Detection of a Common Mutation in Factor V Gene Responsible for Resistance to Activated Protein C Causing Predisposition to Thrombosis

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Hereditary predisposition to thrombosis due to activated protein C resistance (APCR) has been attributed to a missense mutation in the factor V gene at nucleotide 1691 (G to A), causing replacement of arginine at codon 506 with glutamine. Using an RFLP-PCR assay to detect this mutation, we measured a prevalence of 3.3% in healthy Caucasians and 1.25% in healthy African-Americans. In addition, we evaluated a total of 90 consecutive specimens submitted to the coagulation laboratory at the Medical College of Virginia for the presence of this mutation. We compared our results for 78 of these specimens with the values measured by a modified partial thromboplastin assay, the COATEST. Twelve of the 90 samples could not be tested using the COATEST because the patients were undergoing anticoagulant therapy. One of the latter 12 specimens was positive by the RFLP-PCR test. Using the genetic test

as the definitive assay and the cutoff value established for distinguishing between normal and abnormal results by the COATEST, the COATEST had a sensitivity of 50% and specificity of 93% for the detection of factor V mutation. Analysis of the 90 samples stratified by ethnic groups revealed a frequency of mutation of 13.3% for Caucasians and 6.88% for African-Americans, although with the present sample size, the difference was not statistically significant. Although the COATEST is technically simpler to perform than the genetic test for diagnosing the presence of the factor V mutation, its use for this purpose is limited due to low sensitivity. Thus where this disorder is clinically suspected, submission of the specimen directly for genetic testing by RFLP-PCR or equivalent assay should be considered. *J. Clin. Lab. Anal.* 11:328–335, 1997. ©1997 Wiley-Liss, Inc.

Key words: hereditary activated protein C resistance; RFLP-PCR; hereditary thrombosis; APCR

INTRODUCTION

Venous thromboembolism is a serious health problem affecting ~ 1 per 1,000 individuals annually (1). Venous thrombosis generally occurs in two types of patients, those with an acquired predisposing condition such as surgery, oral contraceptive treatment, complicated pregnancy, immobilization, and fracture and those with some type of genetic predisposition as revealed by family history or previous history of venous thrombosis (1). Until recently, the genetic defects known to predispose to thrombosis could account for no more than 1–5% of the cases of venous thrombosis (2). These genetic risk factors included deficiencies of antithrombin III, protein C, and protein S (1). Recently, hereditary resistance to activated protein C (APCR) has been identified as a cause of familial thrombosis (3–6). The genetic cause of this disorder in most affected individuals is a missense mutation in the factor V gene at nucleotide 1691. This mutation, also known as the Leiden mutation, or FV R506Q, arises from substitution of guanine for adenine causing replacement of arginine (R) at

codon 506 by glutamine (G) (7,8). Cleavage of factor V by activated protein C occurs between amino acids 506 and 507 (9). Substitution of glutamine for arginine at this location confers resistance to cleavage of factor V by activated protein C shifting the coagulation/anticoagulation balance toward coagulation and increasing the risk for venous thrombosis (9).

In contrast to other genetic defects associated with thrombosis, FV R506Q is highly prevalent in the general population (10–13). A prevalence of 3–5% was initially described in a healthy Dutch population (5,14). Later, this mutation was shown to be present in British, Swedish, Canadian, and North American Caucasian populations with a prevalence ranging from 2–7% (15–17). Originally, this disorder was linked only

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to the Caucasian ethnic group, but recently several reports have found the presence of this disorder in other ethnic groups. A prevalence similar to that of the U.S. and northern European Caucasians has been observed in healthy individuals in Brazil, Australia, Japan, and India (18–21). More recently, the presence of FV R506Q was described in a population of African-Americans with similar prevalence as the control Caucasian population (22).

From the standpoint of a genetic disorder, the FV R506Q is expressed in autosomal dominant fashion, but the clinical penetrance of this disorder is variable (23). Heterozygous carriers of this mutation have a 5–10-fold increased risk of developing a venous thrombotic episode (24). In individuals homozygous for the mutation, the risk of thrombosis is 50–100-fold greater (25). This mutation has been estimated to be present in 21–64% of patients who are diagnosed with an episode of venous thrombosis (3,5,6,23,26). Deep vein thrombosis of the legs, and its major complication pulmonary embolism, account for the majority of thrombotic episodes associated with this mutation, but retinal vein thrombosis and relapsing nephrotic syndrome also have been described (27,28). Moreover, it was found that 60% of pregnant women and 30% of women using oral contraceptive therapy who present with thrombotic episodes possess this mutation (29–31).

FV R506Q does not seem to play a role in arterial thrombosis and myocardial infarction (32–34). The role of this mutation in ischemic cerebrovascular disease is still unclear. The mutation was not associated with this disorder in an elderly patient population or in the Physicians' Health study in which the mean age for stroke patients was 62.9 years (23,35). However, a recent report described an 8-month-old patient homozygous for this mutation who presented with ischemic stroke (32). Additional case control studies will be necessary to assess any association with cerebrovascular disease.

Basic to furthering our understanding of the clinical significance of this disorder is accurate cost-effective diagnosis. In the present study, modifications of the genetic assay to decrease cost and improve turnaround time were performed. The prevalence of the FV R506Q in a randomly selected population of Caucasian and African-American healthy individuals was measured, and the clinical utility of a commonly used functional coagulation assay, the COATEST, was compared with a genetic assay for detecting this mutation.

MATERIAL AND METHODS

Blood Specimens and Sample Preparation

Thirty EDTA anticoagulated venous blood samples from healthy Caucasian individuals and 161 EDTA anticoagulated peripheral blood specimens from healthy African-American individuals were obtained to measure the prevalence of the FV R506Q in the local general population. In addition, a total of 90 venous blood specimens submitted to the Coagulation

Laboratory at the Medical College of Virginia from January 1995 to September 1995 were collected to compare the utility of the COATEST as a screening assay for detection of APCR with an RFLP-PCR assay. Of the 90 samples, 47 were from Caucasians and 43 were from African-Americans. Samples were centrifuged at 2,000g for 30 minutes and the plasma was removed. Isolation of lymphocytes was performed using Ficoll-Hypaque gradient centrifugation or by lysing the red cell fraction in presence of TE buffer (10 mM Tris-HCl, 1 mM EDTA, Sigma, St. Louis, MO). Standard Ficoll-Hypaque treatment was performed according to manufacturer's specifications (Organon Technika, Research Triangle Park, NC). After centrifugation, the cells were washed twice in PBS and transferred to a 1.5 ml microcentrifuge tube and stored at –80°C until ready for DNA extraction. Red cell lysis was performed using TE buffer. Cell pellets were diluted with 3 volumes of TE buffer, mixed by inversion, and centrifuged at 2,000 rpm for 5 minutes at room temperature. The pellet was washed twice with PBS under the same condition and transferred to a 1.5 ml microcentrifuge tube. Samples were stored at –80°C until nucleic acid extraction.

DNA Extraction

Samples were removed from –80°C storage and thawed at room temperature. DNA was extracted from cell pellets according to two different procedures. A standard Proteinase K, phenol/chloroform method was used as previously described (36). A second method using silica based chromatography columns was used to isolate the DNA according to manufacturer's instructions (QiAmp Tissue kit, Qiagen, Chatsworth, CA). DNA quantification was performed using a UV spectrophotometer reading at 260 nm.

RFLP-PCR for FV R506Q Leiden

The method used for the detection of the mutation at codon 506 nucleotide 1691 of the factor V gene was a modification of the method described by Bertina et al. (8). The upstream primer, APCA (5' primer) TGC CCA GTG CTT AAC AAG ACC A, was located in exon 10 and the downstream primer, APB (3' primer) TGT TAT CAC ACT GGT GCT AA, was located in intron 10 of the factor V gene. Primers were synthesized in a PCRmate DNA synthesizer (Perkin-Elmer Applied Biosystems, Foster City, CA) using phosphoramidite base chemistry. The reaction mix contained 1X PCR buffer (10 mM Tris-HCl pH: 8.3, 50 mM KCl, 1.5 mM MgCl₂), 0.200 mM dNTPs (Promega, Madison, WI), 0.78 µM of each primer and 2 U of AmpliTaq DNA Polymerase (Perkin-Elmer) in a final volume of 100 µl. Eighty ml of master mix were added to each reaction tube, followed by 20 µl containing 0.1 µg of DNA. PCR was performed in a 9600 Perkin Elmer Thermal Cycler with the following conditions: a hold at 95°C for 5 minutes, followed by 36 cycles of 91°C for 30 seconds, 55°C for 30 seconds and 72°C for 30 seconds, and a hold at

72°C for 5 minutes. Two negative controls and two positive controls were included with every run. The negative controls included a reagent control with no template DNA, and a normal DNA control with wild-type sequence. The positive controls included a sample of DNA carrying a heterozygous and one carrying a homozygous FV R506Q.

The resulting PCR product was 267 bp in length and contained two recognition sites for the restriction enzyme MnlI. An aliquot of each PCR product was incubated with 10U of MnlI (New England Biolabs, Boston, MA) in the presence of 1X buffer for 1 hour at 37°C. Subsequently, aliquots of digested and undigested sample were loaded onto a 10% polyacrylamide gel and electrophoresed for 1 hour at 200V. After electrophoresis, the gels were stained with ethidium bromide and bands were visualized under UV exposure. Thermoprints of digitized images were obtained using the IS1000 photo documentation system (Alpha Inotech, Alameda, CA). Digestion of PCR product from individuals carrying the wild-type sequence resulted in three fragments. The molecular weight of the fragments were 37 bp, 67 bp, and 163 bp. In contrast, PCR product from individuals homozygous for the FV R506Q generated only two fragments after digestion since they had lost one restriction enzyme site, resulting in 67 bp and 200 bp fragments. Individuals heterozygous for the FV R506Q presented four fragments after digestion (37 bp, 67 bp, 163, and 200 bp). These four fragments were the result of the combination of one wild-type allele and one mutated allele. The PCR reaction master mix was set up in a dead air box in a laboratory separate from where amplification and analysis of PCR product was performed.

APCR Functional Assay (COATEST)

APCR measurements were obtained using the COATEST® kit (Chromogenix, Molnall, Sweden) according to manufacturer's specifications. This functional assay is based on the measurement of the activated partial thromboplastin time (aPTT) with and without addition of exogenous activated protein C. aPTT times were measured in a ST4 analyzer (American Bioproducts Co., Parsippany, NJ). The value chosen to discriminate normal from abnormal results was 2.1 as suggested by the manufacturer.

DNA Sequencing

DNA sequencing was performed using a biotinylated primer according to a method previously described (37). Briefly, aliquots of DNA (0.5 µg) from patients that showed the presence or absence of FV R506Q by RFLP-PCR were again amplified using the same components and conditions described above, except that in this PCR reaction one of the primers contained a biotin molecule at the 5' end (38). After amplification, removal of unused primers and dNTPs was achieved using a silica-based chromatography column (QiaQuick PCR Purification kit). The eluate from the col-

umn was incubated with magnetic beads coated with streptavidin (Dynabeads M-280 beads, Dynal, Lake Success, NY) and the captured PCR product was denatured in the presence of NaOH. The single-stranded PCR product remaining attached to the magnetic bead was washed and resuspended in 7 µl of HPLC water. Determination of the base change was performed by direct sequencing of the single-stranded PCR product. Samples were sequenced by a modification of the method already described using the Sequenase Version 2.0 sequencing kit according to manufacturer's specifications (USB Biochemicals, Cleveland, OH) with [α -³²P] dCTP (3000 Ci/mmol, NEN Dupont, Boston, MA) (37). The sequencing reactions were run in an 8% polyacrylamide, 7 M urea gel and electrophoresed at 1,500 V for 3 hours at room temperature. The gels were transferred to Whatman 3MM filter paper and exposed to X-Ray film (Kodak XAR-5) for 48 hours for the visualization of bands.

Statistical Analysis

Receiver operator characteristics (ROC) curve analysis and Fisher exact and Chi-square tests were performed using the STATA statistical package version 4.0 (STATA Corp., College Station, TX).

RESULTS

Optimization of RFLP-PCR Assay for Detection of FV R506Q

In order to enhance clinical utility of the genetic test, different assay parameters, including sample preparation, DNA extraction, thermocycling, and master mix stability, were examined from the perspective of decreasing cost and improving turnaround time. Regarding DNA extraction, the yield and quality of DNA obtained using a standard proteinase K phenol/chloroform (P/C) procedure were compared with a silica-based chromatography spin column procedure (Qiagen column). Six aliquot containing 3×10^6 cells of the K562 cell line were extracted by each method. The total amount of DNA recovered using the Qiagen column was $9.03 \mu\text{g} \pm 0.93 \mu\text{g}$, and the total amount of DNA recovered using P/C method was $19.38 \mu\text{g} \pm 1.53 \mu\text{g}$. The amount of DNA recovered using the silica-based method was lower than the P/C method ($P < 0.0001$, Fisher exact test). Despite this, however, both methods provided sufficient DNA for the PCR assay and both methods provided DNA of equivalent purity as judged by OD ratios (260nm/280nm) with values between 1.7–2.0 for both procedures. We also analyzed the integrity of the DNA by applying 1 µg of undigested DNA extracted by each method in duplicate to an 0.8% regular agarose gel followed by electrophoreses for 18 hours. As judged both by the amount of DNA retained in the agarose gel wells as well as the proportion migrating in the region of the 23KB lambda DNA molecular weight marker, the P/C method yielded DNA showing

slightly less degradation than the silica-based method (Fig. 1). Nevertheless, the quality of the DNA obtained from the silica-based material was quite adequate for PCR. The quality of the DNA was judged in both methods using serial dilutions of DNA ranging from 10^{-1} – 10^{-6} μ g. Both dilution series showed detectable DNA down to the 10^{-4} μ g dilution (data not shown). Based on the preceding data, the silica-based DNA extraction method was judged superior since it yielded adequate quality and amount of DNA, and the time involved in extracting samples was ~30 minutes with a hands on time of 15 minutes, compared to the P/C method, which required at least 4 hours with a hands-on time of 1 hour over a 2-day interval (Table 1A).

Next, the use of purified peripheral blood leukocytes was compared with crude whole blood cellular lysate as starting material for the isolation of DNA. Peripheral blood leukocytes were isolated using a Ficoll-Hipaque method which took a minimum of 2 hours (Table 1B). However, the lysis method was rapid and easy to perform and took 30 minutes from start to finish (Table 1B). Anticoagulated peripheral blood was obtained from normal individuals, pooled, and divided into separate equal aliquots. Sample preparation methods were tested in triplicate using the same number of cells. DNA was extracted using the silica-based extraction method. We tested the quality of DNA extracted from both sample preparation methods by amplifying 0.1 μ g of DNA from each method for the factor V gene. Amplification of DNA extracted using both sample preparation methods demonstrated almost equal efficiency of amplification as shown by the intensity of the PCR product bands run on a 10% acrylamide gel and stained by ethidium bromide (data not shown). Thus the lysis method was selected because of its simplicity and reduced cost.

Fig. 1. DNA extraction methods comparison. Panel A: Lanes 1–6. DNA from K564 cell line extracted using phenol/chloroform method. Panel B: Lanes 1–6, DNA from K562 cell line extracted using silica-based chromatography columns. Gels were stained in ethidium bromide and photographed under UV exposure.

TABLE 1. Time Required for Each Step in Comparative Methods Tested

A. Pellet preparation				
	Isolation peripheral blood leukocytes		Whole blood cellular lysate	
	Hands on	Procedure	Hands on	Procedure
Blood dilution	5 minutes	5 minutes	2 minutes	2 minutes
Gradient	10 minutes	75 minutes	—	—
Wash	10 minutes	40 minutes	10 minutes	28 minutes
Total	25 minutes	120 minutes	12 minutes	30 minutes
B. DNA Extraction				
	Phenol/chloroform		Silica based	
	Hands on	Procedure	Hands on	Procedure
Proteinase K digestion	5 minutes	65 minutes	5 minutes	15 minutes
DNA isolation	50 minutes	90 minutes	10 minutes	15 minutes
Precipitation	4 minutes	60 minutes	—	—
Resuspension	1 minute	25 minutes	—	—
Total	60 minutes	240 minutes	15 minutes	30 minutes

The third parameter examined was the stability of master mixes. Two different types of master mixes were tested. One master mix contained all reagents but Taq DNA polymerase and was stored at 4°C, whereas the other contained all reagents but MgCl₂ and was stored at –20°C. Each master mix was prepared and divided into aliquot of 10 reactions each. The stability of the aliquot was monitored over a period of 6 months. All master mixes were tested using one homozygous, one heterozygous, one negative, and one reagent control at intervals of 2 weeks. Figure 2 shows a representation of the results obtained. As clearly seen, the master mix stored at –20°C without the MgCl₂ produced reproducible results over the period tested (Fig. 2, lanes 1–7). However, the master mix stored at 4°C without Taq DNA polymerase did not produce reproducible results, since by the fourth month it had started to produce nonspecific bands during amplification (Fig. 2, lanes 8–13). The results demonstrated that the use of a single master mix during a period of 6 months was feasible, but conditions of storage were critical to maintaining reproducibility of results. An obvious advantage of using a single master mix over a prolonged period is that it reduces the variability between assays and decreases the time necessary to set up each assay. In our procedure the setup time was reduced from 45 minutes to 10 minutes.

Taken together, the above results demonstrate that substantial savings in time and effort can be achieved without reduction in quality of analytical results. The total hands-on time for the technologist could be reduced 2.5 hr and the turnaround time shortened from 3 days to 1 day. The net result of the above modifications was a savings of ~\$95 per analytical run of 3 controls and 7 specimens and an associated decrease in turnaround time of 48 hours.

Fig. 2. Effect of master mix composition and storage conditions in PCR-RFLP for detection of FV R506Q Leiden. Each pair of lanes represents undigested and MnlI-digested PCR products, respectively. Amplification using master mix stored without $MgCl_2$ for 2 months (lanes 1 and 2), 4 months (lanes 3 and 4), and 6 months (lanes 5 and 6) showed deterioration of product formation due to storage. Amplification using master mix stored without Taq DNA polymerase for 2 months (lanes 7 and 8), 4 months (lanes 10 and 11), and 6 months (lanes 12 and 13) showed loss of fidelity in amplification (production of nonspecific bands) by 4 months of master mix storage. Lane 9 contains 10 bp DNA ladder.

Prevalence of FV R506Q in Normal Population

Samples were obtained from 30 nonrelated healthy Caucasian individuals to assess prevalence of this mutation in this population. DNA was analyzed using the RFLP-PCR assay (Fig. 3). Of the 30 specimens tested, one individual was identified who was heterozygous for the FV R506Q (prevalence = 3.3%). DNA was also obtained from 161 blood specimens from healthy African-Americans. Heterozygous FV R506Q was detected in two individuals using the RFLP-PCR assay resulting in a prevalence of 1.25% in the African-American population studied.

Utility of Genetic and Functional Tests for Diagnosis of FV R(506)Q Leiden

Ninety patient specimens submitted to the coagulation laboratory for diagnostic evaluation were tested for FV R(506)Q using the RFLP-PCR genetic assay and the functional assay, COATEST. Of the samples, 13% could not be tested using the COATEST because the patients were undergoing anticoagulant therapy, which interferes with measurement of resistance to activated protein C using this assay. All mutations detected using the RFLP-PCR method were heterozygous. As might be expected, the prevalence of FV R506Q was significantly higher in the group of samples submitted for evaluation in the coagulation laboratory than the group of healthy subjects (8.8% vs. 1.6%; $P = 0.008$, Fisher exact test). Analysis of the 90 samples stratified by ethnic groups revealed a

Fig. 3. APCR RFLP-PCR. Representative PAGE for FV R506Q Leiden. Lanes 2, 4, 6, 9, 11, 13, 17, 20, 22: undigested PCR product. Lanes 3, 5, 12, 18, 21, 23: digested PCR product for wild-type specimens. Lanes 7, 10, 14:

digested DNA heterozygous positive. Lane 15: undigested no template control, Lane 16: digested no template control. Lanes 1, 8, 19: 10bp DNA ladder (BRL, Gaithersburg, MD).

frequency of mutation of 10.64% and 6.88% for Caucasians and African-Americans, respectively, although these two frequencies were not statistically significantly different for this sample size.

Table 2 shows the results for sensitivity and specificity of the functional assay using the genetic assay as the definitive assay. Of eight samples that showed enhanced coagulability by the COATEST, only three revealed the FV R506Q by the genetic assay. In contrast, six samples demonstrated heterozygous genotype, but three of these samples were within the normal range for the COATEST. Sixty-seven samples demonstrated negative results for the mutation and were normal by the functional assay. Thus the COATEST had a sensitivity of 50% and specificity of 93% for the detection of FV R506Q (Table 2). It is interesting that among the five false positives and three false negatives identified by the COATEST, all but three were within 10% of the latter's normal cutoff value.

All samples that were positive by the genetic assay or the functional assay and 10 samples negative by both assays were subjected to direct sequencing to verify the presence of FV R506Q (data not shown). All samples positive by the genetic assay showed the presence of the mutation, and all negative samples showed the wild-type sequence. All samples positive only by the functional, but not by the RFLP-PCR assay, showed wild-type sequences for the region amplified. These data confirmed that the genetic assay is more accurate for detecting FV R506Q. We performed a ROC analysis to assess the utility of the COATEST as a screening assay for FV R506Q (Fig. 4). The ROC figure displays the sensitivity of the COATEST, that is, the fraction of true positives detected by the COATEST as a function of false positives (1-specificity) detected by the test. A hypothetical test with no predictive power is represented by a 45° line between (0,0) and (1,1) with a resultant area under the curve of 0.5. A test with perfect predictive power passes from (0,0) to (0,1) to (1,1) with a resultant area under the curve of 1.0. The area shown in ROC analysis for the COATEST was 0.79 (Fig. 4). The highest COATEST value (3.7) is represented in the (1,1) point, and the lowest COATEST value is represented in the (0,0) point. As stated previously, the point on the ROC curve that reflects the COATEST cutoff value of 2.1 coincides with a sensitivity of 50% and specificity of 93% (false positive rate of 7%). The implication of these values for the use of the COATEST as a screening assay in our population is seen from

Fig. 4. ROC. Sensitivity and specificity of COATEST for detection of the FV R506Q Leiden.

the following considerations. First, the COATEST will miss half of the patients that possess the mutation. Thus since the prevalence of the mutation was 10% in our patients, only five out of a total of 10 true positives would be identified per 100 patients examined by the COATEST. Moreover, as the false positive rate is 7%, seven additional patients also would be selected per 100 examined by the COATEST for the confirmation by the genetic test. Increasing the cutoff value of the COATEST above the normal cutoff value, such that the COATEST achieved a sensitivity of 75% for identifying patients with a FV R506Q, would greatly increase the number of false positives. It would result in the need to examine a total of 48 patients by the genetic test in order to identify 7–8 patients with the mutation and still 25% of patients with FV R506Q would be missed. These results reflect a less than optimum performance for a screening test.

DISCUSSION

In the present study we evaluated an RFLP-PCR assay for the detection of the FV R506Q, which is responsible for hereditary resistance to activated protein C. One of the major aims of the study was to optimize the assay to keep turnaround time to a minimum of 2 days and to reduce the cost of the assay. Use of a lysis method for collecting nuclei with a silica-based chromatography column method and a master mix stored at -20°C that included Taq DNA polymerase but not MgCl_2 contributed to the achievement of these aims. The final protocol proved to be rapid, easy to perform, and very robust. After optimization, the assay could be performed in 6 hours from sample preparation to origination of the report.

FV R506Q has been identified as a major cause of hereditary resistance to activated protein C, which predisposes an individual to deep vein thrombosis (3–6). An increased risk between 5–10-fold has been described for individual heterozygous for the FV R506Q, and this risk is increased 50–100-fold in individuals homozygous for the FV R506Q. The

TABLE 2. Sensitivity^a and Specificity^b of APCR Functional Assay

	PCR (+)	PCR (–)	Total
COAtest(+)	3 (TP)	5 (FP)	8
COAtest(–)	3 (FN)	67 (TN)	70
	6	72	78

^aSensitivity: $\text{TP}/(\text{TP}+\text{FN}) = 3/(3+3) = 50\%$.

^bSpecificity: $\text{TN}/(\text{TN}+\text{FP}) = 67/(67+5) = 93\%$.

mutation has been reported to be present in 2–5% of the general population (10–13), a figure that is inclusive of the value measured in this report. We measured a higher frequency of this mutation in Caucasians than in African-Americans, both in normal and in patients evaluated by the coagulation laboratory, but in neither case was the difference statistically significant (3.3 vs. 1.25, $P = 0.38$; 13.3 vs. 6.88%, $P = 0.07$).

The issue of how individuals identified with this genetic change should be treated is currently evolving. Dahlback (3,5) suggests that screening for APC resistance before surgery, during pregnancy, and before the use of oral contraceptives may be warranted. There appears to be some consensus that individuals with this disorder should be managed similar to those that have deficiencies in antithrombin III, protein C, and protein S. Under these circumstances, heterozygous individuals with no personal or family history of thrombosis and with no other anticoagulant defect would receive prophylactic anticoagulant therapy only in situations known to provoke thrombosis, such as major surgery. In homozygous cases of FV R506Q, preventive therapy would be given liberally for all at risk situations, even if the patient had no history of thrombosis.

Appropriate management, however, clearly necessitates accurate diagnosis. The most widely available test for screening for FV R506Q is an activated partial thromboplastin time that is performed with and without the presence of added activated C protein (14). Although this assay is rapid and technically simple to perform, the accuracy of the assay for identifying patients with factor V mutation has been questioned (14). Our findings in this report emphasize this issue. In the present study, one such functional assay, the COATEST, had a sensitivity of only 50% together with a specificity of 93%. Clearly, the term “false positive” in this circumstance means only that the cause for the patient’s hypercoagulability is not related to the presence of the FV R506Q. Other causes exist for hypercoagulability, including other genetic disorders (4). Of greater concern, however, in this matter is the percentage of false negatives. For every patient with FV R506Q that the COATEST identified, it would be expected to miss an additional patient with the mutation. This type of performance is not ideal for a screening test. In addition, the COATEST cannot be performed on patients undergoing anticoagulant therapy, some of whom will possess the FV R506Q. Recently a new biochemical assay using tissue factor-dependent factor V has been reported that better identifies FV R506Q in otherwise healthy individuals (38). Further analysis of this latter test in comparison to the genetic assay will be needed.

Accordingly, although the COATEST is technically simpler to perform than the genetic test for diagnosing the presence of the FV R506Q, its use for this purpose is limited due to low sensitivity. Thus where this disorder is suspected clinically, submission of the specimen directly for genetic testing by RFLP-PCR or equivalent assay should be considered.

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