Can screening for genetic markers improve peripheral artery bypass patency?

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Objective: **Three genetic mutations have been associated with an increased risk of thromboembolic events: factor V Leiden R506Q, prothrombin G20210A, and methylenetetrahydrofolate reductase C677T (MTHFR) mutations. The aim of this study was to determine the effect of these mutations on patency of peripheral bypass procedures and preoperative and postoperative thromboembolic events.**

Methods: **Two hundred forty-four randomly selected volunteers participating in the Veterans Affairs Cooperative Study #362 were tested for factor V Leiden, prothrombin, or MTHFR mutations with polymerase chain reaction. Patients enrolled in the study were randomized to receive aspirin therapy or aspirin and warfarin therapy after a peripheral bypass procedure. The frequencies of preoperative and postoperative thromboembolic events and primary patency (PP), assisted primary patency (APP), and secondary patency (SP) rates were compared among carriers of the various mutations.**

Results: **Fourteen patients (5.7%) were heterozygous for the factor V Leiden mutation, seven (2.9%) were heterozygous for the prothrombin mutation, and 108 (44.6%) were heterozygous and 15 (6.2%) homozygous for the MTHFR mutation. After surgery, patients homozygous for the MTHFR gene mutation had increased graft thrombosis, compared with patients who were heterozygous (33.3% versus 11.1%; $P = .01$), and lower PP, APP and SP rates ($P < .05$). Furthermore, patients heterozygous for the MTHFR mutation had fewer graft thromboses (11.1% versus 24.4%; $P = .01$), fewer below-knee amputations (0.9% versus 7.6%; $P = .02$), and higher PP, APP, and SP rates (PP, 79.6%; APP, 88.9%; SP, 90.7%; $P < .05$) compared with wild-type control subjects (PP, 63%; APP, 75.6%; SP, 76.5%; $P < .05$).**

Conclusion: **Patients with either factor V Leiden or prothrombin mutations were not at an increased risk for postoperative graft occlusion or thromboembolic events. Patients heterozygous for MTHFR mutation had a lower risk of graft thrombosis and higher graft patency rates compared with both homozygous and wild-type control subjects. Patients homozygous for the MTHFR mutation had lower graft patency rates compared with patients who were heterozygous, and a trend was seen toward lower patency rates compared with wild-type control subjects. Therefore, screening for the MTHFR gene mutation before surgery may identify patients at an increased risk of graft thrombosis. (J Vasc Surg 2002; 36:1198-206.)**

Thromboembolic events, most commonly in the form of deep venous thrombosis (DVT), pulmonary embolism, myocardial infarction (MI), or stroke, cause considerable yearly mortality and morbidity.[1,2] Currently, effective forms of therapy are directed toward anticoagulation therapy, thrombolysis, or circumvention of the occluded vascular segment through arterial reconstruction. However, a significant number of coronary artery and peripheral artery bypass grafts fail because of early thrombotic occlusion.[3,4] The ability to identify patients at increased risk of graft thrombosis could significantly alter their management and hopefully improve their outcome.

In the past decade, our knowledge about the genetic polymorphisms that predispose patients to thrombosis has expanded. Currently, the most common genetic cause of a prothrombotic phenotype is the factor V Leiden mutation in which one nucleotide change at position 1691 of the gene results in the substitution of adenine for guanine in the structure of factor V.[5,6] This mutation results in a delay in the inactivation of activated factor V.[5] It is present in approximately 5% of the US population and increases the risk of venous thrombosis three-fold to eight-fold for heterozygous carriers[7-10] and 80-fold for homozygous carriers.[8] The factor V Leiden mutation also has been linked to a variety of arterial events.[11-13] The second most common genetic cause of thrombophilia is a mutation in the prothrombin gene, resulting in excess circulating levels of serum prothrombin (factor II).[14] This single point mutation in the 3′ untranslated region of the prothrombin gene (on chromosome 11) at nucleotide 20210 is present in 2% of the general population and confers an odds ratio (OR) of thrombosis of 2.1 to 2.8.[7,14,15] Furthermore, a single point mutation in the methylenetetrahydrofolate reductase (MTHFR) gene has been described at nucleotide 677 involving a change of cytosine to thymine.[16] Approxi-

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mately 45% of the general population is heterozygous for the mutation, and only 10% are homozygous.[17-19] Depending on the serum folate level and other factors, carriers of the MTHFR mutation may manifest hyperhomocysteinemia.[20] Hyperhomocysteinemia and the presence of the MTHFR mutation itself have both been independently shown to be risk factors for venous thrombosis, although their role in arterial thrombosis remains controversial.[21-25] Given the prevalence of these genetic polymorphisms in the general population and their association with venous thrombosis and less often with arterial thrombosis, the aim of this study was to determine whether the existence of any one of these genetic mutations would increase the risk of graft thrombosis or postoperative thromboembolic events in a patient population undergoing peripheral bypass procedures.

METHODS

Veterans Affairs Cooperative Study #362. This study was a subset of the Veterans Affairs (VA) Cooperative Study #362 that prospectively enrolled 831 patients undergoing arterial revascularization of the lower extremities between October 1, 1991, and September 30, 1996. Patients were stratified into two arms: those receiving autologous vein (femoral-popliteal or femoral-tibial, femoral-peroneal, or femoral-pedal bypass) or prosthetic grafts (axillo-femoral-femoral, femoral-femoral, femoral-popliteal above-knee, or femoral-popliteal below-knee). All participants were prospectively randomized to one of two treatment groups that consisted of aspirin therapy (325 mg) or aspirin (325 mg) plus low-dose warfarin therapy (prothrombin time, 1.2 to 1.5; or international normalized ratio, 2 to 3). Treatment with aspirin was initiated before surgery, and warfarin therapy was initiated on postoperative day 1. Both agents were intended to be continued throughout the duration of the study. All data were analyzed on an intent-to-treat basis. The study was conducted at 15 medical centers according to the program policies set forth in the Guidelines for VA Cooperative Studies. All patients signed an approved informed consent form for the VA Cooperative Study #362.

The primary endpoint was the initial occlusion of the vascular reconstruction.[26] Additional endpoints were amputation, stroke, MI, major hemorrhage, and death. Significant intercurrent events, including hemorrhage, arterial thromboembolic or occlusive events, and venous thromboembolic events, were recorded. Patients with endpoints were observed until death within the study period.

The mean follow-up period was 3 years, with a range of 2 to 6 years. All patients underwent clinical evaluation during their hospitalization, in the immediate postoperative period at 2 weeks, and every 3 months thereafter. Study protocol forms were completed during the hospitalization and at each outpatient visit. Graft patency was evaluated with clinical examination of the pulses distal to the bypass graft and with color-flow duplex scanning at each of the follow-up visits. Bypass graft occlusion was confirmed with either duplex scan or arteriographic evaluation. *Primary patency* (PP) was defined as patency that was maintained without any operative or angiographic manipulations to extend patency, and *assisted primary patency* (APP) was defined as patency maintained with remedial surgery or angiographic technique to correct a defect, such as stenosis, with the graft remaining patent.

Genetic analysis. Of the 831 patients enrolled into VA Cooperative Study #362, blood samples from 244 volunteers consecutively evaluated in the postoperative period from January 1, 1995, to March 31, 1996, were collected and analyzed with polymerase chain reaction (PCR) for factor V Leiden, prothrombin G20210A, or MTHFR gene mutations. All 244 patients signed an additional approved informed consent form before the collection of the blood samples for the purposes of genetic analysis.

Polymerase chain reaction. Testing for the factor V Leiden mutation was performed as previously described by Bontempo et al.[6] Primers for prothrombin G20210A mutation were designed to generate Taq I susceptible PCR products for the wild-type allele: PTH-4 5′-CAATAAAAGTGACTCTCATC-3″ (20190-20209) and PTH-5 5′-AGGTGGTGGATTCTTAAGTC-3′ (20307-20288).[27] Primers for MTHFR C677T gene variant were as described by Frosst et al[16]: CyF-1 5′-TGAAGGAGAAGGTGTCTGCGGGA 3′ and CyF-2 5′-AGGACGGTGCGGTGAGAGTG-3′. Amplification of 50 to 500 ng of genomic DNA was performed in a 30-cycle PCR reaction for each primer pair. Cycles consisted of denaturaton of 94° C for 15 seconds, annealing at 55° C for 15 seconds, and extension at 72° C for 30 seconds in a 9600 Perkin Elmer thermal cycler (Norwalk, Conn). PCR products were electrophoresed on a neutral 3% agarose gel for inspection.

PTH-4 and PTH-5 amplify a 118-base pair (bp) fragment of the gene encoding the prothrombin protein. The 118-bp fragment includes the G20210A transition site. After Taq I treatment, the wild-type allele yields fragments of 98-bp and 20-bp and the mutant allele that is not susceptible to Taq I yields a 118-bp fragment. Repeat testing of mutant alleles is routinely performed.

CyF-1 and CyF-2 amplify a 198-bp fragment of the MTHFR gene containing the C677T transition site. Hinf I digestion of a mutant allele yields fragments of 175 and 23 bp, and the wild-type allele is not susceptible to digestion (198 bp). All extractions and amplifications were performed in a molecular diagnostics facility. To ensure the absence of amplicon contamination, water negative controls were included in each test.

Thromboembolic variables. The association of these mutations with several venous and arterial thromboembolic events, and other atherosclerotic risk factors, was evaluated. The preoperative variables evaluated included a history of hypertension, diabetes mellitus, MI, transient ischemic attack (TIA), reversible ischemic neurologic deficit, stroke, current tobacco use, and any prior lower extremity amputations. After surgery, correlation was made to graft thrombosis, MI, TIA, stroke, ocular ischemic event, and DVT.

Table I. Patient demographics by gene mutations

Preoperative variable	*Factor V heterozygote*	*Factor V wild-type*	*Prothrombin heterozygote*
Overall*	14/244 (5.7%)	230/244 (94.3%)	7/239 (2.9%)
Race			
Black	0/48	48/48 (100.0%)	0/47
White	14/196 (7.1%)	182/196 (80.3%)	7/192 (3.7%)
Hypertension	6/14 (42.9%)	125/230 (54.4%)	1/7 (14.3%)
Diabetes mellitus	2/14 (14.3%)	82/230 (35.7%)	2/7 (28.6%)
Current tobacco use	9/14 (64.3%)	135/230 (58.7%)	6/7 (85.7%)
Age (mean ± standard error)	59.7 ± 10.6	63.5 ± 7.7	63.6 ± 8.5

*Note: *Overall* also reflects six patients of other races.

PP, APP, and secondary patency (SP) rates were calculated for all groups.

Statistics. Group differences for postoperative thromboembolic events were analyzed with the Fisher exact test, two-tail. Group differences for patency rates were analyzed with the Cox proportional hazards model. Independent predictors were determined with Cox regression. The Kaplan-Meier method was used in the construction of the patency plots. Figures are represented as the mean ± the standard error. Significance was assumed at *P* values of less than .05.

RESULTS

Gene polymorphism and patient demographics. Of the 244 patients evaluated for this study, 14 (5.7%) were heterozygous carriers of the factor V Leiden mutation and seven (2.9%) were heterozygous carriers of the prothrombin G20210A gene mutation. No patients who were homozygous for these mutations were encountered. One hundred eight patients (44.6%) were heterozygous and 15 (6.2%) were homozygous for the MTHRF gene mutation (Table I). All of the mutations were more frequent among white than black patients. No age differences were seen among the various mutation groups. Similarly, the incidence rate of diabetes mellitus, hypertension, and tobacco use was comparable among groups (Table I).

Preoperative thromboembolic events. No differences were found within the three gene polymorphisms and a preoperative history of MI, reversible ischemic neurologic deficit, or stroke (Table II). Heterozygous carriers of the MTHFR mutation reported more TIA compared with MTHFR wild-type control subjects (15% versus 2.5%; Table II). Heterozygous carriers of the factor V Leiden mutation reported more preoperative amputations compared with factor V Leiden wild-type control subjects (37.5% versus 13.9; Table II). No trend in type of amputation was noted.

Postoperative thromboembolic events. Of the postoperative thromboembolic events monitored, including MI, TIA, ocular ischemic events, stroke, DVT, or pulmonary embolism, no significant differences were seen within mutation groups. However, a trend was seen toward graft thrombosis with homozygous carriers of the MTHFR mutation compared with the MTHFR wild-type and heterozygous control subjects (Table III). Unexpectedly, heterozygous carriers of the MTHFR gene mutation had significantly less graft thrombotic events when compared with both MTHFR homozygous and wild-type control subjects (11.1% versus 33.3% and 24.4%, respectively; $P = .010$; Table III). Moreover, heterozygous MTHFR carriers were also less like to undergo below-knee amputation compared with MTHFR wild-type control subjects (0.9% versus 7.6%; $P = .020$).

Graft patency. Cumulative PP, APP, and SP rates, with a mean follow-up period of 3 years, were determined for all patients and were analyzed according to gene mutation (Table IV). Overall, for the 244 patients in this study, the PP rate was 69.7%, the APP rate was 81.1%, and the SP rate was 82.4%. Analysis of patency rates with gene mutation revealed statistically similar data with the exception of carriers of the MTHFR mutation (Table IV and Fig). Patients who were heterozygous had significantly higher PP (79.6%), APP (88.9%), and SP (90.7%) rates compared with both wild-type control subjects (63.0%; $P = .019$; 75.6%; $P = .018$; and 76.5%; $P = .009$, respectively) and homozygous carriers (46.7%; $P = .005$; 66.7%; $P = .021$; and 66.7%; $P = .009$, respectively). Homozygous carriers of the MTHFR mutation had patency rates lower than wild-type control subjects, but given the small number of patients in this group, statistical significance was not reached.

Primary patency, APP, and SP rates among the various genetic make-ups were further analyzed according to study arm and treatment group (Table V). Among the study arms, the same pattern persisted when evaluating patients who received prosthetic grafts. Patients with the heterozygous MTHFR mutation had higher PP, APP, and SP rates compared with both MTHFR homozygous and wild-type control subjects. However, these differences were not observed in patients with similar gene mutations receiving autologous vein as their conduit (Table V). Analysis by treatment group revealed that heterozygous carriers of the MTHFR mutation who received aspirin therapy alone did better than both MTHFR homozygote and wild-type control subjects with respect to PP, APP, and SP rates (Table V). However, among patients with similar genetic muta-

Prothrombin wild-type	*MTHFR heterozygote*	*MTHFR homozygote*	*MTHFR wild-type*
232/239 (97.1%)	108/242 (44.6%)	15/242 (6.2%)	119/242 (49.2%)
47/47 (100.0%)	8/48 (16.7%)	0/48	40/48 (88.3%)
185/192 (96.4%)	100/196 (51.0%)	15/196 (7.7%)	79/196 (40.3%)
128/232 (55.2%)	56/108 (51.9%)	6/15 (40.0%)	68/119 (57.1%)
80/232 (34.5%)	37/108 (34.3%)	4/15 (26.7%)	42/119 (35.3%)
135/232 (58.2%)	64/108 (59.3%)	10/15 (66.7%)	69/119 (58.0%)
63.3 ± 7.8	62.8 ± 8.1	65.0 ± 5.8	63.4 ± 7.9

Table II. Preoperative thromboembolic events by gene mutation

Preoperative variable	*Factor V heterozygote*	*Factor V wild-type*	*Prothrombin heterozygote*	*Prothrombin wild-type*	*MTHFR heterozygote*	*MTHFR homozygote*	*MTHFR wild-type*
MI	2 (14.3%)	50 (21.7%)	2 (28.6%)	49 (21.1%)	22 (20.4%)	3 (20.0%)	26 (21.9%)
TIA	1 (7.1%)	19 (8.3%)	1 (14.3%)	19 (8.2%)	16 (15.0%)	1 (6.7%)	3 (2.5%)
RIND	0	4 (1.8%)	0	4 (1.7%)	2 (1.9%)	1 (6.7%)	1 (0.8%)
Stroke	2 (14.3%)	26 (11.3%)	1 (14.3%)	26 (11.2%)	12 (11.1%)	1 (6.7%)	15 (12.6%)
Prior amputation*	5 (35.7%)	32 (13.9%)	2 (28.6%)	35 (15.1%)	14 (13.0%)	1 (6.7%)	22 (18.5%)
Above-knee	1 (7.1%)	5 (2.2%)	1 (14.3%)	5 (2.2%)	1 (0.9%)	0	5 (4.2%)
Below-knee	1 (7.1%)	4 (1.7%)	0	5 (2.2%)	2 (1.9%)	0	3 (2.5%)
Symes	0	0	0	0	0	0	0
Transmetatarsal	1 (7.1%)	3 (1.3%)	0	4 (1.7%)	1 (0.9%)	0	3 (2.5%)
Digit	1 (7.1%)	18 (7.8%)	1 (14.3%)	18 (7.8%)	6 (5.6%)	1 (6.7%)	12 (10.1%)
Other	1 (7.1%)	7 (3.0%)	0	8 (3.5%)	4 (3.7%)	0	4 (3.4%)
No. (total)	14	230	7	232	108	15	119

*Six patients had more than one amputation.
RIND, Reversible ischemic neurologic deficit.

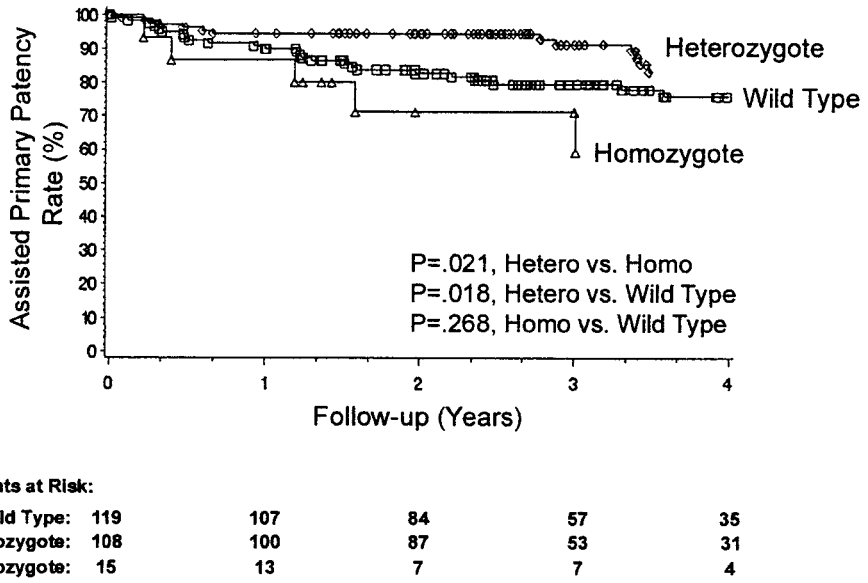
Table III. Postoperative thromboembolic events by gene mutation

Postoperative variable	*Factor V heterozygote*	*Factor V wild-type*	*Prothrombin heterozygote*	*Prothrombin wild-type*	*MTHFR heterozygote*	*MTHFR homozygote*	*MTHFR wild-type*
Graft thrombosis	4 (28.6%)	42 (18.3%)	2 (28.6%)	42 (18.1%)	12 (11.1%)	5 (33.3%)	29 (24.4%)
TIA	0	3 (1.3%)	0	3 (1.3%)	1 (0.9%)	0	2 (1.7%)
Stroke	0	22 (9.6%)	1 (14.3%)	21 (9.1%)	13 (12.0%)	0	8 (6.7%)
Ocular ischemic event	0	3 (1.3%)	0	3 (1.3%)	3 (2.8%)	0	0
MI	0	9 (3.9%)	0	9 (3.9%)	6 (5.6%)	1 (6.7%)	2 (1.7%)
DVT	0	1 (0.4%)	0	1 (0.4%)	1 (0.9%)	0	0
Venous thromboembolism	0	2 (0.9%)	0	2 (0.9%)	1 (0.9%)	0	1 (0.8%)
Amputation	2 (14.3%)	42 (18.3%)	0	44 (19.1%)	16 (14.8%)	4 (26.7%)	24 (20.3%)
Above-knee	2 (14.3%)	7 (3.1%)	0	9 (3.9%)	4 (3.7%)	2 (13.3%)	3 (2.5%)
Below-knee	0	11 (4.8%)	0	11 (4.8%)	1 (0.9%)	1 (6.7%)	9 (7.6%)†
Symes	0	0	0	0	0	0	0
Transmetatarsal	0	7 (3.1%)	0	7 (3.0%)	3 (2.8%)	0	4 (3.4%)
Digit	0	20 (8.7%)	0	20 (8.7%)	9 (8.3%)	2 (13.3%)	9 (7.6%)
Other	0	3 (1.3%)	0	3 (1.3%)	2 (1.9%)	0	1 (0.9%)
No. (total)	14	229	7	231	108	15	118

*$P = .01$ heterozygous versus wild-type.
†$P = 0.02$ heterozygous versus wild-type.

tions receiving both aspirin and warfarin therapy, patency rates were similar across groups from improved patency in the wild-type and homozygote cohorts (Table V).

To determine whether the lack of difference of patency observed between the MTHFR gene mutation groups with respect to the use of autologous vein or among those patients receiving aspirin and warfarin therapy were from a lack of power in our study given the small number of patients in some of the groups, a power analysis was performed. With respect to Table IV, to achieve a statistically significant difference with a two-sided type I error of 0.05 and a power of 80% for the differences observed in APP, the



Life-table analysis of APP patency rates according to **(A)** MTHFR gene mutations, **(B)** treatment arm, and **(C)** treatment group.

Table IV. Graft patency rate by gene mutation

	Factor V heterozygote	*Factor V wild-type*	P *value*	*Prothrombin heterozygote*	*Prothrombin wild-type*	P *value*	*MTHFR heterozygote*	*MTHFR homozygote*	*MTHFR wild-type*	P *value*
PP	9 (64.3%)	161 (70.0%)	.445	4 (57.1%)	164 (70.7%)	.359	86 (79.6%)	7 (46.7%)	75 (63.0%)	.005* .019† .139‡
APP	10 (71.4%)	188 (81.7%)	.199	5 (71.4%)	190 (81.9%)	.461	96 (88.9%)	10 (66.7%)	90 (75.6%)	.021* .018† .268‡
SP	11 (78.6%)	190 (82.6%)	.455	5 (71.4%)	193 (83.2%)	.399	98 (90.7%)	10 (66.7%)	91 (76.5%)	.009* .009† .233‡
No. (total)	14	230		7	232		108	15	119	

*Heterozygote versus homozygote.
†Heterozygote versus wild-type.
‡Homozygote versus wild-type.

following sample sizes would be necessary: heterozygote versus homozygote, n = 56; heterozygote versus wild-type, n = 122; and homozygote versus wild type, n = 403. With respect to Table V, for the differences observed in the aspirin treatment group for APP, the following sample sizes would be necessary to achieve a statistically significant difference with a two-sided type I error of 0.05 and a power of 80%: heterozygote versus homozygote, n = 23; heterozygote versus wild-type, n = 35; and, homozygote versus wild-type, n = 258. With respect to Table V, for the differences observed in the prosthetic study arm for APP, the following sample sizes would be necessary to achieve a statistically significant difference with a two-sided type I error of 0.05 and a power of 80%: heterozygote versus homozygote, n = 31; heterozygote versus wild-type, n = 69; and homozygote versus wild-type, n = 189. As such, our study is underpowered because of the rarity of the mutations and the small sample size in the groups positive for the genetic aberration. All the differences should be interpreted with caution.

DISCUSSION

Thrombosis of peripheral artery bypass grafts remains a significant clinical concern. Approximately 40% of lower extremity reconstructions fail by 4 years.[28] Current therapeutic methods directed toward prevention of graft thrombosis involve antiplatelet therapy or anticoagulation therapy or a combination of both. This, however, implies that patients who are not at an increased risk of thrombosis are treated along with those who are. The ability to identify patients at increased risk of graft occlusion would be beneficial because it would result in the treatment of only those

patients who need anticoagulation, thereby limiting the number of patients exposed to the risks associated with anticoagulant therapy. Although our current knowledge of the hypercoaguable state is expansive, our ability to predict which patients are more prone to have postoperative graft occlusion remains limited. Genetic studies have revealed the existence of multiple mutations that are associated with an increased risk of thrombotic events mostly in the venous system (review[7,29]). Their role if any in arterial bypass failure has never been accurately described. The mere presence of such a genetic mutation and a hypercoaguable state also does not necessarily imply a benefit from postoperative anticoagulation therapy. The aim of this study was to determine whether patients who are carriers of factor V Leiden, prothrombin G20210A, or MTHFR gene mutations were at an increased risk of postoperative thromboembolic events or graft thrombosis.

The factor V Leiden mutation was first described in 1994 in association with resistance to activated protein C and is present in approximately 5% of the US population.[5] Since the original description, it is now widely accepted as a major risk factor for the development of venous thrombosis. Approximately 40% to 60% of patients with a history of familial thrombophilia are carriers of the factor V Leiden mutation, and it is also present in 20% of patients with spontaneous DVT for the first time.[30] Its role in arterial thrombosis, however, is controversial. Most large-scale evaluations found no increased prevalence of the factor V Leiden gene mutation among patients with arterial disease.[10] Smaller studies, however, have associated the mutation with arterial thromboembolic events. Moor et al[12] found that the factor V mutation tended to be associated with early saphenous vein graft occlusion after coronary artery bypass grafting ($P = .06$), and our group[11] found an association between the factor V Leiden mutation and the development of arterial thromboembolic events (MI and stroke).

The association of the factor V Leiden mutation with peripheral vascular disease is less well characterized. Donaldson et al[13] concluded that the presence of the factor V mutation was associated with early cerebrovascular events and late graft thrombosis ($P < .030$). However, Foley et al[31] reported a prevalence of the factor V Leiden mutation in patients with peripheral vascular disease of 17.8%, which is much higher than the 3.5% in the United Kingdom population. But they failed to identify an association with graft occlusion after arterial reconstruction. Our current data found no increase in the prevalence of the factor V Leiden mutation in patients with severe peripheral vascular disease and no association with postoperative graft failures. The patient populations studied may explain the differences among the various reports. Each patient population has its own preexisting diseases and independent risk factors for thromboembolic events, resulting in different risks for thrombosis. Screening for the factor V Leiden mutation would thus be of little benefit in this context.

The body of literature on the prothrombin G20210A gene mutation is much less extensive than that on the factor V Leiden mutation. Originally described in 1996, this less frequent genetic mutation has also been established as a risk factor for venous thromboembolisms.[14] Although its prevalence in the general population is 2%, it is found in 6.2% of patients with DVT and in 18% of patients with a family history of thrombophilia.[14,32] Similar to factor V Leiden mutation, its role in arterial thromboembolism is less well established. The studies evaluating the association between the prothrombin G20210A gene mutation and arterial thromboembolism are few. They have linked the gene to an increased risk of MI[32] and cerebral infarction.[33] Other reports found no increased risk of arterial thrombosis.[34] These studies are limited in number and have small patient enrollments. Our data revealed a prevalence in patients with severe peripheral vascular disease similar to the general population and no clear effect on graft thrombosis or arterial events. With the rarity of the mutation, and until additional data are encountered, screening for this gene in association with arterial reconstruction would not be useful.

The role of the MTHFR gene mutation in venous and arterial thrombosis is much more complex. The presence of the MTHFR mutation has been associated with high serum homocysteine levels in some studies but not in others.[18,19,35-37] It has been postulated that the serum folate level, in addition to other factors, may determine whether the MTHFR mutation will manifest itself with high or normal homocysteine levels.[20] Hyperhomocysteinemia has been independently associated with an increased risk of venous thrombosis, and its role in arterial thrombosis and coronary artery disease remains debatable.[21-25] Because not all patients with the MTHFR mutation have high homocysteine levels, and not all patients with high homocysteine levels have MTHFR mutations, and because there are many other causes of hyperhomocysteinemia, much remains to be learned about this genetic mutation. Although a plethora of studies has evaluated hyperhomocysteinemia and thromboembolic events, few studies have analyzed the association of the genetic mutation with an increased risk of thrombosis. To our knowledge, the relative importance of the heterozygous MTHFR mutation has not been documented before. We noted that heterozygous carriers of the MTHFR gene mutation actually had less graft thrombosis, higher patency rates, and fewer below-knee amputations. If this finding were corroborated with additional studies, it would be reasonable to speculate that the gene in a heterozygous state actually confers a survival benefit, resulting in a selection bias with propagation of the gene. This would explain the relatively high prevalence of this mutation. The heterozygote state may also be a marker of a yet unidentified biochemical or physiologic change that is protective against thrombus formation.

We also observed a trend in the homozygous carriers of the MTHFR mutation toward more graft thromboses and lower patency rates. However, this trend failed to reach statistical significance possibly because of the small number of patients with this particular mutation. A larger cohort of patients may reveal a significant association between the

Table V. Patency rate by treatment group/study arm

	Factor V heterozygote	*Factor V wild-type*	P *value*	*Prothrombin heterozygote*
PP by treatment group				
Aspirin	4/6 (66.7%)	76/109 (69.7%)	.300	2/5 (40.0%)
Aspirin + warfarin	5/8 (62.5%)	85/121 (70.3%)	.691	2/2 (100.0%)
PP by study arm				
Prosthetic	5/7 (71.4%)	88/118 (74.6%)	.535	3/4 (75.0%)
Vein	4/7 (57.1%)	73/112 (65.2%)	.575	1/3 (33.3%)
APP by treatment group				
Aspirin	4/6 (66.7%)	85/109 (78.0%)	.276	3/5 (60.0%)
Aspirin + warfarin	6/8 (75.0%)	103/121 (85.1%)	.326	2/2 (100.0%)
APP by study arm				
Prosthetic	5/7 (71.4%)	91/118 (77.1%)	.471	3/4 (75.0%)
Vein	5/7 (71.4%)	97/112 (86.6%)	.214	2/3 (66.7%)
SP by treatment group				
Aspirin	4/6 (66.7%)	85/109 (78.0%)	.276	3/5 (60.0%)
Aspirin + warfarin	7/8 (87.5%)	105/121 (86.8%)	.859	2/2 (100.0%)
SP by study arm				
Prosthetic	6/7 (85.7%)	92/118 (78.0%)	.920	3/4 (75.0%)
Vein	5/7 (71.4%)	98/112 (87.5%)	.182	2/3 (66.7%)

*Heterozygote versus homozygote.
†Heterozygote versus wild-type.
‡Homozygote versus wild-type.

presence of the homozygote mutation and an increased risk of graft thrombosis.

In this study, we compared the patency rates within the different gene mutation groups for the different treatment groups and study arms. We did not find a statistically significant difference among the MTHFR gene mutation populations with respect to patency with warfarin therapy or a vein conduit. One potential explanation for this pattern in the data could be a type I error from overanalysis of the data through multiple statistical comparisons. A larger prospective study would be necessary to confirm or refute these data. Furthermore, although multiple statistical comparisons were made with the data in this manuscript, large enough sample sizes did not exist for most of the gene mutation groups to achieve 80% power. The intent of this study was not to definitively determine the predictive value of the presence of these gene mutations with respect to graft patency. Rather, the intent was to determine whether a relationship existed that would be worth pursuing with further investigations.

Another shortcoming of this study was the limited number of patients with more than one mutation. Because only nine such patients existed in our study, an analysis of the effect of multiple mutations with respect to patency and graft occlusion was not possible. In a study by Salomon et al,[38] the presence of one, two, or three of these gene mutations was evaluated for the prevalence and thrombotic risk associated with these prothrombotic gene polymor-

Prothrombin wild-type	P *value*	*MTHFR heterozygote*	*MTHFR homozygote*	*MTHFR wild-type*	P *value*
77/107 (72.0%)	.058	37/43 (86.1%)	4/9 (44.4%)	38/62 (61.3%)	.002* .020† .112‡
87/125 (69.6%)	.994	49/65 (75.4%)	3/6 (50.0%)	37/57 (64.9%)	.537* .264† .799‡
89/118 (75.4%)	.993	46/53 (86.8%)	5/9 (55.6%)	41/62 (66.1%)	.002* .013† .150‡
75/114 (65.8%)	.097	40/55 (72.7%)	2/6 (33.3%)	34/57 (59.7%)	.351* .354† .601‡
84/107 (78.5%)	.226	41/43 (95.4%)	5/9 (55.6%)	42/62 (67.7%)	.003* .007† .192‡
106/125 (84.8%)	.993	55/65 (84.6%)	5/6 (83.3%)	48/57 (84.2%)	.985* .868† .911‡
92/118 (78.0%)	.896	47/53 (88.7%)	5/9 (55.6%)	43/62 (69.4%)	.002* .016† .138‡
98/114 (86.0%)	.299	49/55 (89.1%)	5/6 (83.3%)	47/57 (82.5%)	.909* .429† .994‡
84/107 (78.5%)	.226	41/43 (95.4%)	5/9 (55.6%)	42/62 (67.7%)	.003* .007† .192‡
109/125 (87.2%)	.994	57/65 (87.7%)	5/6 (83.3%)	49/57 (86.0%)	.821* .703† .819‡
94/118 (79.7%)	.834	48/53 (90.6%)	5/9 (55.6%)	44/62 (71.0%)	.0008* .012† .106‡
99/114 (86.8%)	.264	50/55 (90.9%)	5/6 (83.3%)	47/57 (82.5%)	.805* .282† .994‡

phisms. Patients with a single mutation in the factor V gene were at the highest risk of venous thrombosis (OR, 16.3), followed by the prothrombin G20210A gene mutation (OR, 3.6) and the MTHFR gene mutation (OR, 2.1). With two mutations, possession of both the factor V and prothrombin mutations increased the OR to 58.6. The presence of all three genetic mutations was associated with an OR of 125.8.

This study is a hypothesis-generating study, designed to examine the association between the different genetic mutations and graft failure. The data are fairly preliminary but seem to point to an important role of the MTHFR gene in postreconstruction graft failure. Widespread screening for the gene cannot be recommended until a larger scale study confirms these findings.

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