544.

2. Koeleman BPC, Reitsma PH, Allaart CF, Bertina RM. Activated protein C resistance as an additional risk factor for thrombosis in protein C deficient families. *Blood.* 1994;84:1031.

3. Zöller B, Berntsdotter A, Garcia de Frutos P, Dahlbäck B. Resistance to activated protein C as an additional genetic risk factor in hereditary deficiency of protein S. *Blood.* 1995;85:3518-3523.

4. Radtke K-P, Lane DA, Greengard JS, Ehrenforth S, Griffin JH, Scharrer I. Combined hereditary APC resistance and ATIII type II deficiency associated with venous thrombosis. *Thromb Haemost.* 1995;73:1377. Abstract.

5. Dahlbäck B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C: prediction of a cofactor to activated protein C. *Proc Natl Acad Sci U S A.* 1993;90:1004-1009.

6. Bertina RM, Koeleman BP, Koster T, Rosendal FR, Dirven RJ, de Ronde H, Van der Velde PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature.* 1994;369:64-67.

7. Dahlbäck B. Inherited thrombophilia: resistance to activated protein C as a pathogenic factor of venous thromboembolism. *Blood.* 1995;85:607-614.

8. Falcon C, Cattaneo M, Panzeri D, Martinelli I, Mannucci PM. High prevalence of hyperhomocyst(e)inemia in patients with juvenile venous thrombosis. *Arterioscler Thromb.* 1994;14:1080-1083.

9. Fermo I, Viganò D'Angelo S, Paroni R, Mazzola G, Calori G, D'Angelo A. Prevalence of moderate hyperhomocysteinemia in patients with early-onset venous and arterial occlusive disease. *Ann Intern Med.* 1995;123:747-753.

10. den Heijer M, Blom HJ, Gerrits WBJ, Rosendaal FR, Haak HL, Wijermans PW, Bos GMJ. Is hyperhomocysteinemia a risk factor for recurrent venous thrombosis? *Lancet.* 1995;345:882-885.

11. den Heijer M, Koster T, Blom HJ, Bos GMJ, Briët E, Reitsma PH, Vandenbrouke JP, Rosendaal FR. Hyperhomocysteinemia as a risk factor for deep-vein thrombosis. *N Engl J Med.* 1996;334:759-762.

12. McCully KS. Homocysteine and vascular disease. *Nat Med.* 1996;2:386-389.

13. Rees MM, Rodgers GM. Homocysteinemia: association of a metabolic disorder with vascular disease and thrombosis. *Thromb Res.* 1993;71:337-359.

14. Kang S-S, Zhou J, Wong PWK, Kowalisyn J, Strokosh G. Intermediate homocysteinemia: a thermolabile variant of methylenetetrahydrofolate reductase. *Am J Hum Genet.* 1988;43:414-421.

15. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJH, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet.* 1995;10:111-113.

16. Kluijtmans LAJ, van den Heauvel LPWJ, Boers GHJ, Frosst P, Stevens EMB, van Oost BA, den Heijer M, Trijbels FJM, Rozen R, Blom HJ. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Hum Genet.* 1996;58:35-41.

17. Frezzato M, Tosetto A, Rodeghiero F. Validation of a questionnaire for the diagnosis of venous thromboembolism. *Br J Haematol.* 1994;87:79-84.

18. de Ronde H, Bertina RM. Laboratory diagnosis of APC-resistance: a critical evaluation of the test and the development of diagnostic criteria. *Thromb Haemost.* 1994;72:880-886.

19. Ubbink JB, Vermaakl WJH, Bissburt S. Rapid high performance liquid chromatographic assay for total homocysteine levels in human serum. *J Chromatogr.* 1991;565:441-449.

20. Faioni EM, Franchi F, Asti D, Sacchi E, Bernardi F, Mannucci PM. Resistance to activated protein C in nine thrombophilic families: interference with a protein S assay. *Thromb Haemost.* 1993;70:1067-1071.

21. Brandt JT, Triplett DA, Alving B, Scharrer I. Criteria for the diagnosis of lupus anticoagulants: an update. *Thromb Haemost.* 1995;74:1185-1190.

22. Triplett DA. Antiphospholipid-protein antibodies: laboratory detection and clinical relevance. *Thromb Res.* 1995;78:1-31.

23. Wilcken DE, Wang XL, Sim AS, McCredie RM. Distribution in healthy and coronary populations of the methylenetetrahydrofolate reductase (MTHFR) C677T mutation. *Arterioscler Thromb Vasc Biol.* 1996;16:878-882.

24. Mannucci PM, Duca F, Peyvandi F, Tagliabue L, Merati G, Martinelli I, Cattaneo M. Frequency of factor V Arg506 Gln in Italians. *Thromb Haemost.* 1996;75:694.

25. Jaques 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. Miller JW, Nadeau MR, Smith D, Selhub J. Vitamin B6 deficiency vs folate deficiency: comparison of responses to methionine loading in rats. *Am J Clin Nutr.* 1994;59:1033-1039.

27. Mandel H, Brenner B, Berant M, Rosenberg N, Lanir N, Jakobs C, Fowler B, Seligson U. Coexistence of hereditary homocystinuria and factor V Leiden: effect on thrombosis. *N Engl J Med.* 1996;334:763-768.