Circulation

(*Circulation*. 1997;95:1777-1782.)

Interrelation of Hyperhomocyst(e)inemia, Factor V Leiden, and Risk of Future Venous Thromboembolism

Paul M. Ridker, MD; Charles H. Hennekens, MD; Jacob Selhub, PhD; Joseph P. Miletich, MD; M. Rene Malinow, MD; Meir J. Stampfer, MD

From the Division of Preventive Medicine (P.M.R., C.H.H.), the Cardiovascular Division (P.M.R.), and the Channing Laboratory (M.J.S.), Department of Medicine, Brigham and Women's Hospital, and the Department of Ambulatory Care and Prevention (C.H.H.), Harvard Medical School, Boston, Mass; the Departments of Nutrition (M.J.S.) and Epidemiology (M.J.S., C.H.H.), Harvard School of Public Health, Boston, Mass; the Laboratory Medicine Division (J.P.M.), Washington University School of Medicine, St Louis, Mo; the Oregon Regional Primate Research Center (M.R.M.), Beaverton; and the Jean Mayer Human Nutrition Research Center at Tufts University (J.S.), Boston, Mass.

Correspondence to Paul M. Ridker, Department of Medicine, Brigham and Women's Hospital, 900 Commonwealth Ave E, Boston, MA 02115. E-mail PMRIDKER@BICS.BWH.HARVARD.EDU

Abstract

Background Because patients with rare familial homocystinuria who also carry factor V Leiden have an increased incidence of venous thromboembolism (VTE), we hypothesized an interrelation of moderate hyperhomocyst(e)inemia, factor V Leiden, and risk of VTE in the general population.

Methods and Results In a large prospective cohort, we determined total homocysteine level and factor V Leiden mutation in baseline blood samples from 145 initially healthy men who subsequently developed VTE and among 646 men who remained free of vascular disease during a 10-year follow-up period. Hyperhomocyst(e)inemia was defined as a total homocysteine level above the 95th percentile (17.25 μmol/L). Compared with men with normal total homocysteine levels, those with hyperhomocyst(e)inemia had no increase in risk of any VTE but were at increased risk of idiopathic VTE (relative risk [RR]=3.4, *P*=.002). Compared with men without Leiden mutation, those with mutation were at increased risk of developing any VTE (RR=2.3, *P*=.005) as well as idiopathic VTE (RR=3.6, *P*=.0002). Compared with men with neither abnormality, those affected by both disorders had a 10-fold increase in risk of any VTE (RR=9.65, *P*=.009) and a 20-fold increase in risk of idiopathic VTE (RR=21.8, *P*=.0004).

Conclusions Apparently healthy men with coexistent hyperhomocyst(e)inemia and Leiden mutation are at substantially increased risk of developing future VTEs, particularly those events considered idiopathic. In these data, the risk of VTE among doubly affected individuals was far greater than the sum of the individual risks associated with either abnormality alone.

Key Words: factor V Leiden • thrombosis, venous • homocysteine • embolism, pulmonary

Introduction

Although marked elevations of tHcy are found in patients with rare inherited defects of methionine metabolism,[1] [2] [3] many cases of moderate hyperhomocyst(e)inemia result from less severe genetic defects and from inadequate intake of folate and vitamins B_6 and B_{12}.[4] Elevated levels of tHcy are associated with increased risk of arterial occlusions and correlate with extent of atherosclerotic disease.[5] [6] [7] [8] Recent data suggest that hyperhomocyst(e)inemia may also be a risk factor for VTE. In two recent case-control studies,[9] [10] patients with tHcy levels above the 95th percentile were found to have a risk of VTE twice that of patients with lower levels, and a third study[11] reported a high prevalence of hyperhomocyst(e)inemia in young adults with deep venous thrombosis or pulmonary embolism. However, two other studies[12] [13] report no association between tHcy level and risk of VTE.

One possible explanation for these apparently conflicting results is that tHcy level may be a relatively weak risk factor for VTE unless a second defect of endogenous anticoagulation coexists. In this regard, the most common inherited factor thus far recognized that predisposes patients to venous thrombosis is activated protein C resistance,[14] [15] [16] a defect usually caused by a single point mutation in the gene coding for coagulation factor V.[17] [18] [19] This mutation, commonly referred to as factor V Leiden, is present in 3% to 7% of the white population and is associated with increased risks of both first and recurrent VTEs, particularly those of idiopathic origin.[20]

Almost no data are available assessing risks of venous thrombosis among individuals who carry the factor V Leiden mutation and who also have moderate hyperhomocyst(e)inemia. However, in a recent report of seven families with clinically severe inherited homocystinuria, the coexistence of factor V Leiden was associated with an increased incidence of thromboembolism.[21] We therefore hypothesized that apparently healthy men with elevations of tHcy who also carry the factor V Leiden mutation might be at increased risk of developing future deep venous thromboses and pulmonary emboli.

Methods

We evaluated the roles of hyperhomocyst(e)inemia and factor V Leiden as markers of risk for VTE (deep venous thrombosis or pulmonary embolism) among 14 916 apparently healthy men participating in the PHS who provided baseline blood and plasma samples and were prospectively followed up for the future occurrence of vascular diseases and cancer. Detailed descriptions of the PHS, a randomized, double-blind, placebo-controlled trial of aspirin and ß-carotene in the primary prevention of cardiovascular disease and cancer, have been presented elsewhere.[22] [23]

All participants in the PHS completed annual questionnaires concerning risk factors and disease outcomes. For any self-report of either pulmonary embolism or deep venous thrombosis, clinic and hospital records, death certificates, and autopsy reports were requested and reviewed by an end-points committee of physicians who used standardized criteria to confirm or reject the diagnosis of each reported case. The

diagnosis of pulmonary embolism was confirmed only when a positive angiogram or ventilation-perfusion scan showed at least two segmental defects without ventilation defects. The diagnosis of deep vein thrombosis was confirmed only if there was documentation of either a positive venographic study or ultrasound study; reported cases of deep vein thrombosis documented by impedance plethysmography or Doppler examination but not by ultrasound were not considered to be confirmed. Deep vein thromboses and pulmonary emboli not associated with cancer, recent surgery, or trauma were considered idiopathic.

During the ≈12 years between randomization in 1982 and August 1994, 158 VTEs were confirmed among the 14 916 study subjects with banked baseline blood samples; of these, 145 (92%) underwent successful analysis for both tHcy level and factor V Leiden status. Plasma levels of tHcy were assessed as the sum of homocysteine and homocysteinyl moieties of the disulfides homocystine and cysteine-homocysteine using high-performance liquid chromatography and electrochemical detection.[8] [24] [25] The mean within-pair coefficient of variation in paired samples run during these analyses was <5%.[6] [26] Genotyping for the presence or absence of factor V Leiden was performed by use of a polymerase chain reaction technique as previously described.[16]

With the use of a nested case-control design, each participant who provided adequate whole blood and plasma samples at baseline and subsequently suffered a confirmed VTE was matched to 1 control subject who was also a study participant, provided adequate baseline blood samples for analysis, and reported no cardiovascular disease during follow-up through the time of matching. To increase the statistical power of the study, we further included as control subjects a group of study participants who remained free of vascular disease during follow-up, provided baseline blood samples for analysis, and had previously served as control subjects for analyses of myocardial infarction and stroke.[6] [16] [26] Thus, in addition to the 145 case subjects, a total of 646 study participants who were free of vascular disease at the time of control assignment also had plasma and DNA assayed for both tHcy level and factor V Leiden.

Statistical Analysis

Means and proportions of baseline vascular risk factors were computed for the case patients and the control subjects. The significance of any difference in means was tested by Student's *t* test, and the difference of any proportions was tested by the χ^2 statistic. On the basis of prior reports,[9] [10] hyperhomocyst(e)inemia (tHcy+) was defined as tHcy levels exceeding the 95th percentile of the study distribution.

To evaluate for evidence of association between tHcy level and risk of future VTE, logistic regression analyses were performed comparing incidence rates for individuals with tHcy levels above and below the 50th, 75th, 80th, 85th, 90th, and 95th percentiles of the study distribution. To evaluate the role of factor V Leiden (Leiden+), similar analyses were performed in which the referent group was those individuals who did not carry the mutation (Leiden-). To evaluate the combined role of hyperhomocyst(e)inemia and factor V Leiden, logistic regression analyses were performed in which the referent group was those individuals with normal tHcy levels who did not carry factor V Leiden (tHcy-,Leiden-). In this latter analysis, RRs of developing VTE were computed for individuals with hyperhomocyst(e)inemia free of factor V Leiden (tHcy+,Leiden-), individuals with normal tHcy levels affected by factor V Leiden (tHcy-,Leiden+), and individuals with both hyperhomocyst(e)inemia and factor V Leiden (tHcy+,Leiden+). Separate analyses were performed for those VTEs considered by the end-points committee to be idiopathic (ie, not associated with

cancer, surgery, or trauma). All analyses were two-tailed, and all CIs were computed at the 95% level.

Results

Table 1 shows baseline characteristics for the 145 study participants who developed confirmed VTE during follow-up (case subjects) and for the 646 study participants who remained free of vascular disease (control subjects). No significant differences were observed between study groups for blood pressure, exercise frequency, alcohol use, or prevalence of diabetes. Of the 145 VTEs, 73 (50.3%) were idiopathic.

Table 1. Baseline Characteristics of Initially Healthy Study Participants Who Developed VTE or Remained Free of Cardiovascular Disease (No VTE) During the Follow-up Period

Characteristic	No VTE (n=646)	VTE (n=145)
Age, y[1]	59.6	57.4
Body-mass index, kg/m^2[1]	24.9	25.8
Weekly exercise, %	72.2	75.2
Diabetes, %	3.6	2.1
Blood pressure, mm Hg		
Systolic	128.2	128.5
Diastolic	79.3	80.8
Smoking status, %[1]		
Never smoked	42.8	50.3
Past smoker	41.4	42.8
Current smoker	15.8	6.9
Alcohol use, %		
Daily	30.1	29.2
Weekly	45.0	48.6
Monthly	10.4	6.9
Rarely/Never	14.6	15.3

[1] $P<.05$.

Mean plasma tHcy levels were similar in the case (11.5±7.6 µmol/L) and control groups (10.9±4.1 µmol/L; $P=.4$), and the 95th percentile for tHcy in the study population was 17.25 µmol/L.

Table 2 shows the RR of developing VTE for study subjects with tHcy levels above and below the 50th, 75th, 80th, 85th, 90th, and 95th percentiles of the study distribution. No statistically significant association was observed between tHcy and VTE of any cause. However, compared with individuals with lower tHcy levels (tHcy-), those with tHcy levels above the 95th percentile (>17.25 µmol/L) (tHcy+) were at significantly increased risk of developing idiopathic VTE (RR=3.4, $P=.002$).

As previously described,[16] individuals with factor V Leiden were at significantly increased risk of developing any VTE (RR=2.3, *P*=.005) as well as idiopathic VTE (RR=3.6, *P*=.0002). These data are summarized in Fig 1.

Table 2. Crude RRs of Developing Future VTE at Prespecified Cutpoints for Baseline tHcy Levels

Cutpoint, Percentile	tHcy Level, µmol/L	Any VTE			Idiopathic VTE		
		RR	95% CI	*P*	RR	95% CI	*P*
50th	>10.08	0.72	0.5-1.1	.1	0.77	0.5-1.3	.3
75th	>12.30	0.82	0.5-1.3	.4	1.02	0.6-1.8	.9
80th	>12.93	0.90	0.6-1.4	.7	1.29	0.7-2.3	.4
85th	>13.69	1.08	0.7-1.8	.8	1.61	0.9-2.9	.1
90th	>14.97	1.15	0.6-2.1	.6	1.64	0.8-3.3	.2
95th	>17.25	1.58	0.8-3.3	.2	3.38	1.6-7.3	.002

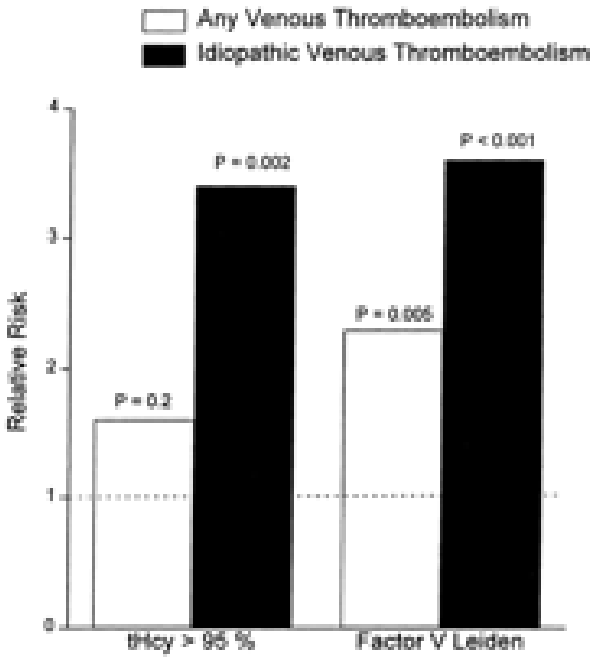

Figure 1. RRs of developing future VTE associated with hyperhomocyst(e)inemia and factor V Leiden in analyses in which each of these parameters was treated as an isolated risk factor. Data are shown for any VTE and for those events determined by the end-points committee to be idiopathic.

Table 3 shows the distribution of case and control subjects defined by the presence or absence of hyperhomocyst(e)inemia and by factor V Leiden status. Fig 2A illustrates the RR of developing any future VTE for each of the four study groups. Compared with individuals without hyperhomocyst(e)inemia or factor V Leiden (tHcy-,Leiden-), the RR of developing any future venous thrombosis among those

with both disorders (tHcy+,Leiden+) was 9.65 (*P*=.009). Lower RRs were found for individuals with only one of these defects (RR=1.07, *P*=.9 for the tHcy+,Leiden- group and RR=1.90, *P*=.045 for the tHcy-,Leiden+ group). As shown in Table 3, adjustment for age, body mass index, and smoking status had no important effects on these relationships.

Table 3. Crude and Adjusted RRs of Developing Any VTE Among Study Subjects According to tHcy Level and Presence or Absence of Factor V Leiden

	tHcy-,Leiden-	tHcy+, Leiden-	tHcy-, Leiden+	tHcy+,Leiden+
Case subjects, n	120	6	15	4
Control subjects, n	579	27	38	2
RR, crude	1.00	1.07	1.90	9.65
95% CI	...	0.43-2.65	1.01-3.57	1.75-53.3
P	...	.88	.045	.009
RR, adjusted[1]	1.00	1.11	2.09	9.70
95% CI	...	0.44-2.81	1.10-3.98	1.64-57.4
P	...	.82	.02	.01

[1] Adjusted for age, body mass index, and smoking status. Covariates are self-reported.

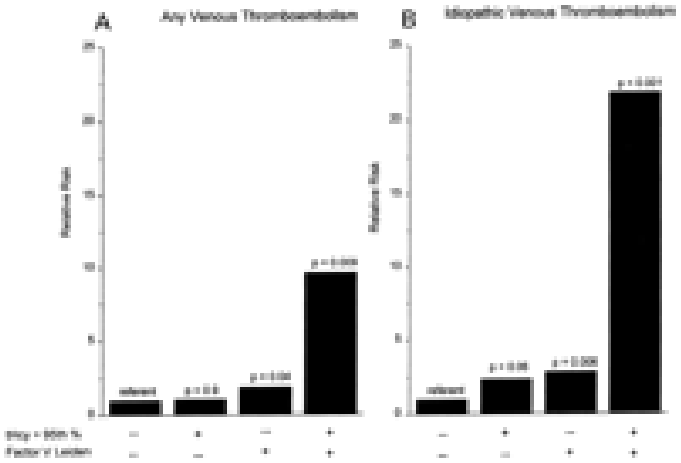

Figure 2. RRs of developing VTE associated with hyperhomocyst(e)inemia and factor V Leiden in analyses in which the coexistence of these parameters was evaluated. Data are shown for any VTE and for those events determined by the end-points committee to be idiopathic, stratified by the presence (+) or absence (--) of hyperhomocyst(e)inemia and by the presence (+) or absence (--) of factor V Leiden.

All of the VTEs that occurred among doubly affected study participants were idiopathic. Thus, as shown in Table 4 and illustrated in Fig 2B, the RRs of VTE not related to surgery, trauma, or cancer for the tHcy-,Leiden-, tHcy+,Leiden-, tHcy-,Leiden+, and tHcy+,Leiden+ groups were 1.00 (referent), 2.43 (*P*=.06), 2.87 (*P*=.006), and 21.8 (*P*=.0004), respectively.

Table 4. Crude and Adjusted RRs of Developing Idiopathic VTE Among Study Subjects According to tHcy Level and Presence or Absence of Factor V Leiden

	tHcy-,Leiden-	tHcy+, Leiden-	tHcy-, Leiden+	tHcy+,Leiden+
Case subjects, n	53	6	10	4
Control subjects, n	579	27	38	2
RR, crude	1.00	2.43	2.87	21.8
95% CI	...	0.96-6.14	1.36-6.09	3.91-122.1
P	...	.06	.006	.0004
RR, adjusted[1]	1.00	2.48	3.05	19.9
95% CI	...	0.97-6.39	1.42-6.51	3.39-117.0
P	...	.06	.004	.001

[1] Adjusted for age, body mass index, and smoking status. Covariates are self-reported.

Discussion

The initiation and propagation of venous thrombosis is a dynamic and multifactorial process that appears to increase in frequency when two or more concomitant defects of hemostasis are present. For example, among patients with deficiencies of protein C or protein S who have suffered venous thromboses, the prevalence of factor V Leiden is increased.[27] [28] [29] Similarly, a recent report[30] described elevated risks of thrombosis among patients with inherited antithrombin deficiencies who carry the Leiden mutation. However, functional deficiencies of protein C, protein S, and antithrombin are rare. In contrast, moderate hyperhomocyst(e)inemia is a common disorder. Because some[9] [10] [11] but not all[12] [13] studies of VTE and hyperhomocyst(e)inemia have reported positive associations, it has been controversial whether elevation of tHcy is an independent risk factor for these events.

The current data indicate that individuals with moderate hyperhomocyst(e)inemia who also carry factor V Leiden are at significantly increased risk of developing future VTE compared with men with neither or only one of these abnormalities. In our prospective cohort of initially healthy men, all VTEs that developed among doubly affected individuals occurred in the absence of cancer, surgery, or trauma. Thus, the risk of developing such idiopathic VTE was >20 times higher among hyperhomocyst(e)inemic patients with factor V Leiden than among individuals without either defect. Our finding that hyperhomocyst(e)inemia achieved statistical significance for the subgroup of idiopathic but not any venous thrombosis also may explain in part why prior studies evaluating the role of tHcy in VTE have been

inconsistent.[9] [10] [11] [12] [13] This observation could also arise due to differing frequencies of factor V Leiden, a hypothesis supported in our data showing an overall increase in risk of any VTE among those with factor V Leiden and among those with factor V Leiden and hyperhomocyst(e)inemia but not among those with hyperhomocyst(e)inemia alone.

Our observation that the coexistence of hyperhomocyst(e)inemia and factor V Leiden results in markedly increased thrombotic risk is consistent with a recent report of individuals affected by hereditary homocystinuria. Specifically, among seven families with inherited defects of methionine metabolism, only those patients with both factor V Leiden and clinical homocystinuria suffered venous thromboembolism.[21] Thus, taken together, these two reports suggest that the marked variability in thrombotic risk among hyperhomocyst(e)inemic patients may be due in part to factor V Leiden status. The biological mechanism of this adverse synergy is uncertain, although elevations of tHcy have been reported to inhibit protein C activation as well as to augment factor V function.[31] [32]

Moderate to intermediate hyperhomocyst(e)inemia can result from poor dietary intake of folate and vitamins B_6 and B_{12}, although recent data indicate that individuals homozygous for a common thermolabile polymorphism in the gene coding for MTHFR also have elevated tHcy levels and may be at increased risk for arterial thrombosis.[33] [34] [35] As described elsewhere,[36] participants in the PHS who are homozygous for this MTHFR polymorphism have significantly higher tHcy levels than do heterozygous carriers or homozygous normal individuals. However, in the current analysis, 57% of study subjects who were defined as hyperhomocyst(e)inemic (tHcy >17.25 μmol/L) were not homozygous for the abnormal MTHFR polymorphism. Thus, it is probable that absolute tHcy level is of greater physiological importance than the presence or absence of a particular MTHFR variant. Because methionine loading was not performed in the PHS and some baseline plasma samples were nonfasting, some individuals with hyperhomocyst(e)inemia may not have been detected in the present study. However, this potential limitation cannot account for our findings because any such misclassification would lead to an underestimation of true risks, if anything.

The current data, combined with those of other investigators, raise several issues and extend prior observations from this cohort.[16] First, because hyperhomocyst(e)inemia can often be corrected by vitamin supplementation, these data raise the possibility that simple dietary interventions might be adequate to reduce long-term risks for some patients.[37] Second, these data provide support for the hypothesis that individuals genetically predisposed to thrombosis due to carriage of factor V Leiden may require additional screening for other concomitant abnormalities of hemostasis and thrombosis, such as tHcy level.[21] Finally, because the prevalence of factor V Leiden is ≈5% and hyperhomocyst(e)inemia has been defined as levels in excess of the 95th percentile, 1 in every 400 healthy individuals is likely to be affected by both of these abnormalities. Given the high RRs of first and recurrent VTEs associated with factor V Leiden[14] [15] [16] [20] and the demonstration in these and other data that concomitant defects can further increase risks, clinicians may consider such patients for long-term anticoagulation. However, because the absolute risks of venous thrombosis associated with factor V Leiden and hyperhomocyst(e)inemia remain uncertain and long-term prophylaxis with full-dose warfarin is associated with higher risks of bleeding, including cerebral hemorrhage, randomized trials of adequate sample size are required

to evaluate the benefit-to-risk ratio of different anticoagulation regimens for these patients.

Selected Abbreviations and Acronyms

Leiden-	= absence of factor V Leiden
Leiden+	= presence of factor V Leiden
MTHFR	= methylenetetrahydrofolate reductase
PHS	= Physicians' Health Study
RR	= relative risk
tHcy	= total plasma homocysteine level
tHcy-	= normal homocysteine level (<17.25 µmol/L)
tHcy-,Leiden-	= normal homocysteine level, absence of factor V Leiden
tHcy-,Leiden+	= normal homocysteine level, presence of factor V Leiden
tHcy+	= hyperhomocyst(e)inemia (total plasma homocysteine >17.25 µmol/L)
tHcy+,Leiden-	= hyperhomocyst(e)inemia, absence of factor V Leiden
tHcy+,Leiden+	= hyperhomocyst(e)inemia, presence of factor V Leiden
VTE	= venous thromboembolism

Received August 8, 1996; revision received November 13, 1996; accepted November 25, 1996.

References

1. Mudd SH, Levy HL, Skovby F. Disorders of transulfuration. In: Scriver CS, Beaudet AL, Sly WL, Valle D, Stanbury JB, Wyngaarden JB, Fredrickson DS, eds. *The Metabolic Basis of Inherited Disease*. New York, NY: McGraw-Hill International Book Co; 1995:1279-1327.
2. Boers GHJ, Smals AGH, Trijbels FJ, Fowler B, Bakkeren JA, Schoonderwaldt HC, Kleijer WJ, Kloppenborg PW. Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. *N Engl J Med.* 1985;313:709-715.
3. Clarke R, Daly L, Robinson K, Naughten E, Cahalane S, Fowler B, Graham I. Hyperhomocysteinemia. *N Engl J Med.* 1991;324:1149-1155.
4. Selhub J, Jacques PF, Wilson PWF, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. *JAMA.* 1993;270:2693-2698.
5. Selhub J, Jacques PF, Bostom AG, D'Agostino RB, Wilson PW, Belanger AJ, O'Leary DH, Wolf PA, Schaefer EJ, Rosenberg IH. Association between plasma homocysteine concentrations and extracranial carotid-artery stenosis. *N Engl J Med.* 1995;332:286-291

6. Stampfer MJ, Malinow MR, Willett WC, Newcomer LM, Upson B, Ullman D, Tischler P, Hennekens CH. A prospective study of plasma homocyst(e)ine and risk of myocardial infarction in US physicians. *JAMA.* 1992;268:877-881.

7. Boushey CJ, Beresford SAA, 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.

8. Malinow MR, Kang SS, Taylor LM, Wong PW, Coull B, Inahara T, Mukerjee D, Sexton G, Upson B. Prevalence of hyperhomocyst(e)inemia in patients with peripheral arterial occlusive disease. *Circulation.* 1989;79:1180-1188.

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

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

11. Falcon CR, 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

12. Brattstrom L, Tengborn L, Lagerstedt C, Israelsson B, Hultberg B. Plasma homocysteine in venous thromboembolism. *Haemostasis.* 1991;21:51-57.

13. Amundsen T, Ueland PM, Waage A. Plasma homocysteine levels in patients with deep venous thrombosis. *Arterioscler Thromb Vasc Biol.* 1995;15:1321-1323.

14. Svensson PJ, Dahlback B. Resistance to activated protein C as a basis for venous thrombosis. *N Engl J Med.* 1994;330:517-522.

15. Koster T, Rosendaal FR, de Ronde H, Briet E, Vandenbroucke JP, Bertina RM. Venous thrombosis due to poor anticoagulant response to activated protein C: Leiden Thrombophilia Study. *Lancet.* 1993;342:1503-1506.

16. Ridker PM, Hennekens CH, Lindpaintner K, Stampfer MJ, Eisenberg PR, Miletich JP. Mutation in the gene coding for coagulation factor V and the risk of myocardial infarction, stroke, and venous thrombosis in apparently healthy men. *N Engl J Med.* 1995;332:912-917.

17. Bertina RM, Koeleman BPC, Koster T, Rosendaal FR, Dirven RJ, deRonde H, van der Velden PA, Reitsma PH. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature.* 1994;369:64-67.

18. Voorberg J, Roelse J, Koopman R, Buller H, Berends F, ten Cate JW, Mertens K, van Mourik JA. Association of idiopathic venous thromboembolism with single point-mutation at Arg^{506} of factor V. *Lancet.* 1994;343:1535-1536.

19. Zoller B, Dahlback B. Linkage between inherited resistance to activated protein C and factor V gene mutation in venous thrombosis. *Lancet.* 1994;343:1536-1538.

20. Ridker PM, Miletich JP, Stampfer MJ, Goldhaber SZ, Lindpaintner K, Hennekens CH. Factor V Leiden and risks of recurrent idiopathic venous thromboembolism. *Circulation.* 1995;92:2800-2802.

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

22. Steering Committee of the Physicians' Health Study Research Group. Final report of the aspirin component of the ongoing Physicians' Health Study. *N Engl J Med.* 1989;321:129-135.

23. Hennekens CH, Buring JE, Manson JE, Stampfer M, Rosner B, Cook NR, Belanger C, LaMotte F, Gaziano JM, Ridker PM, Willett W, Peto R. Lack of effect of long-term supplementation with beta carotene on the incidence of malignant neoplasms and cardiovascular disease. *N Engl J Med.* 1996;334:1145-1149

24. Smolin LA, Schneider JA. Measurement of total plasma cysteamine using high-performance liquid chromatography with electrochemical detection. *Anal Biochem.* 1988;168:374-379.

25. Malinow MR, Sexton G, Averbuch M, Grossman M, Wilson D, Upson B. Homocyst(e)ine in daily practice: levels in coronary artery disease. *Coron Artery Dis.* 1990;1:215-220.

26. Verhoef P, Hennekens CH, Malinow MR, Kok FJ, Willett WC, Stampfer MJ. A prospective study of plasma homocyst(e)ine and risk of ischemic stroke. *Stroke.* 1994;25:1924-1930.

27. 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;84:1031-1035.

28. Koeleman BPC, van Rumpt D, Hamulyak K, Reitsma PH, Bertina RM. Factor V Leiden: an additional risk factor for thrombosis in protein S deficient families? *Thromb Haemost.* 1995;74:580-583

29. Gandrille S, Greengard JS, Alhenc-Gelas M, Juhan-Vague I, Abgrall JF, Jude B, Griffin JH, Aiach M. Incidence of activated protein C resistance caused by the Arg 506 Gln mutation in factor V in 113 unrelated symptomatic protein C deficient patients. *Blood.* 1995;86:219-224.

30. van Boven HH, Reitsma PH, Rosendaal FR, Bayston TA, Chowdhury V, Bauer K, Scharrer I, Lane DA. Interaction of factor V Leiden with inherited antithrombin deficiency. *Thromb Haemost.* 1995;73:1256. Abstract.

31. Rodgers GM, Kane WH. Activation of endogenous factor V by a homocysteine-induced vascular endothelial cell activator. *J Clin Invest.* 1986;77:1909-1916.

32. Lentz SR, Sadler JE. Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. *J Clin Invest.* 1991;88:1906-1914

33. Kang SS, Wong PWK, Zhou J, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase in patients with coronary artery disease. *Metabolism.* 1988;37:611-613.

34. Kang SS, Wong PWK, Susmano A, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. *Am J Hum Genet.* 1991;48:536-545.

35. Kang SS, Passen EL, Ruggie N, Wong PWK, Sora H. Thermolabile defect of methylenetetrahydrofolate reductase in coronary artery disease. *Circulation.* 1993;88:1463-1469

36. Ma J, Stampfer MJ, Hennekens CH, Frosst P, Selhub J, Horsford J, Malinow MR, Willett WC, Rozen R. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. *Circulation.* 1996;94:2410-2416.

37. Stampfer MJ, Malinow MR. Can lowering homocysteine levels reduce cardiovascular risk? *N Engl J Med.* 1995;332:328-329.