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Potential Genetic Markers Predictive of Postoperative Thromboembolism Complicating Total Hip and Knee Arthroplasty

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Abstract: We examined the relationship between two potentially hypercoaguable states, the Factor V Leiden (FVL) mutation and the deletion/deletion polymorphism of the angiotensin-converting enzyme (ACE) gene, and postoperative thromboembolic events. The presence of these genetic profiles was determined for 38 patients who suffered a postoperative symptomatic pulmonary embolus or proximal deep venous thrombosis and for 300 consecutive control patients using molecular biological techniques. The Factor V Leiden mutation was not present in any of the 38 experimental patients and was found in nine (3%) of the 300 controls ($p = 0.28$). Similarly, there was no difference detected in the distribution of polymorphisms for the ACE gene; the deletion-deletion genotype was present in 13 of 38 (36%) experimental patients and in 99 of 300 (33%) controls ($p = 0.343$). Our results suggest that neither of these potentially hypercoaguable states is associated with an increased risk of symptomatic thromboembolic events following total hip or knee arthroplasty in patients receiving pharmacological thromboprophylaxis.

Introduction

Following total hip and knee arthroplasty, patients are at a significant risk for thromboembolic complications. Despite modern prophylaxis against thromboembolism, studies still report a 10–40% frequency of deep venous thrombosis (DVT) and a significant rate of pulmonary embolism (PE) following total hip or knee arthroplasty [6,9,11]. The high incidence of thrombotic disease despite prophylaxis makes early detection imperative, as treatment with anticoagulation is highly effective [1,23].

Both DVT and PE manifest few specific clinical signs or symptoms, making the clinical diagnosis neither sensitive nor specific [2,13,23]. A high index of suspicion based on risk stratification is therefore necessary for the detection and appropriate implementation of diagnostic studies to identify this complication. The ability to identify preoperatively a subset of patients undergoing adult reconstructive surgery who are at a higher risk of developing thromboembolic complications would aid the clinician in making an accurate diagnosis and make possible further research to determine optimal regimes of postoperative detection and prophylaxis.

Although the majority of patients undergoing total hip and knee arthroplasty are subjected to similar perioperative risk factors that predispose to thromboembolism, only a subset of patients develop this complication. The objective of this study was to determine whether a specific genetic profile is associated with a higher risk of developing a postoperative thromboembolic complication. Specifically, we examined the relationship between the Factor V Leiden (FVL) mutation and the deletion polymorphism of the angiotensin-converting enzyme (ACE) gene and postoperative thromboembolic events. The FVL mutation has been associated with an increased risk of idiopathic thromboembolism [8,10,17,21,22] and the deletion polymorphism of the ACE gene has been associated with increased vascular tone, attenuated fibrinolysis, and increased platelet aggregation [5,16].

Materials and Methods

Patients

The presence of the FVL mutation and the deletion-deletion polymorphism of the ACE gene were determined for 38 patients following elective total hip or knee arthroplasty at our institution between February 1997 and July 1999: 30 patients developed symptomatic PE and eight patients developed proximal DVT. Using an unmatched case-control design, the prevalence of these genetic profiles was compared to a control cohort of 300 consecutive patients. These patients had undergone similar procedures between November 1997 and March 1998 at the same institution and their postoperative course was not complicated by symptomatic thromboembolism. A total of 321 elective total hip and knee arthroplasties were performed during the time period that samples for the control group were collected. However, 14 patients chose not to participate in the study and seven patients were discharged to home prior to sample collection.

PE was diagnosed on the basis of clinical symptoms and signs combined with a high probability ventilation-perfu-

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sion scan in 20 of the 30, a positive pulmonary angiogram in six, a positive high-resolution chest computed tomography (CT) in two, and an intermediate probability ventilation-perfusion scan combined with a high clinical suspicion in two. The eight patients with DVT were diagnosed by duplex ultrasonography in seven and contrast venography in one. Thirty-one of the 38 patients were treated with intravenous heparin followed by oral warfarin, five by placement of an inferior vena caval filter and oral warfarin, and two by placement of an inferior vena caval filter followed by intravenous heparin and oral warfarin. Fifty-nine of the 300 control patients were clinically suspected to have had a DVT based on clinical signs and symptoms but had a negative duplex ultrasound of the deep venous system of the lower extremities. Similarly, 16 of the 300 control patients were clinically suspected to have had a PE but had a negative workup that included 13 low probability ventilation-perfusion scans, two intermediate probability ventilation-perfusion scans, and two negative high-resolution chest CTs. Five of these 16 patients (including the two who had an intermediate probability ventilation-perfusion scan) also had a negative pulmonary angiogram.

Demographic and operative information including relevant past medical history and the type of thromboembolic prophylaxis utilized was collected for the experimental and control groups as summarized in Table 1. Approval was obtained from the Institutional Review Board at our hospital prior to initiating this study and all patients signed informed consent prior to participating in the study.

Determination of the FVL mutation

Two-milliliter samples of whole blood were collected in buffered sodium citrate and high-molecular weight genomic DNA was obtained from the peripheral blood leukocyte fraction (QIAamp Blood Tissue Kit, Qiagen, Valencia, CA). The FVL mutation is located in exon 10, 11 nucleotides 5′ of the start of intron 10 at nucleotide 1691, where an adenosine replaces guanidine [7]. A 169-base pair (bp) DNA fragment of the factor V gene that includes nucleotide 1691 was amplified utilizing the polymerase chain reaction (PCR) with the forward primer 5′CATACTACAGTGACGTGGAC3′ and the reverse primer 5′GACCTAACATGTTCTAGCCAGAAG3′. PCR was performed using a standard protocol as follows with a final volume of 50 μl; 5 μl 10 × PCR buffer, 5 μl 2 mM dNTP, 5 μl forward primer (concentration 20 ng/μl), 5 μl reverse primer (concentration 20 ng/μl), 1.5 μl 50 mM MgCl, 0.25 μl Taq polymerase (5 U/μl), and 1 μl sample purified genomic DNA (concentration approximately 30 ng/μl; PCR reagents, Gibco-BRL, Bethesda, MD). Thirty-five cycles of the PCR utilizing a microprocessor-controlled thermal cycler (Perkin-Elmer,

Table 1. Demographic and operative data

	Study group (N = 38)	Control group (N = 300)	*p* value
Mean age (years)	67	64	0.25
Female sex	33 (87%)	199 (66%)	0.01
Thromboembolic prophylaxis			0.24
Enoxaparin sodium	37 (97%)	283 (94%)	
Warfarin	1 (3%)	17 (6%)	
History of prior thromboembolism	5 (13%)	3 (1%)	<0.001
Family history of thromboemolism	3 (8%)	7 (2.3%)	<0.001
Procedure			0.29
Primary total hip arthroplasty	10 (26%)	112 (37%)	
Primary total knee arthroplasty	25 (66%)	152 (51%)	
Revision total hip arthroplasty	1 (3%)	22 (7%)	
Revision total knee arthroplasty	2 (5%)	14 (5%)	
Preoperative diagnosis			
Hips			0.8
Osteoarthritis	6 (55%)	81 (61%)	
Rheumatoid arthritis	1 (9%)	6 (4%)	
Failed implant	1 (9%)	21 (16%)	
Other	3 (27%)	26 (19%)	
Knees			0.8
Osteoarthritis	22 (81%)	133 (80%)	
Rheumatoid arthritis	2 (7%)	8 (5%)	
Failed implant	1 (4%)	14 (8%)	
Other	2 (8%)	11 (7%)	
Estimated blood loss (ml)	323	443	0.14
Operative time (minutes)	128	148	0.17
Anesthetic			0.09
Neuraxial	30 (78%)	174 (58%)	
General	8 (22%)	126 (42%)	

Norwalk, CT) were then performed to amplify the desired segment utilizing the following parameters: 94°C for denaturation for 45 seconds, 63°C for 60 seconds for annealing, and 72°C for 90 seconds for extension.

The amplified 169-bp fragment was digested with 0.4 U of the restriction enzyme *Mnl* I (New England Bio Labs, Beverly, MA) at 37°C for 6–12 hours. The resulting fragments were subjected to electrophoresis on 4% Nu-Sieve GTG agarose gels (FMC Bioproducts, Rockland, ME) and the nucleotide bands visualized by ethidium bromide fluorescence and photography. Digestion yields three fragments (86, 46, and 37 bp) in the normal allele and two fragments (123 and 46 bp) in the mutant allele as the point mutation at position 1691 is associated with loss of the recognition site for *Mnl* I (Figs. 1, 2) Control digestions were performed with fragments amplified from cloned DNA with and without the FVL mutation.

Determination of ACE polymorphisms

The insertion/deletion genotype of subjects was performed using purified genomic DNA (prepared as above) and the PCR using the forward primer 5′CTGGAGACCACTCCCATCCTTTCT3′and the reverse primer 5′GATGTGGCCATCACATTCGTCAGAT3′ as described by Rigat et al. [16]. PCR was performed using a standard protocol as follows with a final volume of 50 μl; 5 μl 10 × PCR buffer, 5 μl 2 mM dNTP, 5 μl forward primer (concentration 20 ng/μl), 5 μl reverse primer (concentration 20 ng/μl), 1.5 μl 50 mM MgCl, 0.25 μl Taq polymerase (5 U/μl), 1 μl sample purified genomic DNA (concentration approximately 30 ng/μl), and 5μl dimethyl sulfoxide. Thirty-five cycles of the PCR utilizing a microprocessor-controlled thermal cycler (Perkin-Elmer) were then performed to amplify the desired segment utilizing the following parameters: 94°C for denaturation for 60 seconds, 63°C for 90 seconds for annealing, and 72°C for 90 seconds for extension. The PCR products were subjected to electrophoresis on 1.2% agarose gels and the nucleotide bands visualized by ethidium bromide fluorescence and photography. The deletion polymorphism is charachterized by a 190-bp fragment, whereas the presence of the insertion leads to a 490-bp fragment. Heterozygotes exhibit an intermediate band that is most likely a heteroduplex DNA fragment (Fig. 3).

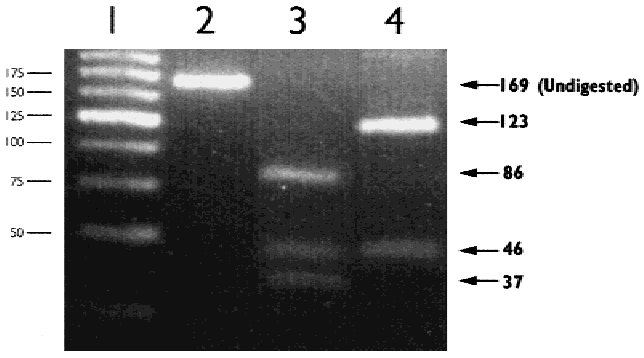

Fig. 1. 169-bp fragment from the factor V gene amplified using the PCR (lane 2). Digestion with the restriction enzyme *Mnl* I yields three fragments in the wild-type subject (lane 3). The mutant allele has the higher molecular weight 123-bp fragment, secondary to loss of the second restriction site (lane 4). Molecular weight markers are seen in lane 1.

Statistical analysis

Statistical analysis was performed using a two-tailed student's *t* test or chi-square analysis where appropriate with a significance set at $p = 0.05$. Assuming an unmatched case-control design (alpha = 0.05 and beta = 0.80) and a 5% prevalence of the FVL mutation in the general population [4,8,10,21], this study, given the sample size, had sufficient statistical power to detect a significant difference in the risk of thromboembolic events if the mutation was associated with an eightfold risk of symptomatic thromboembolic events. Using the same statistical assumptions and a prevalence of the deletion/deletion polymorphism of the ACE gene of 20% in the general population [15,16], a fourfold risk of thromboembolic events would have been detected as significant.

Results

A comparison between the experimental and control subjects revealed that they were of comparable age, but the experimental group consisted of significantly more women ($p = 0.01$, Table 1). Operative indications, procedures, and surgical variables were also similar for the two groups of patients (Table 1). A significant difference noted was that the experimental group had a significantly higher percentage of patients with a personal or family history of thromboembolism ($p < 0.001$ for both).

The FVL mutation was not present in any of the 38 patients but was found in nine of the 300 (3%) controls ($p = 0.28$). Similarly, no difference was detected in the distribution of polymorphisms for the ACE gene, with the deletion-deletion genotype present in 13 of the 38 (36%) patients and in 99 of the 300 (33%) controls ($p = 0.343$).

Discussion

Until recently, the only known hypercoagulable states were several rare genetic disorders of the coagulation cascade (antithrombin III, protein C, and protein S deficiency) that accounted for only a small percentage of all patients with venous thrombosis [14]. In 1993, Dahlback et al. [3] described a previously unreported hypercoagulable state among members of three families who suffered from recur-

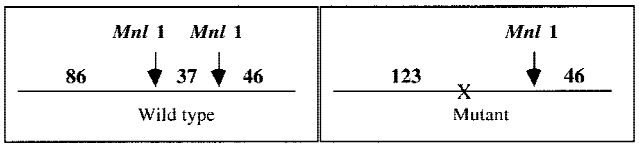

Fig. 2. Schematic representation of digestion. The wild-type gene (left) produces three fragments. The FVL mutation (X) causes loss of the second restriction site of *Mnl* I, resulting in the production of only two fragments.

rent venous thrombosis. Further investigation revealed an autosomal dominant inherited defect in the anticoagulant function of factor V, resulting in resistance to the anticoagulant action of activated protein C (APC) [4]. Formal evidence for this association came from a large population-based patient-control study, the Leiden Thrombophilia Study. The study followed 474 consecutive patients younger than 70 years with a first episode of objectively confirmed DVT [10]. Twenty-eight percent of patients in the study group and 5.7% of controls were found to be APC resistant. Furthermore, it was estimated that these patients have a sevenfold greater risk of developing a DVT. The abnormal factor V that causes APC resistance was subsequently termed factor V Leiden. Later studies confirmed a seven to eightfold increased risk for patients heterozygous for the factor V mutation and an 80-fold increased risk in homozygous individuals [1–10]. Therefore, FVL is the most common thrombophilic disorder described. It is 10 times more common than all the other genetic coagulopathies combined, with an estimated prevalence of 5% in the general population [7,10,21].

ACE digests angiotensin I to angiotensin II (a potent vasoconstrictor) and is thus involved with the regulation of vascular tone. It has also been shown to attenuate fibrinolysis and to affect both platelet activation and aggregation [5]. The ACE gene has been found to have a polymorphism consisting of an insertion and a deletion of a 287-bp fragment of intron 16 [16]. Patients may thus be of one of three separate genotypes: insertion/insertion, deletion/deletion, or insertion/deletion. Patients with the deletion/deletion genotype have been shown to have mean plasma ACE levels of approximately twice that of patients with the insertion/insertion genotype [16]. Thus, patients with the deletion/deletion genotype may be at increased risk for thromboembolic events.

Others have examined the relationship between inherited hypercoagulable states and thromboembolism following total hip and knee arthroplasty, with mixed results. Lowe et al. [12] found that the FVL mutation was associated with an increased risk of DVT (as determined by routine bilateral ascending venography) in 480 European patients who had undergone total hip arthroplasty. However, only 41 of the 120 patients with DVT had proximal thrombi. Svensson et al. [20] found that among a cohort of 100 Swedish patients who had undergone hip arthroplasty, female patients who were heterozygous for the FVL mutation had a fourfold increased risk of thrombosis. However, they believed that based on their data, no definite association between the FVL mutation and postoperative thrombosis could be made. In contrast, Ryan et al. [18] studied 825 patients who had routine bilateral ascending venography following total hip and knee arthroplasty. They found that the prevalence of the FVL mutation was no different between patients who did and did not have venographic evidence of DVT. Similarly, Woolson et al. [24] studied 36 patients who had a proximal DVT after total hip arthroplasty (detected by routine predischarge compression duplex ultrasound). They found that the prevalence of the FVL was no different in that population compared to 45 controls. In contrast to the aforementioned studies, the present report studied patients who had developed symptomatic thromboembolic events (the majority of which were PE), which may be a more relevant endpoint for the orthopaedic surgeon. Our results support the findings of others. In our patient population receiving pharmacological prophylaxis against postoperative thrombosis, the FVL mutation is not associated with an increased risk of symptomatic thromboembolism following total hip or knee arthroplasty.

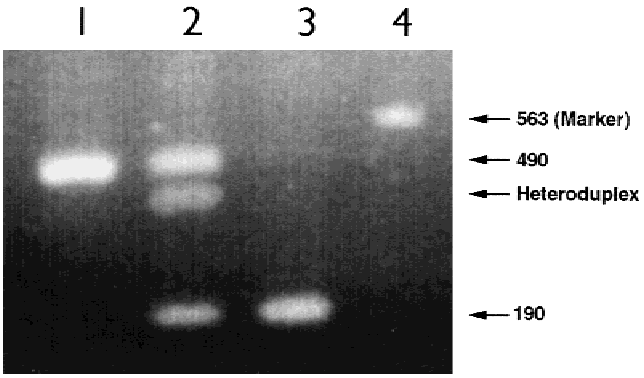

Fig. 3. Lane 1: insertion/insertion homozygote (490 bp); lane 2: insertion/deletion heterozygote with DNA heteroduplex of intermediate size; lane 3: deletion/deletion homozygote (190 bp); lane 4: molecular weight marker (λDNA/HindIII fragments).

Although Philipp et al. [15] found no association between the FVL mutation and DVT, they did find that the deletion-deletion genotype of the ACE was strongly associated with postoperative venous thrombosis in 85 patients who had undergone total hip arthroplasty (30 of whom had a thromboembolic event as detected by routine compression duplex ultrasound). They concluded that patients with the deletion/deletion genotype were at a 10-fold increased risk for a thromboembolic event following total hip arthroplasty as compared to patients with the insertion-insertion genotype. However, 12 of the 30 subjects had isolated distal DVT (which is of questionable clinical significance) and only 10% had a PE. The results of this study had encouraged us to screen our patient population for these polymorphisms. When utilizing a more relevant clinical endpoint (symptomatic PE or DVT), we were unable to confirm this association.

Due to the relative infrequence of symptomatic thromboembolic events while using pharmacological agents as prophylaxis, an unmatched case-control study design was employed. This type of study design has the advantage of increased statistical power for studying relatively rare events [19]. However, its disadvantages include the possibility that other variables that were not controlled for could have affected our results. The patients in both the case and control groups were operated on during overlapping time periods and were found to be demographically similar. Therefore, we do not believe that such confounding variables affected our results. Our power analysis reveals that a relatively strong association between these genetic profiles and postoperative thromboembolism (eightfold increased

risk for the FVL mutation and a fourfold increased risk for the deletion/deletion polymorphism of the ACE gene) would have been required to detect a significant difference between the prevalence of these mutations in our case and control groups. Likewise, a larger number of patients would have been required to find a significant difference if a weaker association was assumed. However, no trend was detected in our data to suggest that such an association was present. Furthermore, such a weak association would not make preoperative screening and identification of such patients cost effective.

It was noted, however, that a significantly greater percentage of patients who suffered a thromboembolic event had a personal or family history of thromboembolism ($p = 0.001$ for both). The report by Woolson et al. [24] included similar findings. Residual abnormalities of the deep venous system could account for the higher prevalence of a personal history of prior DVT or PE. However, the higher prevalence of a family history of thromboembolic events suggests that an as yet undescribed genetically determined hypercoagulable state or predisposition may be present in these patients.

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