Association between factor V Leiden, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T mutations and events of the arterial circulatory system: A meta-analysis of published studies

Robert J. Kim, MD,[a] and Richard C. Becker, MD[b] *Worcester, Mass, and Durham, NC*

Background The association between the inherited gene mutations of factor V, prothrombin, and homocysteine metabolism and venous thromboembolic events is accepted widely; however, their influence on the arterial circulatory system remains controversial.

Methods We performed a MEDLINE search to identify published case-control and cohort studies correlating the factor V Leiden, prothrombin (PT) G20210A, and methylenetetrahydrofolate reductase (MTHFR) C677T (TT genotype) mutations with myocardial infarction, ischemic stroke, or peripheral vascular disease. Studies were included only when they adhered to specific diagnostic criteria for ischemic events and met the published methodological criteria. Odds ratios (ORs) with accompanying 95% CIs were calculated for each mutation and clinical end points with a random-effects model (DerSimonian and Laird method).

Results The association between inherited gene mutations and arterial ischemic events was modest: factor V Leiden mutation (OR, 1.21; 95% CI, 0.99–1.49), PT G20210A mutation (OR, 1.32; 95% CI, 1.03–1.69), and MTHFR TT mutation (OR, 1.20; 95% CI, 1.02–1.41). Subgroup analyses of younger patients (<55 years old) and of women revealed slightly stronger associations overall.

Conclusions Genetic abnormalities specific to factor V, prothrombin ,and homocysteine metabolism increase the risk for myocardial infarction and ischemic stroke, particularly among younger patients and women. Because the overall association is only modest, screening studies should be limited to carefully selected patient populations. The individual propensity for arterial and venous thrombosis is likely influenced by differing local mechanisms, systemic mechanisms, or both. (Am Heart J 2003;146:948−57.)

In 1994, Bertina et al[1] described a common variation (G1691A) in the factor V gene as a molecular defect responsible for activated protein C (APC) resistance,[2] a previously unrecognized mechanism of inherited thrombophilia. Through the years, several additional mutations within the genes coding for coagulation proteases were subsequently identified. The factor V Leiden and prothrombin G20210A mutations are 2 such examples; each has been found to be associated strongly with spontaneous and recurrent venous thromboembolism.[3,4] Similarly, a thermolabile variant of methylenetetrahydrofolate reductase, an enzyme involved in the folate-dependent metabolism of homocysteine, increases the risk for deep vein thrombosis and pulmonary embolism.[5]

It remains less well characterized, however, whether the presence of these relatively common mutations poses risk for thrombosis localized to the arterial circulatory system, resulting in acute myocardial infarction (MI), ischemic stroke, or complicated peripheral vascular occlusive disease (PVD). Because most previously published studies examining such potential relationships investigated either an isolated gene mutation or a single clinical event, we performed a comprehensive meta-analysis of the 3 most prevalent genetic mutations predisposing to venous thromboembolism and

From the [a]Department of Internal Medicine, University of Massachusetts-Memorial Medical Center, Worcester, Mass, and [b]Cardiovascular Thrombosis Center, Duke University Medical Center, Durham, NC.
Submitted March 6, 2003; accepted July 3, 2003.
Reprint requests: Richard C. Becker, MD, Professor of Medicine, Director, Cardiovascular Thrombosis Center, Duke University Medical Center, Durham, NC 27715.
E-mail: beckerr@ummhc.org

0002-8703/2003/$30.00 + 0
doi:10.1016/S0002-8703(03)00519-2

determined their potential association with acute arterial occlusive events.

Materials and methods

Literature search

Using a systematic search of MEDLINE electronic database between 1990 and 2002, we identified all studies correlating the factor V Leiden, prothrombin G20210A, or methylenetetrahydrofolate reductase (MTHFR) C677T gene mutations with MI, ischemic stroke, or PVD. Terms used for the search were Medical Subject Heading (MeSH) terms and the text words "myocardial infarction," "ischemic stroke," or "peripheral vascular disease," combined with "factor V," "prothrombin," "methylenetetrahydrofolate reductase," or "hyperhomocysteinemia," combined with "polymorphism," "mutation," or "genetics." We limited our search to "human" and "English language."

Literature screening

We considered studies acceptable for inclusion when they were written as full-length articles, published in peer-reviewed journals, and correlated the presence of any of the 3 genetic mutations being considered with the risk of MI, ischemic stroke, or PVD. We examined the abstract, methods, and results sections of all initially identified publications to establish the pertinence of each article to our study. We then inspected all relevant articles for compliance with 2 recently published criteria for methodological quality.[6,7] Objectivity and reproducibility were not used as reasons for exclusion; however, we excluded a study when other remaining criteria were not satisfied (inadequate spectrum or delineation of cases, inadequate spectrum or delineation of comparison groups).[6] Studies in which either a portion of or all the chosen criteria appeared within an appropriately referenced article were considered for inclusion.[47,58,61,75] We contacted the authors directly to clarify missing information or to request additional unpublished data that would be relevant to our study.

Subject description

Each study included in our analysis required a group of unrelated patients and a comparable group of control subjects that was ethnically and geographically representative of the population from which the patients were selected (Table I). Patients were required to have experienced an acute MI or ischemic stroke, or have documented peripheral vascular occlusive disease (see definition). For patients who had experienced >1 arterial event, only the index event (that the original study was designed to investigate) was included in the analysis. Control subjects were defined as individuals who had not experienced an arterial event during the designated study period. All patients underwent genetic testing for the prespecified mutation. On the basis of prior observations and published studies, women, men, and patients <55 years old were grouped and analyzed separately.

Outcome definitions

The diagnosis of MI required at least 2 of these 3 criteria: clinical symptoms, cardiac biomarker elevation, and a diagnostic electrocardiogram. We also accepted autopsy confirmation. The diagnosis of ischemic stroke required at least 1 of these 2 criteria: 1) acute onset of focal neurologic deficit lasting at least 24 hours or 2) demonstrated ischemic abnormality on computed tomography or magnetic resonance imaging. When a study included a subgroup of patients with a transient ischemic attack (TIA) rather than a stroke, this subgroup was excluded from the analysis.[57,59,61,67] Patients who sustained hemorrhagic or cavernous venous strokes were also excluded. The prespecified diagnostic criteria for PVD included the presence of any of these 3 criteria: 1) rest pain or tissue gangrene (Fontaine stage III or IV), 2) a history of infrainguinal arterial bypass graft surgery or angioplasty, or 3) a history of any non-traumatic extremity amputation caused by vascular insufficiency.

Data extraction and statistical analysis

Data were extracted and entered into separate databases at 2 different times by a single investigator (R.J.K.). The results were compared, and any disagreement was resolved by reviewing the data for a third time. Data were analyzed with the Meta-Analyst software version 0.991 (Lau J, New England Medical Center, Boston, Mass, 1990–1997).[8] Raw prevalence data from each population were entered separately. Odds ratios (ORs) and 95% CIs for dichotomous data were calculated with a random-effects model according to the DerSimonian and Laird method,[9] which incorporates variability both within and between studies. This method produces a wider confidence interval when heterogeneity between studies exists.

Results

Excluded studies

We excluded 1 study[10] because it used a duplicate set of patients in separate studies.[66] Four studies were excluded because we could not ascertain the absence of prior ischemic events in control subjects (inadequate control delineation).[11–14] Two studies were excluded because of an inadequate control population.[15,16] We eliminated 13 additional studies from consideration[17–29] because adequate definitions or diagnostic criteria for cited ischemic events were not provided (inadequate case delineation). A total of 56

Table I. Individual studies and raw data listed by category

Mutation	Event	Age group	Study (ref)	Sex	Prevalence	
					Patients	Controls
FV Leiden	MI	All	Doggen (32)	M	38/560	32/646
			Ridker (34)	M	23/374	42/704
			Cushman (39)	M/F	5/147	34/482
			Gardemann (40)	M	40/1038	59/1172
			Makris (42)	M/F	16/80	10/124
			Gowda (44)	M/F	9/109	5/112
			Russo (45)	M/F	6/244	15/224
			Feng (46)	M/F	3/32	1/25
			Kontula (47)	M/F	6/71	1/87
			Burzotta (50)	M/F	6/190	7/247
			Juul (77)	M/F	79/962	629/7907
			Psaty (82)	F	9/232	39/718
			Celik (81)	M/F	11/135	7/95
FV Leiden	MI	Under *55*	Rosendaal (33)	F	8/84	16/388
			Ardissino (37)	M/F	1/100	2/100
			Inbal (38)	M	7/112	12/187
			Ardissino (43)	M/F	9/200	8/200
			Kontula (47)	M/F	1/51	3/50
			Mansourati (49)	M/F	24/351	20/400
			Junker (51)	M	21/241	12/179
FV Leiden	IS	All	Ridker (34)	M	9/209	42/704
			Cushman (39)	M/F	8/149	34/482
			Markus (57)	M/F	13/138	5/70
			Fisher (58)	M/F	0/63	0/31
			Lalouschek (61)	M/F	8/62	4/81
			Hankey (63)	M/F	10/219	4/179
			Juul (77)	M/F	40/641	629/7907
			Szolnoki (80)	M/F	64/664	13/199
FV Leiden	IS	Under *55*	Lopaciuk (54)	M/F	3/100	10/238
			Longstreth (55)	F	0/41	16/388
			de Stefano (56)	M/F	5/72	6/198
			Nabavi (59)	M/F	18/194	12/200
			Margaglione (60)	M/F	30/202	43/1036
			Madonna (64)	M/F	8/132	19/262
			Voetsch (69)	M/F	5/153	8/225
FV Leiden	PVD	All	Renner (78)	M/F	7/85	27/300
PT G20210A	MI	All	Ridker (30)	M	12/404	69/1774
			Croft (31)	M/F	11/539	14/498
			Doggen (32)	M	10/560	8/646
			Gardemann (40)	M	27/1038	35/1172
			Russo (45)	M/F	11/244	7/224
			Feng (46)	M/F	3/32	1/25
			Burzotta (50)	M/F	13/190	7/247
			Smiles (52)	M/F	19/682	24/686
			Psaty (82)	F	13/232	18/721
PT G20210A	MI	Under *55*	Eikelboom (35)	M/F	9/402	22/679
			Rosendaal (36)	F	4/79	6/381
			Inbal (38)	M	7/112	6/187
			Ardissino (43)	M/F	11/200	8/200
			Franco (48)	M/F	7/173	4/400
PT G20210A	IS	All	Ridker (30)	M	11/259	69/1774
			Smiles (52)	M/F	9/407	24/686
			Ferraresi (62)	M/F	1/40	3/70
			Hankey (63)	M/F	8/219	4/179
PT G20210A	IS	Under *55*	Lopaciuk (54)	M/F	2/100	5/238
			Longstreth (55)	F	1/41	6/382
			de Stefano (56)	M/F	9/72	5/198
			Margaglione (60)	M/F	10/202	43/1036
			Madonna (64)	M/F	14/132	21/262
			Voetsch (69)	M/F	7/153	5/225

Table I. continued

Mutation	Event	Age group	Study (ref)	Sex	Prevalence	
					Patients	Controls
PT G20210A	PVD	All	Renner (78)	M/F	1/85	13/300
MTHFR TT	MI	All	Anderson (41)	M/F	23/200	59/554
			Gardemann (53)	M	110/1152	151/1301
			Girelli (70)	M/F	30/183	25/137
			Ma (71)	M	33/293	39/290
			Adams (72)	M/F	32/310	29/222
			Schmitz (75)	M/F	29/190	27/188
			Brugada (76)	M/F	6/79	12/155
			Fernandez (83)	M/F	61/272	90/472
			Thogersen (84)	M/F	5/69	7/129
MTHFR TT	MI	Under 55	Inbal (38)	M	27/112	20/187
			Ardissino (43)	M/F	35/200	38/200
			Schwartz (73)	F	7/69	43/338
			van Bockxmeer (74)	M/F	15/139	15/143
			Gulec (85)	M	15/96	5/100
MTHFR TT	IS	All	Lalouschek (61)	M/F	6/62	9/81
			Morita (66)	M/F	55/256	33/325
			Markus (67)	M/F	30/271	22/161
			Harmon (68)	M/F	27/174	19/183
MTHFR TT	IS	Under 55	Lopaciuk (54)	M/F	12/100	26/238
			de Stefano (56)	M/F	17/72	35/198
			Margaglione (60)	M/F	50/202	196/1036
			Madonna (64)	M/F	33/132	50/262
			Pezzini (65)	M/F	4/31	4/36
			Voetsch (69)	M/F	21/153	16/225
			Kristensen (79)	M/F	11/80	3/41

studies and 54,547 persons served as the basis of our final analysis.

Factor V Leiden mutation

The pooled analysis of studies investigating an association between factor V Leiden mutation and arterial ischemic events is summarized in Table II. The overall relationship, on the basis of 33 studies including 25,053 patients, was modest with an OR of 1.21 (95% CI, 0.99–1.49; Figure 1). Considering the clinical end points individually, the relationships with MI, ischemic stroke, and PVD were 1.10 (95% CI, 0.88–1.36), 1.27 (95% CI, 0.86–1.87), and 0.91 (95% CI, 0.38–2.16), respectively. Patients <55 years old were at a greater risk for arterial ischemic event (OR, 1.37; 95% CI, 0.96–1.97) than older patients with factor V Leiden mutation.

Prothrombin G20210A mutation

The pooled analysis of studies investigating an association between the prothrombin G20210A mutation and arterial events appears in Table II. The relationship with MI and ischemic stroke was modest (MI: OR, 1.28; 95% CI, 0.94–1.73; OR, 1.30; ischemic stroke: 95% CI, 0.91–1.87). Only 1 study investigating the association with PVD met our selection criteria; however, no association was present (OR, 0.26, 95% CI, 0.03–2.04). To estimate the strength of association between the prothrombin G20210A mutation and overall arterial events, we pooled the data from all studies across the 3 events groups (Figure 2). Despite the large number of patients (n = 16,945), the relationship remained modest (OR, 1.32; 95% CI, 1.03–1.69). When the analysis was limited to patients <55 years old, the association was slightly more robust (OR, 1.66; 95% CI, 1.13–2.46).

MTHFR C677T mutation

A pooled analysis of studies including an evaluation of MTHFR (C677T variant) and arterial events failed to reveal an association with MI (OR, 1.05; 95% CI, 0.86–1.27); the relationship with ischemic stroke was more robust, but still modest (OR, 1.46; 95% CI, 1.19–1.79). There were no studies evaluating a potential association with PVD. To estimate the strength of association between MTHFR TT and overall arterial ischemic events, we pooled the data from all studies (n = 12,099 patients) in the 2 available event groups (Figure 3). The relationship remained modest. When the analysis was limited to subjects <55 years old, the as-

Table II. Quantitative summary of pooled analyses

Category	No. studies	No. patients	No. controls	Total	OR	95% CI	*P*
FVL-MI	20	5313	14,047	19,360	1.10	0.88–1.36	.41
FVL-IS	15	3039	12,200	15,239	1.27	0.86–1.87	.22
FVL-PVD	1	85	300	385	0.91	0.38–2.16	NS
FVL (<55)	14	2033	3663	5696	1.37	0.96–1.97	.084
FVL-Total	33	8437	17,066	25,503	1.21	0.99–1.49	.068
PT-MI	14	4887	7840	12,727	1.28	0.94–1.73	.11
PT-IS	10	1625	5050	6675	1.30	0.91–1.87	.15
PT-PVD	1	85	300	385	0.26	0.03–2.04	NS
PT (<55)	11	1666	3806	5472	1.66	1.13–2.46	.011
PT-Total	23	6597	10,348	16,945	1.32	1.03–1.69	.029
M-MI	14	3364	4416	7780	1.05	0.86–1.27	.66
M-IS	11	1533	2786	4319	1.46	1.19–1.79	<.001
M (<55)	12	1386	3004	4390	1.41	1.13–1.76	.0021
M-Total	25	4897	7202	12,099	1.20	1.02–1.41	.029
FVL-male	6	2639	3328	5967	1.22	0.82–1.81	.33
FVL-female	4	454	1702	2156	1.79	0.54–5.88	.34
PT-male	6	2848	4525	7373	0.97	0.73–1.30	.85
PT-female	5	618	1890	2508	1.73	0.99–3.02	.052
M-male	7	2184	2657	4841	1.24	0.85–1.82	.26
M-female	3	218	1209	1427	1.04	0.71–1.53	.84

sociation increased minimally (OR, 1.41; 95% CI, 1.13–1.76).

Sex differences

Of the studies that included and reported male and female subjects, 3[31,60,83] also provided sex-differentiated data. The OR for arterial events was higher in women than men with respect to the factor V Leiden and prothrombin G20210A mutations (Table II).

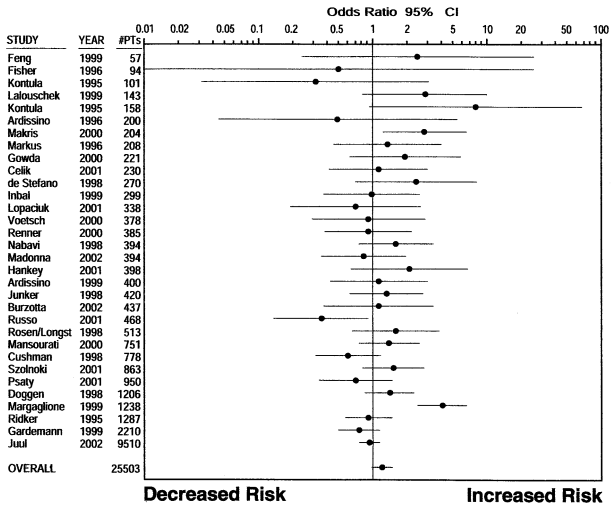
Discussion

In our meta-analysis of >17,000 patients with coronary, cerebrovascular, or peripheral vascular events, several observations were made. First, the 3 common gene anomalies associated with venous thromboembolism (factor V Leiden mutation, prothrombin G20210A mutation, and MTHFR C677T mutation) increase the risk of arterial thrombotic events to comparatively modest degree. Second, the association is more robust in patients <55 years old and in women (for factor V Leiden and PT G20210A mutations).

Thrombosis, a fundamental and teleologically life-sustaining response that successfully stems blood loss after vascular injury, can also be responsible for potentially life-threatening events involving the venous circulatory system, arterial circulatory system, or both. Venous thrombosis, although most often occurring in the regions of static blood flow, is most strongly influenced by the presence of activated coagulation proteases that lead to thrombin generation.[86] Accordingly, and as evidenced by the predisposition to venous thromboembolism observed among individuals with factor V Leiden and prothrombin G20210A mutations, an intact and fully functional system of intrinsic vascular thromboresistance, such as provided by activated protein C, antithrombin, and tissue factor pathway inhibitor, is of importance. In contrast, arterial thrombosis, responsible for acute MI, ischemic stroke, and peripheral vascular occlusive disease, occurs at the site of vessel wall injury on a template of activated endothelial cells, monocytes (tissue factor bearing cells), and platelet aggregates.[87] This environment is minimally influenced by small decreases in either the level or functionality of the vascular surface anticoagulant system. The only exception may be among young individuals in whom a primary thrombotic event is more likely to occur than in an older person in whom chronic atherosclerotic disease and its risk factors are primarily operative. For example, arterial thrombosis is present in 70% of patients <40 years old who experience sudden cardiac death, as compared with an incidence of ≤30% among patients >70 years.[88] It has also been shown that patients without flow-limiting stenosis after MI have increased frequencies of thrombophilic mutations.[96]

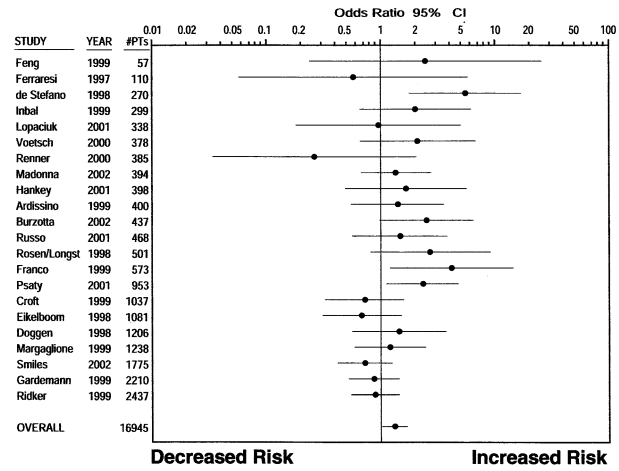
In our meta-analysis, patients <55 years old were at greater risk for arterial events than older individuals. The association between the mutations and MI, ischemic stroke, or peripheral vascular events might have been even stronger had we restricted the analysis to patients <40 years old, thus excluding potentially confounding variables encountered with advanced age and

Figure 1



Prevalence of factor V Leiden mutation in patients with ischemic arterial events compared with control subjects. ORs are shown with corresponding 95% CIs for individual studies and pooled data. Two studies[33,55] (Rosen/Longst) shared a common set of control subjects but investigated different ischemic events—the prevalence data of patients (but not control subjects) from the 2 studies were combined. In each of the 3 studies,[34,39,77] 2 ischemic events were investigated with a single set of control subjects—the prevalence data of the 2 event groups in each study were combined.

Figure 2

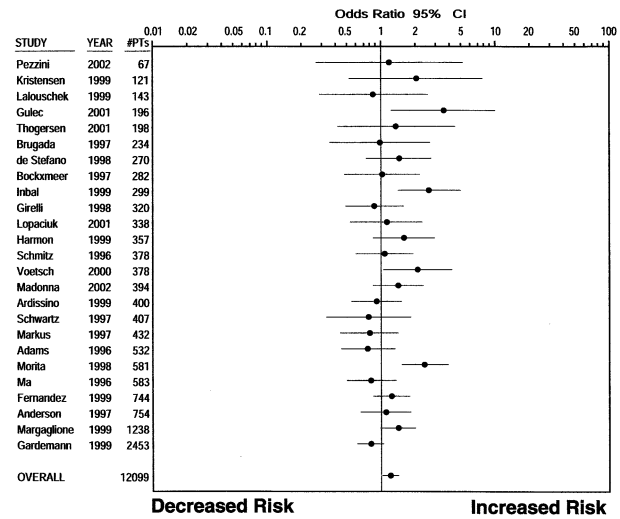


Prevalence of prothrombin G20210A mutation in patients with ischemic arterial events compared to controls. ORs are shown with corresponding 95% CIs for individual studies and pooled data. Two studies[36,55] (Rosen/Longst) shared a common set of control subjects but investigated different ischemic events—the prevalence data of patients (but not control subjects) from the 2 studies were combined. In each of the 2 studies,[30,52] 2 ischemic events were investigated with a single set of control subjects—the prevalence data of the 2 event groups in each study were combined.

permitting a more accurate risk estimate derived from the inherited mutations themselves. Whether atherosclerosis, through localized compensatory mechanisms, reduces the inherent risk imparted by the gene mutations of surface coagulation proteases is unknown. The importance of age as a risk factor for thrombosis may also dilute the effect of inherited thrombophilias on the arterial circulatory system. APC resistance, recognized as the most common inherited cause for venous thromboembolism, portends a particularly high risk for patients <70 years old.[89] In addition, APC resistance resulting from factor V Leiden mutation has been associated with stroke in pediatric populations.[90]

Several investigators have reported an association between inherited thrombophilias and arterial thrombosis involving the coronary vasculature, cerebral vasculature, or both in women, particularly women who smoke or take oral contraceptive agents.[33,82,91] Similarly, we found a greater risk for arterial events among women than men with either factor V Leiden or PT G20210A mutation. These observations support an important genetic-environmental factor interaction and suggest that tobacco and estrogen[92] exert their pro-

Figure 3



Prevalence of MTHFR CC→TT mutation in patients with ischemic (arterial) events compared with control subjects. ORs are shown with corresponding 95% CIs for individual studies and pooled data.

thrombotic effects on endothelial cells, platelets, and/or tissue factor-bearing cells.[93]

The relatively modest association between MTHFR C677T mutation and arterial events comes as somewhat of a surprise because of the recognized detrimental and prothrombotic effects of homocysteine on endothelial cells (reducing nitric oxide and prostacyclin synthesis and activity, while concomitantly increasing tissue factor expression).[94] However, our findings are consistent with prior observations and closely approximate the result of a similar meta-analysis[97] when the geographic origins of subjects are taken into account. It seems likely that concomitant enzyme defects in homocysteine metabolism, plasma homocysteine levels (phenotypic expression, nutritional deficiencies, coexisting disease states), and acquired thrombophilic factors (oral contraceptives, pregnancy, trauma) dominate the overall clinical risk profile.

Study limitations

Several potential limitations of our study are similar to those often cited for meta-analyses. First, it is important to acknowledge heterogeneity within patient demographics and study designs. Second, the calculated OR does not reflect the sole risk estimate of a mutation and includes risk contributions from concomitant (and more traditional) cardiovascular risk factors. Third, the population with PVD was underrepresented because of the lack of published studies meeting our rigorous inclusion criteria. Fourth, significant potential for publication bias exists because letters, abstracts, presentations, and other non-full length articles were not considered.

Conclusions

The association between genetic mutations specific to factor V, prothrombin, and homocysteine metabolism and acute arterial thrombosis is modest. Routine screening for factor V Leiden, prothrombin G20210A, and/or MTHFR C677T mutations among patients with MI, ischemic stroke, or peripheral vascular occlusion is probably not warranted. When performed, it should be restricted to patients <55 years old, particularly in the absence of traditional atherosclerosis risk factors, women receiving oral contraceptives or hormone replacement therapy, patients with early saphenous vein graft occlusion,[95] or when paradoxical embolism is strongly suspected.

Anticoagulant rather than platelet-directed therapy may be preferable in patients with factor V Leiden, prothrombin G20210A, or MTHFR C677T mutations who are experiencing acute arterial events; however, further investigation is needed.

We thank Dr Joseph Lau for his expert technical assistance with statistical analyses and data preparation; those who provided information about their past publications (Drs V. Arruda, A.J. Catto, V. de Stefano, J. Eikelboom, J. Genest, J. Holm, C. Irvine, R. Junker, W. Lalouschek, P.M. Manucci, G. Marchetti, M. Margaglione, A. Pezzini, W. Prohaska, W. Renner, P. Ridker, N. Samani, T. Tatlisumak, X.L. Wang, S. Whitehead, and D. Wilcken); and Robert Goldberg, PhD, and Frederick Spencer, MD, for their review of the manuscript and comments.

References

1. Bertina RM, Koeleman BPC, Koster T. Mutations in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64–7.
2. Dahlback B, Carlsson M, Svensson PJ. Familial thrombophilia due to a previously unrecognized mechanism characterized by poor anticoagulant response to activated protein C. Proc Natl Acad Sci U S A 1993;90:1004–8.
3. Svensson PJ, Dahlback B. Resistance to activated protein C as a basis for venous thrombosis. N Engl J Med 1994;330:517–22.
4. Poort SR, Rosendaal FR, Reitsma PH. 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;88:3698–703.
5. Ray JG. Meta-analysis of hyperhomocysteinemia as a risk factor for venous thromboembolic disease. Arch Intern Med 1998;158: 2101–6.
6. Bogardus ST, Concado J, Feinstein AR. Clinical epidemiological quality in molecular genetic research: the need for methodological standards. JAMA 1999;281:1919–26.
7. Little J, Bradley L, Bray MS. Reporting appraising and integrating data on genotype prevalence and gene-disease associations. Am J Epidemiol 2002;156:300–10.
8. Egger M, Sterne JAC, Smith GD. Meta-analysis software. Brit Med J 1998;316:7126.
9. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177–88.
10. Morita H, Kurihara H, Sugiyama T. Polymorphism of the methionine synthase gene: association with homocysteine metabolism and late-onset vascular diseases in the Japanese population. Arterioscler Thromb Vasc Biol 1999;19:298–302.
11. Rassoul F, Richter V, Janke C. Plasma homocysteine and lipoprotein profile in patients with peripheral arterial occlusive disease. Angiology 2000;51:189–96.
12. Iniesta J, Corral J, Fernandez-Pardo J. Factor-V (Arg506→Gln) mutation in ischemic cerebrovascular disease. Haemostasis 1997; 27:105–11.
13. Catto A, Carter A, Ireland H. Factor V Leiden gene mutation and thrombin generation in relation to the development of acute stroke. Arterioscler Thromb Vasc Biol 1995;15:783–5.
14. Araujo F, Santos A, Araujo V. Genetic risk factors in acute coronary disease. Haemostasis 1999;29:212–8.
15. Arruda V, Annichino-Bizzacchi JM, Gonçalves MS. Prevalence of the prothrombin gene variant (nt20210A) in venous thrombosis and arterial disease. Thromb Haemost 1997;78:1430–3.
16. Arruda VR, Siquiera LH, Chiaparini LC. Prevalence of the prothrombin gene variant 20210G→A among patients with myocardial infarction. Cardiovasc Res 1998;37:42–5.

17. Wu Y, Tomon M, Sumino K. Methylenetetrahydrofolate reductase gene polymorphism and ischemic stroke: sex difference in Japanese. Kobe J Med Sci 2001;47:255–62.
18. Virgos C, Joven J, Simo JM. Homocysteine and the C677T mutation of methylenetetrahydrofolate reductase in survivors of premature myocardial infarction. Clin Biochem 2000;33:509–12.
19. D'Angelo A, Coppola A, Madonna P. The role of vitamin B12 in fasting hyperhomocysteinemia and its interaction with the homozygous C677T mutation of the methylenetetrahydrofolate reductase (MTHFR) gene. Thromb Haemost 2000;83:563–70.
20. Dilley A, Hooper WC, El-Jamil M. Mutations in the genes regulating methylenetetrahydrofolate reductase (MTHFR C→T677) and cystathione β-synthase (CBS G→A919, CBS T→c833) are not associated with myocardial infarction in African Americans. Thromb Res 2001;103:109–15.
21. Zhang G, Dai C. Gene polymorphisms of homocysteine metabolism-related enzymes in Chinese patients with occlusive coronary artery or cerebral vascular diseases. Thromb Res 2001;104:187–95.
22. Press RD, Liu XY, Beamer N. Ischemic stroke in the elderly: role of the common factor V Leiden mutation causing resistance to activated protein C. Stroke 1996;27:44–8.
23. Wang XL, Wang J, McCredie RM. Polymorphisms of factor V, factor VII, and fibrinogen genes: relevance to severity of coronary artery disease. Arterioscler Thromb Vasc Biol 1997;17:246–51.
24. Redondo M, Watzke HH, Stucki B. Coagulation factors II, V, VII, and X, prothrombin gene 20210G→A transition, and factor V Leiden in coronary artery disease. Arterioscler Thromb Vasc Biol 1999;19:1020–5.
25. Holm J, Zoller B, Berntorp E. Prevalence of factor V gene mutation amongst myocardial infarction patients and healthy controls is higher in Sweden than in other countries. J Intern Med 1996;239:221–6.
26. Baranovskaya S, Kudinov S, Fomicheva E. Age as a risk factor for myocardial infarction in Leiden mutation carriers. Mol Genet Metab 1998;63:155–7.
27. Corral J, Gonzalez-Conejero R, Lozano ML. The venous thrombosis risk factor 20210A allele of prothrombin gene is not a major risk factor for arterial thrombotic disease. Br J Haematol 1997;99:304–7.
28. Dilley A, Harland A, Hooper WC. Prevalence of the prothrombin 20210 G-to-A variant in blacks: infants, patients with venous thrombosis, patients with myocardial infarction, and control subjects. J Lab Clin Med 1998;132:452–5.
29. Prohaska W, Mannebach H, Schimidt M. Evidence against heterozygous coagulation factor V 1691 G→A mutation with resistance to activated protein C being a risk factor for coronary artery disease and myocardial infarction. J Mol Med 1995;73:521–4.
30. Ridker PM, Hennekens CH, Miletich JP. G20210A mutation in prothrombin gene and risk of myocardial infarction, stroke, and venous thrombosis in a large cohort of US men. Circulation 1999;99:999–1004.
31. Croft SA, Daly ME, Steeds RP. The prothrombin 20210A allele and its association with myocardial infarction. Thromb Haemost 1999;81:861–4.
32. Doggen CJM, Cats VM, Bertina RM. Interaction of coagulation defects and cardiovascular risk factors. Circulation 1998;97:1037–41.
33. Rosendaal FR, Siscovick DS, Schwartz SM. Factor V Leiden (resistance to activated protein C) increases the risk of myocardial infarction in young women. Blood 1997;89:2817–21.
34. Ridker PM, Hennekens CH, Lindpainter K. 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;332:912–7.
35. Eikelboom JW, Baker RI, Parsons R. No association between the 20210G/A prothrombin gene mutation and premature coronary artery disease. Thromb Haemost 1998;80:878–80.
36. Rosendaal FR, Siscovick DS, Schwartz SM. A common prothrombin variant (20210 G to A) increases the risk of myocardial infarction in young women. Blood 1997;90:1747–50.
37. Ardissino D, Peyvandi F, Merlini PA. Factor V (Arg506→Gln) mutation in young survivors of myocardial infarction. Thromb Haemost 1996;75:701–2.
38. Inbal A, Freimark D, Modan B. Synergistic effects of prothrombotic polymorphisms and atherogenic factors on the risk of myocardial infarction in young males. Blood 1999;93:2186–90.
39. Cushman M, Rosendaal FR, Psaty BM. Factor V Leiden is not a risk factor for arterial vascular disease in the elderly: results from the Cardiovascular Health Study. Thromb Haemost 1998;79:912–5.
40. Gardemann A, Arsic T, Katz N. The factor II G20210A and factor V G1691A gene transitions and coronary heart disease. Thromb Haemost 1999;81:208–13.
41. Anderson J, King GJ, Thomson MJ. A mutation in the methylenetetrahydrofolate reductase gene is not associated with increased risk for coronary artery disease or myocardial infarction. J Am Coll Cardiol 1997;30:1206–11.
42. Makris TK, Krespi PG, Hatzizacharias AN. Resistance to activated protein C and FV Leiden mutations in patients with a history of acute myocardial infarction or primary hypertension. Am J Hypertens 2000;13:61–5.
43. Ardissino D, Mannucci PM, Merlini PA. Prothrombotic genetic risk factors in young survivors of myocardial infarction. Blood 1999;94:46–51.
44. Gowda MS, Zucker ML, Vacek JL. Incidence of factor V Leiden in patients with acute myocardial infarction. J Thromb Thrombolysis 2000;9:43–5.
45. Russo C, Girelli D, Olivieri O. G20210A prothrombin gene polymorphism and prothrombin activity in subjects with or without angiographically documented coronary artery disease. Circulation 2001;103:2436–40.
46. Feng YJ, Draghi A, Linfert DR. Polymorphism in the genes for coagulation factors II, V, and VII in patients with ischemic heart disease. Arch Pathol Lab Med 1999;123:1230–5.
47. Kontula K, Ylikorkala A, Miettinen H. Arg506Gln factor V mutation (factor V Leiden) in patients with ischemic cerebrovascular disease and survivors of myocardial infarction. Thromb Haemost 1995;73:558–60.
48. Franco RF, Trip MD, ten Cate H. The 20210G to A mutation in the 3′-untranslated region of the prothrombin gene and the risk for arterial thrombotic disease. Br J Haemotol 1999;104:50–4.
49. Mansourati J, Da Costa A, Munier S. Prevalence of factor V Leiden in patients with myocardial infarction and normal coronary angiography. Thromb Haemost 2000;83:822–5.
50. Burzotta F, Paciaroni K, de Stefano V. Increased prevalence of the G20210A prothrombin gene variant in acute coronary syndromes without metabolic or acquired risk factors or with limited extent of disease. Eur Heart J 2002;23:26–30.
51. Junker R, Heinrich J, Schulte H. Plasminogen activator inhibitor-1 4G/5G-polymorphism and factor V Q506 mutation are not associated with myocardial infarction in young men. Blood Coagul Fibrinolysis 1998;9:597–602.

52. Smiles AM, Jenny NS, Tang Z. No association of plasma prothrombin concentration or the G20210A mutation with incident cardiovascular disease. Thromb Haemost 2002;87:614–21.
53. Gardemann A, Weidemann H, Philipp M. The TT genotype of the methylenetetrahydrofolate reductase C677T gene polymorphism is associated with the extent of coronary atherosclerosis in patients at high risk for coronary artery disease. Eur Heart J 1999;20:584–92.
54. Lopaciuk S, Bykowska K, Kwiecinski H. Factor V Leiden, prothrombin G20210A variant, and methylenetetrahydrofolate reductase C677T genotype in young adults with ischemic stroke. Clin Appl Thromb Haemost 2001;7:346–50.
55. Longstreth WT, Rosendaal FR, Siscovick DS. Risk of stroke in young women and two prothrombotic mutations: factor V Leiden and prothrombin gene variant (G20210A). Stroke 1998;29:577–80.
56. de Stefano V, Chiusolo P, Paciaroni K. Prothrombin G20210A mutant genotype is a risk factor for cerebrovascular ischemic disease in young patients. Blood 1998;91:3562–5.
57. Markus HS, Zhang Y, Jeffery S. Screening for the factor-V Arg 506Gln mutation in patients with TIA and stroke. Cerebrovasc Dis 1996;6:360–2.
58. Fisher M, Fernandez J, Ameriso SF. Activated protein C resistance in ischemic stroke not due to factor V Arg506→Glutamine mutation. Stroke 1996;27:1163–6.
59. Nabavi DG, Junker R, Wolff E. Prevalence of factor V Leiden mutation in young adults with cerebral ischemia: a case-control study on 225 patients. J Neurol 1998;245:653–8.
60. Margaglione M, D'Andrea G, Giuliani N. Inherited prothrombotic conditions and premature ischemic stroke. Arterioscler Thromb Vasc Biol 1999;19:1751–6.
61. Lalouschek W, Aull S, Serles W. C677T MTHFR mutation and factor V Leiden mutation in patients with TIA/minor stroke: a case-control study. Thromb Res 1999;93:61–9.
62. Ferrerasi P, Marchetti G, Legnani E. The heterozygous 20210G/A prothrombin genotype is associated with early venous thrombosis in inherited thrombophilias and is not increase in frequency in artery disease. Arterioscler Thromb Vasc Biol 1997;17:2418–22.
63. Hankey GJ, Eikelboom JW, van Bockxmeer FM. Inherited thrombophilia in ischemic stroke and its pathogenic subtypes. Stroke 2001;321:1793–9.
64. Madonna P, de Stefano V, Coppola A. Hyperhomocysteinemia and other inherited prothrombotic conditions in young adults with a history of ischemic stroke. Stroke 2002;33:51–6.
65. Pezzini A, Del Zotto E, Archetti S. Plasma homocysteine concentration, C677T MTHFR genotype, and 844ins68bp CBS genotype in young adults with spontaneous cervical artery dissection and atherothrombotic stroke. Stroke 2002;33:664–9.
66. Morita H, Kurihara H, Tsubaki S. Methylenetetrahydrofolate reductase gene polymorphism and ischemic stroke in Japanese. Arterioscler Thromb Vasc Biol 1998;18:1465–9.
67. Markus HS, Ali N, Swaminathan R. A common polymorphism in the methylenetetrahydrofolate reductase gene, homocysteine, and ischemic cerebrovascular disease. Stroke 1997;28:1739–43.
68. Harmon D, Doyle RM, Meleady R. Genetic analysis of the thermolabile variant of 5,10-methylenetetrahydrofolate reductase as a risk factor for ischemic stroke. Arterioscler Thromb Vasc Biol 1999;99:208–11.
69. Voetsch B, Damasceno BP, Camargo ESC. Inherited thrombophilia as a risk factor for the development of ischemic stroke in young adults. Thromb Haemost 2000;83:229–33.
70. Girelli D, Friso S, Trabetti E. Methylenetetrahydrofolate reductase C677T mutation, plasma homocysteine, and folate in subjects from northern Italy with or without angiographically documented severe coronary atherosclerotic disease: evidence for an important genetic-environmental interaction. Blood 1998;91:4158–63.
71. Ma J, Stampfer MJ, Hennekens CH. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physician. Circulation 1996;94:2410–6.
72. Adams M, Smith PD, Martin D. Genetic analysis of thermolabile methylenetetrahydrofolate reductase as a risk factor for myocardial infarction. QJM 1996;89:437–44.
73. Schwartz SM, Siscovick DS, Malinow R. Myocardial infarction in young women in relation to plasma total homocysteine, folate and a common variant in the methylenetetrahydrofolate reductase gene. Circulation 1997;96:412–7.
74. van Bockxmeer FM, Mamotte CDS, Vasikaran SD. Methylenetetrahydrofolate reductase gene and coronary artery disease. Circulation 1997;95:21–3.
75. Schmitz C, Lindpainter K, Verhoef P. Genetic polymorphism of methylenetetrahydrofolate reductase and myocardial infarction. Circulation 1996;94:1812–4.
76. Brugada R, Marian AJ. A common mutation in methylenetetrahydrofolate reductase gene is not a major risk of coronary artery disease or myocardial infarction. Atherosclerosis 1997;128:107–12.
77. Juul K, Tybjarg-Hansen A, Steffensen R. Factor V Leiden: the Copenhagen City Heart Study and 2 meta-analyses. Blood 2002; 100:3–10.
78. Renner W, Koppen H, Brodmann M. Factor II G20210A and factor V G1691A gene mutations and peripheral arterial occlusive disease. Thromb Haemost 2000;83:20–2.
79. Krinstensen B, Malm J, Nilsson TK. Hyperhomocysteinemia and hypofibrinolysis in young adults with ischemic stroke. Stroke 1999; 30:974–80.
80. Szolnoki Z, Somogyvari F, Kondacs A. Evaluation of the roles of the Leiden V mutation and ACE I/D polymorphism in subtypes of ischemic stroke. J Neurol 2001;248:756–61.
81. Celik S, Ovali E, Baykan M. Factor V Leiden and its relation to left ventricular thrombus in acute myocardial infarction. Acta Cardiol 2001;56:1–6.
82. Psaty BM, Smith NL, Lemaitre RN. Hormone replacement therapy, prothrombotic mutations, and the risk of incident nonfatal myocardial infarction in postmenopausal women. JAMA 2001;285:906–13.
83. Fernandez-Arcas N, Dieguez-Lucena JL, Munoz-Moran E. The genotype interactions of methylenetetrahydrofolate reductase and renin-angiotensin system genes are associated with myocardial infarction. Atherosclerosis 1999;145:293–300.
84. Thogersen AM, Nilsson TK, Dahlen G. Homozygosity for the C677→T mutation of 5,10-methylenetetrahydrofolate reductase and total plasma homocyst(e)ine are not associated with greater than normal risk of a first myocardial infarction in northern Sweden. Coron Artery Dis 2001;12:85–90.
85. Gule 86 S, Aras O, Akar E. Methylenetetrahydrofolate reductase gene polymorphism and risk of premature myocardial infarction. Clin Cardiol 2001;24:281–4.
86. Thomas DP. Overview of venous thrombogenesis. Semin Thromb Haemost 1988;68:329–37.
87. Mustard JF, Packham MA, Kinlough-Rathbone RI. Platelets, blood flow, and the vessel wall. Circulation 1990;81(1 Suppl):124–7.
88. Burke AP, Farb A, Pestaner J. Traditional risk factors and the incidence of sudden coronary death with and without coronary thrombosis in blacks. Circulation 2002;105:419–24.

89. Dahlback B. Resistance to activated protein C as a risk factor for thrombosis: molecular mechanisms, laboratory investigation, and clinical management. Semin Hematol 1997;34:217–34.
90. Nowak-Gottl U, Strater R, Heinecke A. Lipoprotein(a) and genetic polymorphisms of clotting factor V, prothrombin, and methylenetetrahydrofolate reductase are risk factors of spontaneous ischaemic stroke in childhood. Blood 1999;94:3678–82.
91. Vandenbroucke JP, Koster T, Briet E. Increased risk of venous thrombosis in oral contraceptive users who are carriers of factor V Leiden mutation. Lancet 1994;344:1453.
92. Rosendaal FR, Helmerhorst FM, Vandenbroucke JP. Female hormones and thrombosis. Arterioscler Thromb Vasc Biol 2002;22: 201–10.
93. Tremoli E, Camera M, Toschi V. Tissue factor in atherosclerosis. Atherosclerosis 1999;144:273–83.
94. Cella G, Burlina A, Sbarai A. Tissue factor pathway inhibitor levels in patients with homocysteinuria. Thromb Res 2000;98:375–81.
95. Moor E, Silveira A, van't Hooft F. Coagulation Factor V(Arg^{506}→Gln) mutation and early saphenous vein graft occlusion after coronary artery bypass grafting. Thromb Haemost 1998;80: 220–4.
96. French JK, Van de Water NS, Sutton TM. Potential thrombophilic mutations/polymorphism in patients with no flow-limiting stenosis after myocardial infarction. Am Heart J 2003;145:118–24.
97. Klerk M, Verhoef P, Clarke R. MTHFR 677C→T polymorphism and risk of coronary heart disease. JAMA 2002;288:2023–31.