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Outcomes of Genetic Testing in Adults with a History of Venous Thromboembolism

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Preface

The Agency for Healthcare Research and Quality (AHRQ), through its Evidence-Based Practice Centers (EPCs), sponsors the development of evidence reports and technology assessments to assist public- and private-sector organizations in their efforts to improve the quality of health care in the United States. This report was requested and funded by the Centers for Disease Control and Prevention (CDC), Office of Public Health Genomics (OPHG). The reports and assessments provide organizations with comprehensive, science-based information on common, costly medical conditions and new health care technologies. The EPCs systematically review the relevant scientific literature on topics assigned to them by AHRQ and conduct additional analyses when appropriate prior to developing their reports and assessments.

To bring the broadest range of experts into the development of evidence reports and health technology assessments, AHRQ encourages the EPCs to form partnerships and enter into collaborations with other medical and research organizations. The EPCs work with these partner organizations to ensure that the evidence reports and technology assessments they produce will become building blocks for health care quality improvement projects throughout the Nation. The reports undergo peer review prior to their release.

AHRQ expects that the EPC evidence reports and technology assessments will inform individual health plans, providers, and purchasers as well as the health care system as a whole by providing important information to help improve health care quality.

We welcome comments on this evidence report. They may be sent by mail to the Task Order Officer named below at: Agency for Healthcare Research and Quality, 540 Gaither Road, Rockville, MD 20850, or by e-mail to **epc@ahrq.gov.**

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Structured Abstract

Objective: To address whether Factor V Leiden (FVL) testing alone, or in combination with prothrombin G20210A testing, leads to improved clinical outcomes in adults with a personal history of venous thromboembolism (VTE) or to improved clinical outcomes in adult family members of mutation-positive individuals.

Data sources: Searches of MEDLINE®, EMBASE®, The Cochrane Library, the Cumulative Index to Nursing & Allied Health Literature, and PsycInfo© through December 2008.

Review methods: We focused on the analytic validity, clinical validity, and clinical utility of these tests. Each included article underwent double review for data abstraction and assessment of study quality. We pooled the results of studies addressing the clinical validity of these tests when there were sufficient data. Other evidence was summarized in evidence tables. We graded the evidence by adapting a scheme recommended by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group by assessing the limitations affecting individual study quality, the certainty regarding the directness of the observed effects in the studies, the precision and strength of the findings, and the availability (or lack) of data to answer the relevant key question. Evidence for each sub-question was graded as high, moderate, or low.

Results: We reviewed 7,777 titles and included 124 articles. No direct evidence addressed the primary objective. However, high-grade evidence supported the conclusion that tests for the detection of FVL and prothrombin G20210A have excellent analytic validity. Most clinical laboratories test for these mutations accurately. Heterozygosity [odds ratio (OR) =1.56 (95 percent confidence interval (CI) 1.14 to 2.12)] and homozygosity [OR=2.65 (95 percent C.I. 1.2 to 6.0)] for FVL in probands are predictive of recurrent VTE. Heterozygosity for FVL predicts VTE in family members [OR=3.5 (95 percent C.I. 2.5 to 5.0)] as does homozygosity for FVL [OR=18 (95 percent C.I. 7.8 to 40)]. Heterozygosity for prothrombin G20210A is not predictive of recurrence in probands [OR=1.45 (95 percent C.I. 0.96-2.2)]. Evidence is insufficient about heterozygosity for prothrombin G20210A in family members and insufficient about homozygosity for prothrombin G20210A. A single study supported the hypothesis that clinicians might change management based on test results. There was high-grade evidence that anticoagulation reduces recurrent events in probands with FVL or prothrombin G20210A; however, there was low-grade evidence that the relative reduction with treatment is comparable to that seen in individuals without mutations. There was moderate evidence to support the conclusion that neither harms nor benefits of testing have been demonstrated conclusively. Decision-analysis models suggest that testing may be cost-effective in select individuals.

Conclusions: There is no direct evidence that testing for these mutations leads to improved clinical outcomes in adults with a history of VTE or their adult family members. The literature supports the conclusion that while these assays have high analytic validity, the test results have variable clinical validity for predicting VTE in these populations and have only weak clinical utility.

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Appendixes and Evidence Tables for this report are provided electronically at http://www.ahrq.gov/downloads/pub/evidence/pdf/gentestvt/fvl.pdf.

Executive Summary

Introduction

Venous thromboembolism (VTE) refers to pathologic thrombosis in the venous circulation. Although the most frequent venous thromboembolic event is deep venous thrombosis in the veins of the legs, thromboses can also occur in the veins of the upper extremities, pelvis, abdomen, and cerebral venous sinuses. Pulmonary embolism is the main life-threatening complication of deep vein thrombosis, in which a portion of the venous thrombus is carried to the pulmonary arteries by blood flow, potentially obstructing the pulmonary vasculature. Treatment begins with short-term use of a parenteral anticoagulant (and sometimes thrombolytic therapy) and then usually continues with a vitamin K antagonist, most commonly warfarin. The duration of therapy depends on whether the patient is considered to have continuing risk factors for recurrence.

Much effort has been devoted to quantifying the risk of recurrent thrombosis. If the patient has a persistent risk factor for thrombosis, anticoagulant therapy is often continued, sometimes for the life of the patient. The clinician and patient also try to reduce exposure to any modifiable risk factors and may use mechanical or pharmacological means of preventing thrombosis at high-risk times, such as during hospitalization or pregnancy.

The question of whether a patient has ongoing risk leads to the subject of this report, the value of testing individuals who have had a venous thromboembolic event for Factor V Leiden (FVL) and prothrombin G20210A. The FVL mutation is a base change (from G to A at position 1691) in the gene coding for the Factor V protein; the resulting amino acid substitution eliminates one of three activated protein C cleavage sites in Factor V. As a result, Factor V is inactivated to a lesser extent and persists longer in the circulation, leading to more thrombin generation. In the United States, a single FVL allele is present in about 5, 2.2, and 1.2 percent of the Caucasian, Hispanic, and African American populations, respectively. The prothrombin (Factor II) mutation is the second most common inherited risk factor for thrombosis. The mutation in the prothrombin G20210A gene is associated with an elevation of prothrombin levels to about 30 percent above normal in heterozygotes and to 70 percent above normal in homozygotes. In the United States, the prevalence of this allele is 1.1 percent in Caucasians and Hispanics and 0.3 percent in African Americans.

The *Evaluation of Genomic Applications in Practice and Prevention* (EGAPP) initiative was established by the Office of Public Health Genomics at the Centers for Disease Control and Prevention (CDC) to address the increasingly urgent need for timely and objective information that would allow health care providers and payers, policymakers, and consumers to identify genetic tests that are safe and useful and to provide guidance on their appropriate use in practice, based on available evidence. At their request, we reviewed the evidence regarding the value of testing for these mutations in two specific populations: (1) individuals who have had a venous thromboembolic event (probands), and (2) their family members.

Methods

The overarching question we were asked to address (Key Question [KQ] 1) was: Does FVL testing, alone or in combination with prothrombin G20210A testing, lead to improved clinical outcomes (e.g., avoidance of a recurrent VTE) in adults with a personal history of VTE or to improved clinical outcomes (e.g., avoidance of an initial VTE) in adult family members of mutation-positive individuals? Are testing results useful in medical, personal, or public health decision making? To address this question, we reviewed the literature regarding these tests' analytic validity, clinical validity, and clinical utility when used in probands with VTE and in their family members.

The other KQs were as follows:

KQ2 What is the evidence regarding the analytic validity of existing diagnostic tests for the FVL mutation and the prothrombin G20210A mutation, specifically their analytic sensitivity and specificity, reproducibility, and robustness (sources of variability)?

KQ3a What is the evidence that the presence of FVL alone, prothrombin G20210A alone, or the two in combination predicts the risk of recurrent VTE in individuals (probands) who have had VTE and predicts the risk of VTE in the probands' family members who have been tested? Does the testing add predictive information beyond clinical data?

KQ3b What is the evidence that demographic or clinical factors modify the relationship between the presence of FVL or prothrombin G20210A and the risk of VTE?

KQ4a What is the evidence that clinicians manage patients differently based on the results of testing for FVL or prothrombin G20210A? How do clinicians manage anticoagulation of individuals who have had testing, as compared to those who have not had testing? What other diagnostic tests do clinicians order or not order, based on testing results? What recommendations do clinicians make regarding other therapies and exposures, based on testing results?

KQ4b What is the evidence that testing, and the resultant management, reduces VTE related-outcomes or has other benefits in individuals who have had VTE or in the probands' family members who have been tested?

KQ4c What is the evidence of harms to individuals with VTE or to the probands' family members who are tested for FVL or prothrombin G20210A as a result of testing or as a result of changed management based on the test results?

KQ4d What is the evidence that testing for FVL alone, prothrombin G20210A alone, or the two tests in combination is a cost-effective strategy when caring for a patient with VTE or a family member of a proband?

Our comprehensive search included electronic and hand searching. We searched five databases, MEDLINE® (1950 through May 2008), EMBASE® (1974 through December 2008),

The Cochrane Library (Issue 2, 2008), the Cumulative Index to Nursing & Allied Health Literature (CINAHL®; 1982 through December 2008) and PsycInfo©, to identify primary literature on the analytic validity, clinical validity, and clinical utility of testing for FVL and prothrombin G20210A.

Two independent reviewers, from among six study team members, conducted title scans in parallel. The title review was designed to capture as many studies as possible that reported on the analytic validity, clinical validity, and clinical utility of testing for FVL and prothrombin G20210A. All titles potentially addressing these issues were promoted to the abstract review phase.

Abstracts were reviewed independently by two investigators. Abstracts were excluded if the investigators agreed that the article: (1) was not relevant to any of the key questions; (2) did not include any human data; (3) contained no original data; (4) was not conducted in adults; and (5) was not published in English. Differences of opinion were resolved through consensus adjudication.

Full articles selected for review underwent another independent parallel review by two investigators. In addition to the exclusion criteria used for the abstract review, there were additional exclusion criteria for each KQ. For the question about clinical validity of the mutations (KQ 3), we included only prospective studies of probands, although we permitted retrospective studies of family members because we anticipated few prospective studies. Each article underwent double review by study investigators for full data abstraction and assessment of study quality. We used a sequential review process in which the primary reviewer completed all data abstraction forms, and the second reviewer checked the first reviewer's data abstraction forms for completeness and accuracy. Reviewer pairs were formed to include personnel with both clinical and methodological expertise. All information from the article review process was entered directly into the SRS 4.0 database.

The primary outcome extracted from the studies of analytic validity was concordance between the test and the reference test, as that was most often reported. When there were sufficient data (three or more studies) and the studies were qualitatively homogeneous with respect to key variables (population characteristics, study duration, mutation status, and length of follow-up), we conducted meta-analyses for the studies addressing the clinical validity of the tests. When it was inappropriate to combine studies quantitatively, we qualitatively summarized the results. For pooling, we used the number of events and count of the patients under observation in each group. We calculated a pooled estimate of the odds ratio for VTE in probands and separately in family members. We used a random effects model with the DerSimonian and Laird method for calculating between-study variance.

At the completion of our review, we graded the quantity, quality, and consistency of the best available evidence addressing the KQs by adapting an evidence-grading scheme recommended by the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Working Group. To assess the quantity of evidence, we focused on the number of studies with the strongest design. We also assessed the quality and consistency of the best available evidence, including assessment of the limitations affecting individual study quality (using the individual study quality assessments), certainty regarding the directness of the observed effects in the studies, the precision and strength of the findings, and the availability (or lack) of data to answer the KQ. We classified evidence bodies as: (1) "high" grade, indicating confidence that further research is very unlikely to change our confidence in the estimated effect in the abstracted literature; (2) "moderate" grade, indicating that further research is likely to have an important

impact on our confidence in the estimates of effects and may change the estimates in the abstracted literature; (3) “low” grade, indicating the further research is very likely to have an important impact on confidence in the estimates of effects and is likely to change the estimates in the abstracted literature.

Results

We reviewed 7,777 titles and included 124 articles in the review of one or more of the KQs.

Key Question 1

We identified no evidence to directly address KQ1, which required studies that directly tested the impact of testing on patient outcomes. The articles addressing the questions below, particularly KQ4, provided indirect evidence to answer KQ1.

Key Question 2

The conventional “gold standard” method for FVL and prothrombin G20210A detection is the sequencing of the specific genetic region of the gene of interest. However, a number of other reference methods are used instead because of the complexity and high costs associated with sequencing. Many studies that we reviewed used polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) or allele-specific polymerase chain reaction (AS-PCR) assays as their reference standards, both of which are considered by the Food and Drug Administration to be acceptable reference standards.

Detection of FVL. Forty-one studies compared at least two methods for FVL detection**.** The majority of studies demonstrated 100 percent concordance between the experimental method and the reference method. The least concordance was seen in a study using electrochemical genosensors, which had a concordance of only 93 percent.

Detection of prothrombin G20210A. The concordance rates between the experimental methods and the reference standards for the detection of prothrombin G20210A were 100 percent in nearly all of the 23 studies.

Simultaneous detection of FVL and prothrombin G20210A. All 12 studies that employed multiplex technologies for the simultaneous detection of the two mutations reported 100 percent concordance between this approach and the matched reference standard.

Quality assurance. We identified three studies that addressed external quality assurance or laboratory performance relative to the gold standards. One described the results from the United Kingdom National External Quality Assessment Scheme (UK NEQAS) Thrombophilia Screening Program. Two hundred eighty centers participated in the thrombophilia screening exercises. For the centers performing genetic analysis for FVL and the prothrombin mutation, an

error rate of 3 to 6 percent was identified, with both transcription-related and analytical errors observed.

Another study described results from the Royal College of Pathologists of Australasia external quality assurance (QA) program. This program sent 133 DNA samples with known mutations to laboratories in 10 separate surveys. Of 3,799 responses, the overall successful identification rate was 98.6 percent. Success rates in identifying specific mutations were 98.1 percent for FVL and 98.8 percent for prothrombin G20210A.

Finally, a survey was organized by the Subcommittee on Hemostasis of the Italian Committee for Standardization of Laboratory Tests (CISMEL). The authors concluded that regular quality control programs are warranted to identify the causes of failures to correctly identify specimens.

Key Question 3

Probands. Twenty-two articles examined the rates of recurrent venous thromboembolism in individuals with a history of VTE (probands) and the FVL mutation.

We pooled 13 studies comparing probands with heterozygous FVL to probands without this mutation, yielding an odds ratio for recurrence of 1.56 (95 percent confidence interval [CI], 1.14 to 2.12). The annualized event rates among heterozygous individuals were 3.1 percent to 7.5 percent in three studies. Seven studies described event rates in probands homozygous for FVL and those without mutations. The number of homozygous individuals ranged from 1 to 11 across studies. The odds ratio of recurrence was 2.65 (95 percent CI, 1.2 to 6.0).

Eight studies did not specify if the participants were heterozygous or homozygous for FVL. The pooled odds ratio (from 3 studies) associated with this unspecified status was 1.56 (95 percent CI, 0.75-3.2), very similar to the odds ratio for known heterozygous individuals.

We identified 18 articles that examined rates of recurrent VTE in probands with the prothrombin G20210A mutation. Nine of these compared individuals heterozygous for the mutation to individuals without mutation. The pooled odds ratio was 1.45 (95 percent CI, 0.96-2.2). Two studies described individuals homozygous for this mutation, but there were only 3 individuals with homozygosity. None of the three had a recurrent thrombosis.

Seven studies did not specify whether the individuals had homozygous or heterozygous prothrombin G20210A. The pooled odds ratio in the four studies with available data was 0.73 (95 percent CI, 0.37-1.4).

Doubly heterozygous individuals (with FVL and the prothrombin G20210A mutations) had an odds ratio of 4.8, with a wide confidence interval (95 percent CI, 0.5 to 46.)

When we separately evaluated patients with idiopathic VTE as the index event, we found that the odds ratio associated with heterozygous FVL was close to one (1.17 [95 percent CI, 0.63-2.18]).

Family members. We identified 17 articles that evaluated the occurrence of venous thrombosis in family members of probands. The majority of these were retrospective studies, although three included a prospective component. Nine studies described results for family members who were heterozygous for FVL. Six studies contributed to the pooled odds ratio, which was 3.5 (95 percent CI, 2.5 to 5.0). Homozygosity for FVL among family members had a pooled odds ratio of 18 (95 percent CI, 7.8 to 40) in the five studies pooled. The six studies that did not specify

whether individuals were homozygous or heterozygous had a pooled odds ratio of 2.9 (95 percent CI, 1.8-4.8).

Only three studies evaluated heterozygosity for prothrombin G20210A in family members. The odds ratio was 1.9 (95 percent CI, 0.35-10). There was only a single study of family members with homozygosity for this mutation.

Doubly heterozygous family members had a pooled odds ratio of 6.7 (95 percent CI, 2.9 to 15) in the three studies with data to pool.

A sizeable subgroup of family members had VTE associated with pregnancies. Four articles exclusively addressed the risk of VTE attributable to FVL and prothrombin G20210A during pregnancies of family members, and one additional study of family members had useable pregnancy data.

Two of these studies, which included few women, evaluated the risk associated with homozygous FVL. The odds ratios for venous thrombosis were 16 (95 percent CI, 0.9 - 278) and 41(95 percent CI, 5.5-419). The odds ratios associated with heterozygous FVL in two studies were 5.4 (95 percent CI, 0.65-46) and 3.4 (95 percent CI, 0.35 to 33). The rates of events were low, at 2.5 percent per pregnancy (95 percent CI, 0.9-5.4) and 1.5 percent per pregnancy (95 percent CI, 0.5-4.3), respectively. Two studies evaluated the risk associated with heterozygosity for the prothrombin G20210A mutation. The rates of VTE were low, at 0.3 percent per pregnancy (95 percent CI, 0.1 to 1.6) and 1 percent per pregnancy (95 percent CI, 0.2-3.6), respectively. The odds ratios in these two studies were close to 1.

Individuals who were doubly heterozygous and pregnant were evaluated in two studies. The larger of the two studies reported an odds ratio similar to the odds ratios associated with heterozygosity for either mutation (odds ratio, 4.1; 95 percent CI, 0.37-46). The smaller study reported an odds ratio that was identical to that for homozygous FVL (odds ratio, 16; 95 percent CI, 0.9-278).

Key Question 4

Effect of testing on clinicians' management. We found a single study addressing how physicians' management decisions are affected by FVL testing. Canadian obstetrical care providers (N=662) were asked about management recommendations in response to four clinical scenarios involving pregnant women with FVL. For scenarios involving asymptomatic women, if the patient was described as having a family history of VTE, the percentage of doctors recommending thromboprophylaxis was twice the percentage recommending it for women lacking a family history (58 versus 26-34 percent).

Effect of management, stratified by test results, on VTE-related outcomes. No studies directly addressed the effect of *testing* on outcomes. We therefore also included the four studies that described VTE recurrence rates during anticoagulation among probands with FVL or prothrombin G20210A. Two studies investigated the effect of warfarin on recurrence rates, and one the effect of ximelagatran; one did not specify the treatment that patients received.

One study assessed thromboembolism recurrence rates among individuals with FVL or prothrombin G20210A who received either a low-intensity warfarin regimen or placebo. Low-intensity warfarin reduced the rate of recurrence among thrombophilic patients by 75 percent. This risk reduction, however, was not significantly different than the 58 percent reduction seen among patients without either mutation.

In one study, the recurrence rate for patients with FVL while receiving low- *or* conventional-intensity warfarin therapy was 0.8 percent per year (95 percent CI, 0.2 to 2.2). This rate was not statistically different from the rate among those participants without FVL (hazard ratio 0.7; 95 percent CI, 0.2 to 2.6).

In another study, 2 of 111 FVL carriers receiving ximelagatran had recurrences of VTE, as compared to 16 of the 125 with mutations who were assigned to receive the placebo; this difference in recurrence rates was described as statistically significant. Recurrence rates with treatment were very similar for individuals without FVL or prothrombin G20210A, There was no interaction between FVL or prothrombin G20210A and the effect of ximelagatran in preventing recurrent VTE (p-value for the interaction, 0.92 for FVL and 0.98 for prothrombin G20210A).

Finally, one group studied the rate of recurrence of VTE in a cohort of 304 patients in thrombophilic families, according to whether they were or were not receiving long-term anticoagulation. Long-term anticoagulation decreased the rate of VTE recurrence among these probands. A quantitative estimate of the risk reduction was not provided, nor were the details of the anticoagulation regimens available

Effect of testing and results on other outcomes. Four studies addressed how probands' and family members' knowledge, behaviors, and healthcare experiences were affected by their being tested for FVL or prothrombin G20210A. Two studies employed cross-sectional surveys of convenience samples of probands and family members to assess risk perception and behavioral effects following genetic testing. The other two studies used qualitative, structured interviews of probands and relatives to describe their experience during the process of testing as well as their interpretation of the results.

One group surveyed the perception of VTE risk and changes in behavior following testing for FVL or prothrombin G20210A among first-degree relatives of probands. More mutation carriers recognized trauma as a risk factor for VTE than did non-carriers, but otherwise there were no statistically significant differences between the two groups regarding their recognition of risk factors for VTE. Behavior changes following testing were uncommon in both groups.

One group investigated whether the type of thrombophilic mutation and history of VTE affect the perception of risk and level of worry among probands or their relatives with FVL, as compared to other thrombophilic mutations. Individuals with a history of VTE had an increased perception of risk and worried more about VTE than did individuals without prior VTE. However, worry and risk perception were not measured in non-carriers for comparison.

One study involved a qualitative study of asymptomatic relatives of probands with FVL to assess their overall experience with the testing process and how the results affected their daily lives. Among the 17 participants, most found that the testing process itself was not stressful; all had received written information about the test prior to testing. Although the majority of participants indicated that the testing had not altered their daily lives, many wanted to screen their children to decrease their risk of VTE from pregnancy or oral contraceptive use.

One group (in two studies) assessed the level of understanding of the testing process and the implications of the results among probands and relatives referred for FVL testing by their primary care doctor or specialist. Most participants did not consider thrombophilia testing to be different from other tests ordered by their providers. Most participants did not incorporate behaviors to reduce their risk for VTE into their daily routines as a result of the testing, although most participants who were aware of their positive status stated they had undergone testing to

inform their decision about whether to take hormonal therapies, or to advise relatives on the matter.

Cost-effectiveness of FVL and prothrombin G20210A testing in the care of probands and their relatives. We identified six studies that assessed the cost-effectiveness of genetic testing and resultant changes in management and one study that assessed only effectiveness. The cost-effectiveness studies all used decision analytic models, which can provide support for further investigation of the utility of an intervention if the assumptions in the models are compatible with actual practice. The data ranges explored in the sensitivity analyses demonstrated the variables to which the cost-effectiveness of the interventions were most sensitive.

One group used decision analysis to assess the cost-effectiveness of testing for FVL and extending warfarin anticoagulation for 3 years or for life in carriers following a first VTE in a hypothetical cohort of 35-year-old women. If the rate of recurrence remained constant (7.3 percent/year), lifelong anticoagulation was the more cost-effective strategy (incremental cost-effectiveness ratio [ICER] = $16,823/quality-adjusted life year [QALY]) when compared to no testing and 6 months of anticoagulation). Lifelong anticoagulation was less cost-effective in patient populations with low FVL prevalence, low risk of recurrent VTE, or risk factors for bleeding on anticoagulant therapy.

Another group used decision analysis to assess the cost-effectiveness of testing for FVL and 2 years of warfarin anticoagulation in carriers following a VTE in a hypothetical cohort of 60-year-old men. FVL testing with 2 years of anticoagulation for carriers was a cost-effective strategy (ICER = $12,833/QALY) when compared to no testing and 6 months of anticoagulation. However, this intervention was not cost-effective for individuals with a high risk of fatal bleeding on warfarin, low VTE recurrence rate, low anticoagulation efficacy, or low anticoagulation compliance.

Building on the previous study, the author employed the same model to assess the cost-effectiveness of testing for double heterozygosity, followed by 2 years of warfarin anticoagulation for doubly heterozygous individuals. Testing for both mutations was cost-effective (ICER = $13,624/QALY) when compared to no testing and 6 months of anticoagulation. Testing was not cost-effective for patient populations with a high bleeding risk, low double-heterozygote prevalence, low levels of pulmonary embolism or mortality, or low anticoagulation efficacy.

Another group assessed the cost-effectiveness of a hypercoagulability testing panel and warfarin anticoagulation for 6, 12, 18, 24, or 36 months, or for life, following an apparently idiopathic deep venous thrombosis (DVT) in a hypothetical cohort of 40 year- olds. In the base case analysis, extending anticoagulation for 24 months following a positive test was the most cost-effective option (ICER = $11,100/ QALY) when compared to the least costly option of not testing and treating for 24 months. The authors concluded that tests detecting disorders present in at least 5 percent of the population that confer a relative risk exceeding 1.25, including FVL and prothrombin G20210A, should be included.

Another decision analysis with a 5-year time horizon assessed the effectiveness of extending anticoagulation from 3 months for FVL carriers and non-carriers following an initial lower-limb DVT to 1, 2, 3, 4, or 5 years in a hypothetical cohort. The authors stated that the risk of bleeding must be below 2.5 percent/year in order for prolonged anticoagulation to be the more effective strategy.

Finally, one group used a decision-analytic model with a 12-month time horizon to assess the cost-effectiveness of universal or selective screening for FVL and resultant changes in

management for carriers in four cohorts at high risk of VTE. In all four cohorts, selective screening was more cost-effective than universal screening.

One group used the cost and outcomes data from a prospective cohort of 967 pregnant women in the United Kingdom to assess the cost-effectiveness of FVL testing and enoxaparin anticoagulant prophylaxis to prevent pregnancy-related vascular complications over the 8-month time horizon, from 12 weeks of gestation to 6 weeks postpartum. No women actually received anticoagulant prophylaxis, but the hypothetical impact of treating FVL carriers with an assumed efficacy of 50 percent was modeled. Testing only those women with a personal or family history of VTE was the most cost-effective approach.

Discussion

Based on our review of the evidence, we graded the strength of the evidence for the key questions as follows:

Key Question 1

There was no direct evidence that testing for FVL or the prothrombin G20210A mutations improves clinical outcome in adults with a personal history of VTE or that it improves clinical outcomes in adult family members of mutation-positive individuals. The evidence supporting KQ 2 through 4 can be considered indirect evidence to answer this overarching question.

Key Question 2

- There was high-grade evidence that tests to detect FVL have excellent analytic validity.
- There was high-grade evidence that tests to detect prothrombin G20210A have excellent analytic validity.
- There was high-grade evidence that most, but not all, clinical laboratories can test for FVL and prothrombin G20210A very accurately. There may be some laboratories that, for technical or administrative reasons, report inaccurate results.

We note that the majority of the tested assays are not presently in widespread use. The majority of U.S. laboratories use PCR or invader chemistry technologies.

Key Question 3

- There was moderate-grade evidence that homozygosity for FVL in probands is predictive of recurrent VTE.
- There was moderate-grade evidence that heterozygosity for FVL in probands is predictive of recurrent VTE.
- The evidence is insufficient regarding the predictive value in probands of homozygosity for prothrombin G20210A, which is a rare genotype.
- There was moderate-grade evidence that heterozygosity for prothrombin G20201A in probands is not predictive of VTE.

- The evidence is insufficient regarding the predictive value in probands of double heterozygosity (FVL and prothrombin G20210A).

We note that there may be little predictive value in knowing the mutation status in patients with idiopathic VTE as the index event, since the odds ratios for this subgroup were close to one.

- There was high-grade evidence that homozygosity for FVL in family members is predictive of VTE.
- There was moderate-grade evidence that heterozygosity for FVL in family members is predictive of VTE.
- The evidence is insufficient regarding the predictive value in family members of homozygosity for prothrombin G20210A, which is a rare genotype.
- The evidence is insufficient regarding the predictive value in probands of heterozygosity for prothrombin G20210A.
- There was low-grade evidence that double heterozygosity (FVL and prothrombin G20210A) in family members is predictive of VTE.
- There was low-grade evidence that homozygosity for FVL in pregnant family members is predictive of VTE.
- The evidence was insufficient regarding the predictive value in pregnant family members of heterozygosity for FVL.
- The evidence was insufficient regarding the predictive value in pregnant family members of homozygosity for prothrombin G20210A.
- The evidence was insufficient regarding the predictive value in pregnant family members of heterozygosity for prothrombin G20210A.
- The evidence was insufficient regarding the predictive value in pregnant family members of double heterozygosity (FVL and prothrombin G20210A.)

For clinical context, we note that the annualized rate of venous thromboembolic events for family members without a mutation was approximately 0.1 percent per year. This translates to an event rate in heterozygous family members of 0.3 percent per year, or an absolute increase of 0.2 percent per year (a change from an average of 1/1000 person-years to 3/1000 person-years).

Key Question 4

- There was no direct evidence that management guided by test results reduces VTE related-outcomes in individuals who have had VTE or in the probands' family members who have been tested.
- There was low-grade evidence that physicians may alter patient management based on the results of testing for FVL or prothrombin G20210A.
- There was high-grade evidence that anticoagulation can reduce recurrent events in patients with FVL or prothrombin G20210A; however, there was only low-grade evidence that the relative reduction in risk is comparable to that seen in individuals without these mutations.

- There was moderate-grade evidence that neither harms nor benefits have been conclusively demonstrated in individuals with VTE or in their family members when tested for FVL or prothrombin G20210A.
- There was low-grade evidence, derived from models, that testing for FVL alone, prothrombin G20210A alone, or the two tests in combination may be cost-effective when caring for selected patients with VTE (those with a high risk of recurrence and/or low risk of bleeding) or their family members.

Limitations

In addition to the reported deficits in the literature, there are limitations to this report. In our assessment of clinical validity, we chose to pool odds ratios rather than time-dependent measures of recurrence (such as hazard ratios or incident rate ratios). This approach necessarily excluded some studies from the pooled estimates.

The odds ratios should approximate the relative rates of events in most studies, as these were relatively rare outcomes. We pooled the results using the DerSimonian and Laird random effects methods; this is a conservative method that often results in wide confidence intervals.

Many of these studies were observational studies, and physicians may have altered their management based on their knowledge of mutation status, thereby changing the likelihood of a particular outcome. Most studies mitigated this potential difficulty by excluding patients who were chronically anticoagulated or by using a pre-defined anticoagulation approach. In those studies that reported the duration of anticoagulation after the index event in mutation-positive and -negative subgroups, there was no obvious discordance in anticoagulation duration.

In these cohort studies, ascertainment bias is possible. In the studies of probands, the individuals were not blinded to their mutation status. Patients with mutations may be more likely to seek medical attention for symptoms consistent with VTE and might have been over-diagnosed with recurrence (as a result of false-positive tests), and those without mutations might have been under-diagnosed (because they did not seek medical attention for a thrombotic event that ultimately resolved without therapy). Ascertainment bias would tend to augment the association between the mutations and recurrent thrombosis. None of the studies we included had scheduled periodic radiographic testing to limit the potential for ascertainment bias.

The majority of the observational studies about family members were retrospective, with some notable exceptions. Retrospective studies are prone to important biases, including recall bias. Although such bias can be mitigated by interviewing participants before they have knowledge of their mutation status, this approach was variably described in these studies.

Implications for Future Research

Studies to directly address the overarching question (KQ 1) would ideally be designed as randomized trials, in which participants with venous thrombosis and/or their family members would be randomized to a test arm or a no-test arm. Individuals would be managed by their physicians on the basis of the results of the testing (with evidence-based recommendations). Sufficient follow-up time would be included so that venous thromboembolic events could be witnessed and compared between the tested and untested groups.

Analytic Validity

Although the mutation detection methods have been shown to have high analytic validity, a small minority of laboratories account for a disproportionate percentage of errors in the performance of these tests.

- There is a need for ongoing programs aimed at monitoring molecular diagnostic laboratories, through quality assurance programs, to ensure the consistent provision of high-quality genetic testing services.

Clinical Validity

- Future studies should report event rates over time (and relative rates of recurrence between specified groups), rather than just the number of events.
- Studies should consistently differentiate between heterozygosity and homozygosity.
- Studies should continue to use objectively measured thrombosis (radiographically proven) as a criterion for both the index and recurrent thromboses and should include more detail about both the index events and recurrences, such as the precipitants of these events.
- Additional studies are needed to quantify the effect size more precisely with regard to the prothrombin G20210A mutation (alone and in conjunction with FVL).
- Future research would be appropriate in Caucasian populations outside of Europe or in other populations with appreciable frequencies of mutations.
- Future research could better explore the age-mutation interaction.

Clinical Utility

- Studies should measure how actual clinician practices change in response to results of both FVL and prothrombin G20210A testing.
- Studies should be powered sufficiently to evaluate the *risks* associated with prolonged anticoagulation, as they relate to patients with specific thrombophilic mutations.
- Future studies in both probands and family members might focus on whether management decisions (duration of therapy, use of thromboprophylaxis) affect the rates of VTE, particularly during times of heightened thromboembolic risk.
- Studies based in the United States may give a clearer understanding of how patients here might respond to the testing process and results.
- Larger sample sizes should also be used to increase the ability to detect rarer events, such as stigmatization.
- Efforts should be made to recruit representative patient populations, and relevant comparison groups should be included (e.g., carriers and non-carriers) to increase the practical applications of the study findings. Quantitative studies may be preferable, using standardized, validated questionnaires to evaluate patients' experiences.
- Clinical trials could include an assessment of the costs associated with a testing strategy, as compared to care without testing.

Our literature review included articles through December 2008. We do not anticipate any important secular changes in the event rate that would markedly change the event rates in upcoming years. We also do not expect major changes in the coming years in terms of the methods used to detect mutations. The most anticipated change would be an increasing in options to reduce risk as new drugs become available. Future research will need to include an evaluation of the risks and benefits associated with use of new anticoagulant drugs in probands and family members at high risk of thromboembolic events.

Evidence Report

Chapter 1. Introduction

Venous Thromboembolism

Venous thromboembolism (VTE) refers to pathologic thrombosis in the venous circulation. Although the most frequent venous thromboembolic event is deep venous thrombosis (DVT) in the veins of the legs, thromboses can also occur in the veins of the upper extremities, pelvis, abdomen, and cerebral venous sinuses. Pulmonary embolism is the main life-threatening complication of DVT, in which a portion of the venous thrombus is carried to the pulmonary arteries by blood flow, potentially obstructing the pulmonary vasculature. VTE and its complications are a common cause of morbidity and mortality in the United States. Data from the Rochester Epidemiology Project indicate that the average annual incidence of isolated DVT is 48 per 100,000 individuals, and that of pulmonary embolism (with or without DVT) is 69 per 100,000.[1] Others estimate the incidence rate to be similar, at approximately 100 per 100,000, with approximately one-third manifesting as pulmonary embolism and two-thirds as DVT alone.[2]

Incident VTE is triggered by a confluence of modifiable and unmodifiable risk factors. Some of these risks are situational. Trauma or surgery may lead to direct endothelial injury, exposing circulating clotting factors to the thrombogenic material of the vessel wall (such as tissue factor). Stasis of blood is a precipitant of thrombosis, particularly when blood pools in the deep veins of the legs. Acquired alterations in coagulability are also precipitated by systemic illnesses, particularly malignancies, infection, nephrotic syndrome, and the antiphospholipid syndrome. These conditions are associated with varying degrees of inflammation, which activates hemostasis and/or changes in blood constituents (such as a loss of anti-clotting proteins in nephrotic syndrome, or the activation of the thrombotic cascade by tumor products). Primary hematological disorders, including myeloproliferative disorders such as polycythemia vera and essential thrombocythemia, increase the risk of thrombosis,[3] as do sickle cell anemia, thalassemia, and paroxysmal nocturnal hemoglobinuria.[4] Heparin-induced thrombocytopenia is a unique condition associated with venous or arterial thrombosis in up to 50 percent of patients in whom it develops.[5] Hyperhomocysteinemia is associated with increased risk of both venous and arterial thrombosis and can result from inherited enzymopathies or from acquired disorders of homocysteine metabolism, including renal failure and folate and vitamin B12 deficiency.[6-8] Hormonal therapies, primarily estrogens, are prothrombotic, as is pregnancy and the post-partum state.[3] Advanced age, too, is a potent risk factor for VTE.[9] Genetic risk factors for venous thrombosis include: deficiencies of endogenous anticoagulant proteins, antithrombin III, protein C, or protein S; and elevated levels of clotting factors VIII, IX, and XI; and possibly the 5,10-methylenetetrahydrofolate reductase C677T polymorphism.[3] Although one would expect that disturbances of normal fibrinolytic function (e.g., excessive levels of plasminogen activator inhibitor-1 or α_2-antiplasmin, or deficiencies in Factor XII or tissue plasminogen activator) would contribute to a hypercoaguable state, clinical evidence for such effects is lacking.[10-12] Rarely, dysfibrinogenemia is associated with an increased tendency toward clot formation.[13]

This report focuses on two specific genetic factors associated with an increased risk of VTE, the Factor V Leiden (FVL) mutation and the prothrombin G20210A mutation. These mutations will be described in detail below.

The risk factors for recurrent venous thrombosis differ from the risks for incident events; men are more likely to have recurrent events than are women.[14] In patients who stop taking

anticoagulant medications following treatment for an acute event, recurrent DVT occurs in about 20 percent of patients within 5 years of the first event and in 30 percent within 10 years.[15 16] However, continued anticoagulation treatment is highly effective in suppressing recurrent disease.[17]

Diagnosis and Treatment

Many diagnostic tests and testing strategies have been evaluated with the goal of maximizing both the sensitivity and specificity of the diagnosis, so that an expeditious diagnosis can be made and treatment promptly initiated. Treatment of the incident event happens in two phases: Treatment begins with short-term use of a parenteral anticoagulant (and sometimes thrombolytic therapy) and then usually continues with a vitamin K antagonist, most commonly warfarin. In some instances, parenteral therapy is continued instead of warfarin.[18] The duration of therapy depends on whether the patient is considered to have continuing risk factors for recurrence. Much effort has been devoted recently to quantifying the risk of recurrent thrombosis, with methods aimed at detecting whether a thrombophilic state persists. This effort has included measurement of D-dimer production[19] and assessment of persistence of thrombosis by ultrasonography,[20] as well as assessment of ongoing clinical risk factors. If the patient has a persistent risk factor for thrombosis, anticoagulant therapy is often continued, sometimes for the lifetime of the patient. The clinician and patient also try to reduce exposure to any modifiable prothrombotic risks as a secondary means of preventing additional events, and they may use mechanical or pharmacological means of preventing thrombosis at high-risk times, such as during hospitalization or pregnancy.

The question of whether a patient has ongoing risk is directly associated with the subject of this report, an evaluation of the value of testing for FVL and prothrombin G20210A. A diagnostic test has value only if the process of testing or the results of testing lead to a change in outcome. In the case of venous thrombosis, a test that predicts recurrent events is appropriate if there is an intervention that can modify the risk of events. In this case, use of anticoagulants can modify the risk. Also, removal or avoidance of prothrombotic contributors (such as hormonal therapy) modifies the risk. It is then easily argued that identification of genetic risks may be important if it permits actions can be taken that alter outcomes. Even if the risk factor itself is not modifiable, management of the patient based on knowledge of the risk factor can change outcomes.

Genetic Mutations Associated with VTE: FVL and Prothrombin G20210A

FVL refers to a base change (from G to A at position 1691) in the gene coding for the Factor V protein, an amino acid substitution that eliminates one of three activated protein C cleavage sites in Factor V. As a result, Factor V is inactivated to a lesser extent and persists for a longer period in the circulation, leading to more thrombin generation.[21] In the United States, a single FVL allele is present in about 5, 2.2, and 1.2 percent of the Caucasian, Hispanic, and African American populations, respectively; overall, about 1 in 5,000 individuals is homozygous for the mutation.[22 23] The FVL mutation has been estimated to be present in 15 to 20 percent of patients with first venous thrombosis[21] and is the most common *heritable* prothrombotic risk factor in the

United States. In population-based studies, FVL increases the risk of a first venous thrombosis 4- to 7-fold in heterozygous individuals and 40- to 80-fold in homozygous individuals.[21 22]

The prothrombin (Factor II) mutation is the second most common inherited risk factor for thrombosis. In the United States, the prevalence of this allele is 1.1 percent in Caucasians and Hispanics and 0.3 percent in African Americans.[24] The mutation in the gene, G20210A, is associated with an elevation of prothrombin levels to about 30 percent above normal in heterozygotes and to 70 percent above normal in homozygotes. This mutation is present in about 2 percent of the population and in 6 percent or more of patients who present with a first VTE. Six to 12 percent of individuals who are heterozygous for FVL and have VTE also have the prothrombin G20210A mutation (double heterozygotes).[21 22 25] Heterozygotes are at a two- to four-fold increased risk of an initial thrombosis.[22 26] Individuals who are doubly heterozygous for FVL and prothrombin G20210A (about 1 in 1,000 Americans) have an estimated 20-fold increased risk when compared to individuals without either mutation, suggesting a multiplicative elevation in risk.[21 22 25]

Methods for Identifying Mutations

Testing for these mutations is widely offered in the United States. In December 2003, the U.S. Food and Drug Administration (FDA) approved the first DNA-based laboratory tests specifically designed for FVL and prothrombin G20210A detection, manufactured by Roche Diagnostics Corporation (LightCycler Instrument). In 2007, Autogenomics Inc. received approval for their INFINITI System Assay for detection of the mutations. These tests are FDA-approved for use as an "aid to diagnosis in the evaluation of patients with suspected thrombophilia."[27 28] These commercially available tests have demonstrated, in their pre-approval testing, sufficient analytic validity to receive approval for use. They were not required to demonstrate clinical validity or clinical utility (to be defined below). Laboratories have also developed numerous other methods for detecting these mutations and do not use the commercially available systems; the analytic validity of these tests, however, is not well known.

Objectives of this Evidence Report

Our goal was to review the evidence regarding the value of testing for these mutations in two specific populations: (1) individuals who have had a VTE event (probands), and (2) the family members of probands with mutations. We did not aim to review the evidence regarding testing for these mutations as a screening strategy (for example, in a population with no known risks for disease, or in other clinical settings such as before use of oral contraceptives or hormonal therapy). Similarly, it was not our intention to review the use of these genetic tests in other clinical settings, such as in the investigation of fetal loss.

To achieve our goal, we developed an analytic framework (described in Chapter 2). To understand this framework, one must be familiar with the definitions of *analytic validity, clinical validity,* and *clinical utility.*[29] *Analytic validity* is a measure of an assay's performance, which includes its analytic sensitivity and specificity as well as its robustness. Robustness refers to how resistant the test is to changes in pre-analytic and analytic variables, such as the source of the specimen or the temperature of the environment. Included in the framework of analytic validity

is the assessment of laboratories' quality control activities. These are procedures that ensure that the results fall within specified limits.

Clinical validity is the extent to which the presence of the mutation predicts the clinical condition of interest. Clinical validity, therefore, includes the sensitivity and specificity of the test, as well as the predictive values of positive and negative tests, taking into account the disorder prevalence. Clinical validity may also be affected by penetrance, which is the relationship between the genotype and phenotype. In this report, the clinical condition of interest is recurrent venous thrombosis in the probands (because they have all had an incident event) and a first thrombotic event in the family members. Therefore, our assessment of clinical validity involves identifying the extent to which the presence of the mutations predicts venous thrombosis.

Clinical utility refers to the impact of testing on outcomes. This evaluation involves knowing the availability and effectiveness of interventions employed in response to the test results, and an understanding of the risks and benefits when the test is used in routine practice.

The report is structured as follows: In Chapter 2, we present our methods, which include a description of the Key Questions addressed in this evidence report, the analytic framework we constructed, and the methods used in our literature search, data abstraction, and synthesis of results. In Chapter 3, we present our results and assessment of the strength of the evidence for each Key Question, in terms of the analytic validity, clinical validity, and clinical utility of testing. Chapter 4 is our discussion and includes a review of the grading of the strength of the evidence and the implications of our findings for future research.

Chapter 2. Methods

The *Evaluation of Genomic Applications in Practice and Prevention* (EGAPP) initiative was established by the Office of Public Health Genomics at the Centers for Disease Control and Prevention (CDC) to address the increasingly urgent need for timely and objective information that would allow health care providers and payers, policymakers, and consumers to identify genetic tests that are safe and useful. The CDC expects the EGAPP initiative to provide guidance on the appropriate use of genetic tests in practice, based on available evidence. The independent, multidisciplinary EGAPP Working Group, established in April, 2005, requested this review on the topic of testing for Factor V Leiden (FVL) and prothrombin G20210A.

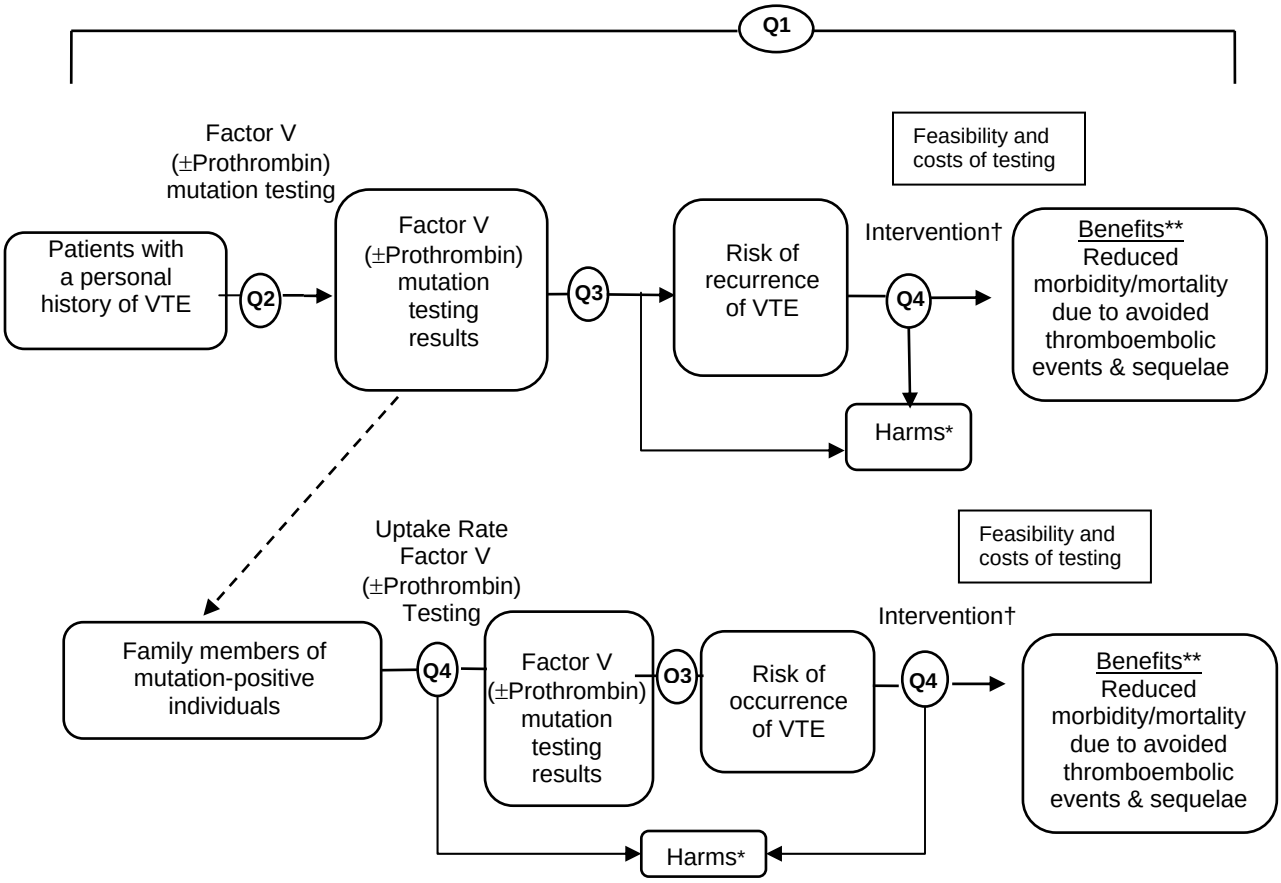
Establishing a Technical Expert Panel

We began by recruiting experts to form a Technical Expert Panel. These experts were asked to provide input that would help us to refine the key questions to be answered with this review, to examine our list of articles identified for inclusion, and to suggest additional sources to search for evidence. These experts were also invited to be the peer reviewers of our finished report. The panel included four faculty members from the Johns Hopkins University. One of the four Hopkins panelists was a bioethicist and the former Assistant Director of the National Human Genome Research Institute, one was a clinical investigator with extensive experience with genetic testing for colon cancer, one was the director of our special coagulation laboratory, and one was the director of our anticoagulation management service and an expert on hypercoagulable states. Our external panelists included experts in thrombophilia and the management of venous thromboembolism (VTE); a genetic counselor; three members of the EGAPP Working Group with expertise in medical screening, genetics, and methodologies of genetic testing; a family physician; and a patient affected by these mutations who is a leader in the National Alliance for Thrombosis and Thrombophilia (see Appendix A).

Analytic Framework

We used an analytic framework to identify the evidence required to answer our Key Questions. The analytic framework (see Figure 1) was developed with the involvement of our Task Order Officer from the Agency for Healthcare Research and Quality (AHRQ) and the Technical Expert Panel.

Figure 1. FVL and prothrombin testing for the prevention of venous thromboembolic events: Analytic framework



* harms include management-related outcomes and emotional/behavioral/social sequelae of testing
** benefits include VTE-related outcomes, management-related outcomes, emotional/behavioral/social sequelae of testing
† intervention (or change in management or recommendations) may or may not vary with test result
Q = Question; VTE = venous thromboembolism

Key Questions

Guided by the analytic framework, we developed the following key questions (KQs). Our population was defined as adults with a personal history of VTE, or adult family members of individuals identified as having an FVL and/or prothrombin G20210A mutation(s).

KQ1 [Overarching question] Does FVL testing, alone or in combination with prothrombin G20210A testing, lead to improved clinical outcome (e.g., avoidance of a recurrent VTE) in adults with a personal history of VTE or to improved clinical outcome (e.g., avoidance of an initial VTE) in adult family members of mutation-positive individuals? Are the testing results useful in medical, personal, or public health decisionmaking?

KQ2 What is the evidence regarding the analytic validity of existing diagnostic tests for the FVL mutation and the prothrombin G20210A mutation, specifically their

analytic sensitivity and specificity, reproducibility, and robustness (sources of variability)?

KQ3a What is the evidence that the presence of FVL alone, prothrombin G20210A alone, or the two in combination predicts the risk of recurrent VTE in individuals (probands) who have had VTE and predicts the risk of VTE in the probands' family members who have been tested? Does the testing add predictive information beyond clinical data?

KQ3b What is the evidence that demographic or clinical factors modify the relationship between the presence of FVL or prothrombin G20210A and the risk of VTE?

KQ4a What is the evidence that clinicians manage patients differently based on the results of testing for FVL or prothrombin G20210A? How do clinicians manage anticoagulation in individuals who have had testing, as compared to those who have not had testing? What other diagnostic tests do clinicians order or not order, based on testing results? What recommendations do clinicians make regarding other therapies and exposures, based on testing results?

KQ4b What is the evidence that testing, and the resultant management, reduces VTE related-outcomes or has other benefits in individuals who have had VTE or in the probands' family members who have been tested?

KQ4c What is the evidence of harms to individuals with VTE or to the probands' family members who are tested for FVL or prothrombin G20210A as a result of testing or as a result of changed management based on the test results?

KQ4d What is the evidence that testing for FVL alone, prothrombin G20210A alone, or the two tests in combination is a cost-effective strategy when caring for a patient with VTE or a family member of a proband?

Search Strategy

Searching the literature involved identifying reference sources, formulating a search strategy for each source, and executing and documenting each search. We also searched for medical subject heading (MeSH) terms that were relevant to the genetics of Factor V and prothrombin. We used a systematic approach for searching the literature, with specific eligibility criteria, to minimize the risk of bias in selecting articles for inclusion in the review. The systematic approach was intended to help identify gaps in the published literature.

Our comprehensive search included electronic and hand searching. We searched five databases, MEDLINE® (1950 through December 2008), EMBASE® (1974 through December 2008), The Cochrane Library (Issue 2, 2008), the Cumulative Index to Nursing & Allied Health Literature (CINAHL®; 1982 through December 2008) and PsycInfo©, to identify primary literature on the analytic validity, clinical validity, and clinical utility of testing for FVL and prothrombin G20210A. Hand searching for possibly relevant citations took two forms. First,

from our electronic search, we identified the 11 journals (see Appendix B) that were most likely to publish articles on this topic (i.e., these journals had the highest number of abstracts and articles included in the review). We scanned the table of contents of each issue of these journals for relevant articles from March 2008 through September 2008. For the second form of hand searching, reviewers reviewed eligible articles and flagged references of interest for the team to compare to the existing database.

Search strategies specific to each database were designed to enable the team to focus the available resources on articles that were the most likely to be relevant to the key questions. We initially developed a core strategy for MEDLINE®, accessed via PubMed®, based on an analysis of the MeSH terms and text words of key articles identified *a priori*. The PubMed® strategy formed the basis for the strategies developed for the other electronic databases (see Appendix C). The results of the searches were downloaded and imported into ProCite® version 5 (the Thompson Corporation, Stamford, CT). We used the duplication scan feature in ProCite® to delete citations already retrieved. From ProCite®, the articles were uploaded to SRS 4.0 (TrialStat! Corporation, Ottawa, Ontario, Canada), a Web-based software package developed for systematic review data management. This database was also used to store citations in portable document format (PDF) and to track the search results at the title review, abstract review, article inclusion/exclusion, and data abstraction levels. A list of excluded articles is presented in Appendix D.

Study Selection

There were a total of six reviewers who were paired, so that each pair included an expert in the content of this review. Two reviewers independently conducted title scans in a parallel fashion. For a title to be eliminated at this level, both reviewers had to indicate that it was ineligible. If the two reviewers did not agree on the eligibility of an article, it was promoted to the next level (see Appendix F, Title Review Form). The title review phase was designed to capture as many studies as possible that reported on the analytic validity, clinical validity, and clinical utility of testing for FVL and prothrombin G20210A. All titles that were identified as potentially addressing these issues were promoted to the abstract review phase.

Abstracts were reviewed independently by two investigators. Abstracts were excluded if both investigators agreed that the article met one or more of the following exclusion criteria: It (1) was not relevant to any of the key questions; (2) did not include any human data; (3) contained no original data; (4) was not conducted among adults; or (5) was not published in English. Differences of opinion regarding abstract eligibility were resolved through consensus adjudication. At this level of review, the reviewers were also asked to identify to which Key Question(s) the article might apply if it was eligible for review.

Because of the broad array of potentially eligible articles obtained at the abstract review phase, full articles selected for review underwent another independent parallel review by two investigators to determine whether the articles should be included in the full data abstraction. In addition to the exclusion criteria used for the abstract review, there were additional exclusion criteria for each Key Question (see Table 1).

Table 1. List of additional exclusion criteria by Key Question

Question	Criteria	Explanation
KQ2	• Published prior to 2000 • Evaluated only APC resistance • Evaluated only DNA extraction methods • Did not report concordance, discordance, or reproducibility • Evaluated fewer than 10 samples	
KQ3	• Did not report results separately for FVL and/or prothrombin G20210A • Studied fewer than 10 probands or fewer than 10 family members • Did not objectively confirm VTE event • Was a case-control or retrospective cohort study of probands • Patients described in another publication	We opted to *exclude* retrospective studies of probands, since this study design is more subject to biases than is a prospective design. We opted to *include* retrospective studies of family members because we expected to have few, if any, prospective studies.
KQ4	• Did not study probands or family members • Fewer than 80% of the probands had the FVL or prothrombin G20210A mutations • Fewer than 80% of the probands had VTE	We required that 80% of the study population had the mutations of interest if it was a study including family members with other hypercoaguable conditions and results were not reported separately by mutation. We required that 80% of the probands had VTE if it was a study of individuals with mutations, in keeping with our stated population of interest.

APC = activated protein C; DNA = deoxyribonucleic acid; FVL = Factor V Leiden; KQ = Key Question; VTE = venous thromboembolism

At this phase of the review, the investigators determined which of the Key Questions each article addressed (see Appendix E, Article Inclusion/Exclusion Form). If the articles were deemed to have applicable information, they were included in the full data abstraction. Differences of opinion regarding article eligibility were resolved through consensus adjudication.

Data Abstraction

We used a systematic approach to extracting data in order to minimize the risk of bias in this process. By creating standardized forms for data extraction, we sought to maximize consistency in identifying all pertinent data available for synthesis.

Each article underwent double review by study investigators for full data abstraction and assessment of study quality. For all data abstracted from studies, we used a sequential review process. In this process, the primary reviewer completed all data abstraction forms. The second reviewer checked the first reviewer's data abstraction forms for completeness and accuracy. Reviewer pairs were formed to include personnel with both clinical and methodological expertise. Reviewers were not masked to the articles' authors, institutions, or journal.[30] In most instances, data were directly abstracted from the article text. If possible, relevant data were also abstracted from figures. Differences of opinion were resolved through consensus adjudication. For assessments of study quality, each reviewer independently judged study quality and rated items on quality assessment forms (see Appendix F, Data Abstraction Review Forms).

For articles that applied to Key Question 2, we abstracted information on the sample selection criteria, the test operators, the characteristics of the DNA extraction process, the experimental test, the reference standard, the commercial instruments used, the concordance rates, and the explanation of any false positives or false negatives, if applicable. For articles that applied to Key Question 3, we abstracted information on study characteristics (i.e., study design, enrollment dates, location, inclusion criteria, genetic test used, duration of follow-up, and radiographic and clinical surveillance), population characteristics (i.e., age, gender, idiopathic VTE, oral contraceptive or hormone use, pregnancy, other precipitating factors, and other thrombotic mutations), and the results. For articles that applied to Key Question 4, we abstracted information on the study characteristics (i.e., study design and location), population characteristics (i.e., patients or providers, age, race, gender, genotype, socioeconomic status, and specialty of the providers), the main objective of the study, and the results.

All information from the article review process was entered into the SRS 4.0 database by the individual completing the review. Reviewers entered comments into the system whenever applicable. The SRS 4.0 database was used to maintain and clean the data, as well as to create detailed evidence tables and summary tables (see Appendix G and Summary Tables).

Study Quality Assessment

The study aspects considered in our quality assessment varied according to the question being addressed and according to the study design. As part of our dual, independent review of study quality, we judged articles on several aspects of each study type's internal validity. Quality assessment of diagnostic studies for Key Question 2 was designed by selecting elements from the Standards of Reporting of Diagnostic Accuracy (STARD) Initiative[31] and included: (1) adequate descriptions of the setting, the experimental test, and the reference standard; (2) a statement about testing being conducted without knowledge of the reference standard results; (3) a statement about all specimens being tested with both the experimental test and reference standard; (4) the reporting of a summary index and a measure of variability; and (5) a description of the funding source. Quality assessment for Key Question 3 was based on quality forms previously developed by our Evidence-based Practice Center (EPC).[32 33] This assessment included items about the setting, inclusion/exclusion criteria, key characteristics of the enrolled subjects, losses to follow-up, and the funding source. Quality assessment of the qualitative studies for Key Question 4 included items from The Joanna Briggs Institute Qualitative Assessment and Review Instrument,[34]supplemented with quality items previously developed by our EPC. The qualitative study quality assessment form included: (1) a statement locating the researcher culturally or theoretically, (2) a statement of ethical approval, (3) a description of the theoretical basis for the study, (4) a description of why patients were selected, (5) adequate representation of the participants and their voices, (6) composition of the interview setting to maximize data gathering, (7) reporting of theme exhaustion, (8) a clear description of the data collection process, (9) addressing the influence of researchers on research and vice versa, (10) the use of triangulation, and (11) the development of a concept, model, or theory based on the data collected. Quality assessment of surveys for Key Question 4 was based on quality forms previously developed by our EPC and included: (1) a description of the theoretical basis for the study; (2) a statement of ethical approval; (3) a description of the setting, inclusion/exclusion criteria, and key characteristics of the study participants; (4) survey completion rates; and (5) a discussion of the validity, reliability, and interpretability of the survey instrument. Quality

assessment of cost-effectiveness analyses for Key Question 4 was based on a review of guidelines for good practice in decision-analytic modeling in health technology assessment[35] and included items regarding the structure, data, and consistency.

A senior reviewer adjudicated any discrepancies in the quality assessment.

Applicability

Throughout the report, we describe the applicability of studies in terms of the extent to which the study population and testing procedure were relevant to the process of testing patients and their family members in the United States**.** We evaluated the applicability of studies in terms of: (1) the study population, (2) the genetic tests used to determine mutation status, (3) the eligibility criteria, (4) the outcome rates and measures, (5) the treatment regimen, (6) the interventions, (7) the follow-up time, and (8) the standards of care.

Data Synthesis

For each Key Question, we created a set of detailed evidence tables containing all the information extracted from the eligible studies. The investigators reviewed the tables and eliminated items that were rarely reported.

In calculating the concordance rates for the studies that examined the analytic validity of the tests for detecting the mutations, we used the initial test results before any resolution by repeat testing. In the tables presenting these results (in Chapter 3), if the concordance was less than 100 percent, we provide details about the discordant results.

We conducted meta-analyses of the studies addressing the clinical validity of the tests, when there were sufficient data (three or more studies) and the studies were qualitatively homogeneous with respect to key variables (population characteristics, study duration, mutation status, and length of follow-up). When it was inappropriate to combine studies quantitatively, we qualitatively summarized the results.

For the pooling, we used the number of events and count of the patients under observation in each group. We calculated a pooled estimate of the odds ratio for VTE in probands and separately in family members. We used a random effects model with the DerSimonian and Laird method for calculating between-study variance.[36] The random effects model was chosen because we anticipated some heterogeneity among the studies. We repeated the pooling using Mantel-Haenszel (M-H) fixed effect methods and Peto fixed effects methods. As the outcome events were not always rare, the Peto methods yielded markedly different pooled estimates for some of the comparisons. The M-H fixed effects and random effects pooled estimates were quite similar.

We assessed heterogeneity among the studies considered using a standard chi-squared test and a significance level of alpha ≤ 0.10. We also examined heterogeneity among studies with an I^2 statistic, which describes the variability in effect estimates that is due to heterogeneity rather than random chance.[37] A value greater than 50 percent may be considered to indicate substantial variability.

We tested for publication bias in two ways. First, we used the Duval and Tweedie nonparametric "trim and fill" method of accounting for publication bias in meta-analysis.[38] This methodology explores whether the inclusion of “missing” studies would alter the pooled odds ratio. We also used the Egger’s test to evaluate the likelihood of missing studies.[39] Finally, we sequentially removed each study from the calculation of the pooled estimates and recalculated

the pooled odds ratios. In this way, we were able to assess the impact on the odds ratios of one particularly influential study.

All statistical analyses were conducted using STATA (Intercooled, version 9.0, StataCorp, College Station, TX).

Data Entry and Quality Control

Initial data were abstracted by the investigators and entered directly into Web-based data collection forms using SRS® 4.0 (TrialStat! Corporation, Ottawa, Ontario, Canada). After a second reviewer reviewed the data, the adjudicated data were re-entered into the Web-based data collection forms by the second reviewer. Second reviewers were generally more experienced members of the research team, and one of their main priorities was to check the quality and consistency of the first reviewers' answers. If problems were recognized in a reviewer's data abstraction, the problems were discussed at a meeting with the reviewers.

Rating the Body of Evidence

At the completion of our review, we graded the quantity, quality, and consistency of the best available evidence addressing the Key Questions by adapting an evidence-grading scheme recommended by the Grading of Recommendations Assessment, Development and Evaluation (GRADE) Working Group.[40] We assessed the strength of the study designs for each question. To assess the quantity of evidence, we focused on the number of studies with the strongest design. We also assessed the quality and consistency of the best available evidence, including assessment of the limitations affecting individual study quality (using the individual study quality assessments), certainty regarding the directness of the observed effects in the studies, the precision and strength of the findings, and the availability (or lack) of data to answer the Key Question. We classified evidence bodies pertaining to the Key Questions into the following categories: (1) "high" grade, indicating confidence that further research is very unlikely to change our confidence in the estimated effect in the abstracted literature; (2) "moderate" grade, indicating that further research is likely to have an important impact on our confidence in the estimates of effects and may change the estimates in the abstracted literature; (3) "low" grade, indicating the further research is very likely to have an important impact on confidence in the estimates of effects and is likely to change the estimates in the abstracted literature. We also noted when there was no available evidence. This framework for grading the body of evidence is consistent with that suggested by the EGAPP working group.[41]

Chapter 3. Results

Results of the Search

The literature search process identified 13,711 citations that were potentially relevant to the Key Questions. An additional 165 articles were found by hand searching; thus, the total number of citations retrieved was 13,876 (see Figure 2). We excluded 6,099 duplicate citations. Most duplicates came from concurrently searching MEDLINE® and EMBASE®. The search strategy we used in EMBASE®was modeled on that which we used in MEDLINE®, with similar search terms. (see Appendix C). Also, the EMBASE® search engine allows the user to search the MEDLINE® database as well as EMBASE®. Using this strategy often leads to many duplicates between the two search sites. However, this Evidence-based Practice Center (EPC) employs this strategy in order to improve the sensitivity of the search.

In the title review process, we excluded 5,553 citations that were ineligible for inclusion. In the abstract review process, we excluded 1,827 citations that did not meet one or more of the eligibility criteria (see the list in the Methods chapter, and Figure 2). At article review, we then excluded an additional 273 articles that did not meet one or more of the eligibility criteria. That left 124 articles eligible for inclusion in the review of one or more of the Key Questions.

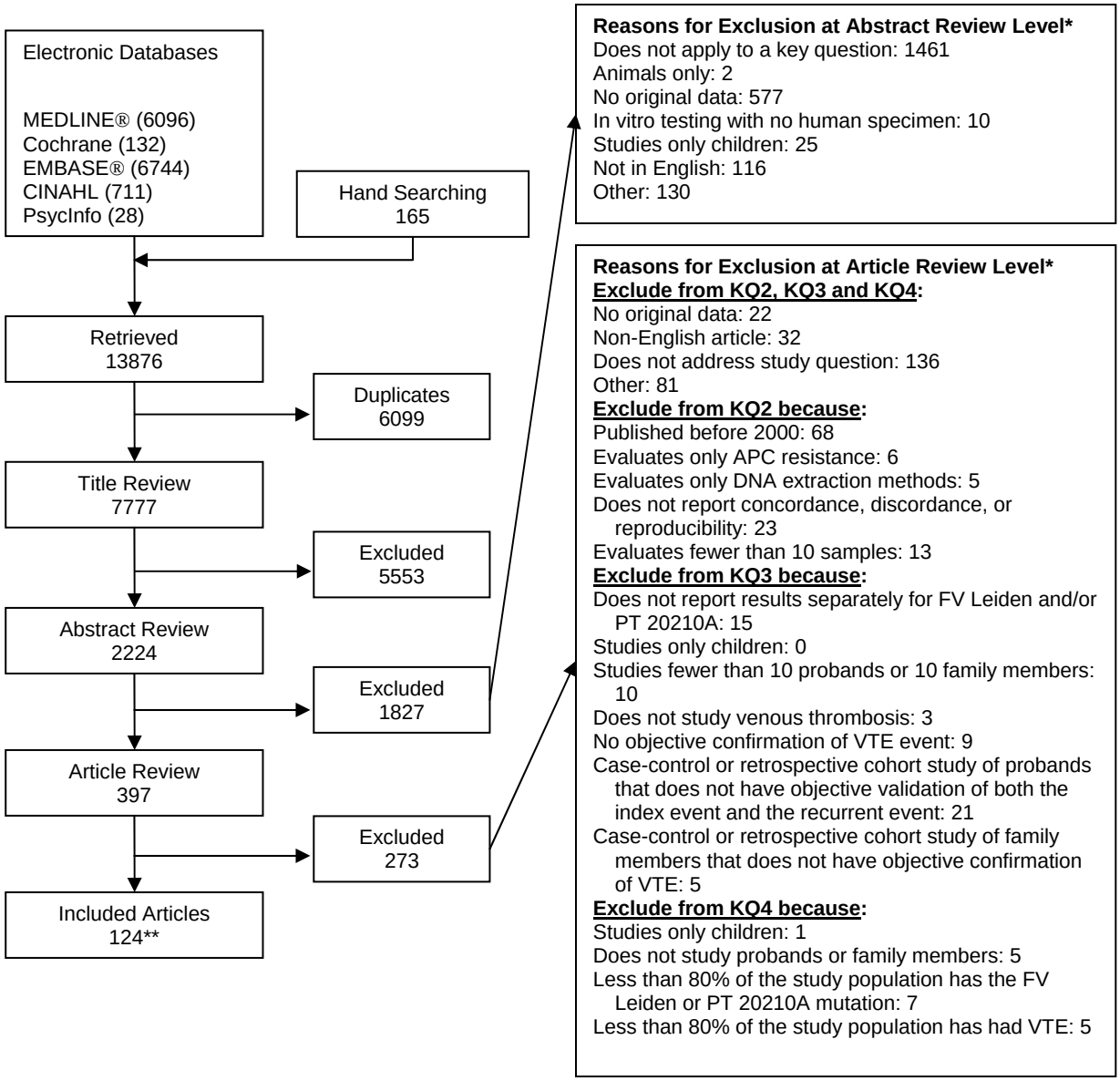
Description of Types of Studies Retrieved

No articles were identified that directly answered Key Question 1. One recent article nearly met our inclusion criteria and is described in Chapter 4.[42] We identified 66 articles that were appropriate for Key Question 2. For Key Question 3, 23 articles were relevant to probands with the mutations, and 22 articles were relevant to family members with mutations. An additional 14 articles were identified relevant to Key Question 4, including 7 decision analyses.

Key Question 1: Association of FVL testing, alone, or in combination with prothrombin G20210A testing, with improved clinical outcomes in adults with a personal history of VTE or in adult family members of mutation-positive individuals as a result of medical, personal, or public health decision making

We found no evidence that allowed us to directly answer this question.

Figure 2. Summary of literature search (number of articles)



* Total may exceed number in corresponding box, as articles could be excluded for more than one reason at this level.
**3 articles addressed both KQ3 and KQ4.
APC = activated protein C; CINAHL = Cumulative Index to Allied Health and Nursing Literature; DNA = deoxyribonucleic acid; FV Leiden = Factor V Leiden; KQ = Key Question; PT 20210A = prothrombin G20210A; VTE = venous thromboembolism

Key Question 2:
Analytic validity of tests to identify FVL and prothrombin G20210A mutations

As described early in this report, we employed the same definition of analytic validity that is used in the Analytic Validity, Clinical Validity, Clinical Utility and Associated Ethical, Legal and Social Implications (ACCE) framework.[29] Accordingly, our review of analytic validity assessed the analytic sensitivity (the analytic detection rate), analytic specificity, laboratory quality control, and assay robustness. Calculations of sensitivity and specificity, however, are most applicable to tests with dichotomous results. Given that there are two alleles, genetic test results have three outcomes: no mutations, a single mutation (heterozygous), or two mutations (homozygous). The studies we reviewed uniformly and appropriately described analytic validity as *concordance* or *discordance* between the experimental test and the reference standard, rather than sensitivity and specificity.

Study quality was generally high (see Appendix G, Evidence Table 1). In our quality assessment, we focused mainly on biases that could result from commercial conflicts of interest. The majority of these studies were published before journals began requiring disclosure of funding sources and potential conflicts of interest. More studies than not, however, did report on the source of funding; we considered these sources of funding to be unlikely to bias the results, since most of the reported funding was from governmental grant support.

Studies varied greatly in the extent to which they described the population from which the participants came; 17 studies adequately reported the setting or population from which the blood samples were obtained.[43-46 46-48 48-57] Most described the experimental test adequately; approximately 60 to 70 percent of the studies gave a detailed description of their reference methods.

An important quality-related consideration was whether the study indicated whether the experimental test was conducted without knowledge of the reference standard results. In only a minority of the studies, the staff members were unaware of the reference standard results at the time they performed the experimental tests.[46 50 52 55 58-64]

The conventional "gold standard" method for Factor V Leiden (FVL) and prothrombin G20210A detection is the bidirectional sequencing of the specific genetic region of the gene of interest. The Food and Drug Administration (FDA) requires that manufacturers of new assays compare the assay to an established method and considers polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) to be an acceptable reference standard. Indeed, many of the studies used PCR-RFLP or allele-specific polymerase chain reaction (AS-PCR) as their reference standards. In the absence of a substantial number of studies comparing the test under evaluation to the gold standard (bidirectional DNA sequencing), we included in our review studies that used traditionally accepted reference tests, typically PCR-RFLP or AS-PCR. We acknowledge that a "methods comparison" may be a lower level of evidence regarding analytic validity than a gold standard comparison would be.

All the studies included used blood as the source of DNA for analysis. In the table below, the studies are grouped according to the underlying chemical/biochemical experimental methods they employed (Tables 2-4; Appendix G, Evidence Tables 2-4). We observed a high level of concordance between the experimental methods and the reference standards, with many studies reporting 100 percent concordance (range of concordance, 98 to 100 percent).

Table 2. Analytic validity of testing for the FVL mutation

Experimental Test	Flap Endonuclease + FRET (Invader Assay)	FRET + Melting Curve Analysis (Light Cycler)	Taqman Real-Time PCR Assay	Electrochemical Genosensors	Temperature Gradient Capillary Electrophoresis
Reference Standard	**AS-PCR or PCR-RFLP**	**PCR or PCR-RFLP**	**PCR-RFLP**	**PCR-RFLP**	**PCR-RFLP**
Studies	Hessner, 2000[43] Ledford, 2000[44] Patnaik, 2004[67]	Schroell-Metzger, 2003[68] Mammo, 2006[69] Cooper, 2003[70] Nauck, 2000[45] Parks, 2001[48]	Louis, 2004[62] Benson, 2001[47] Behrens, 2004[71]	Ozkan, 2002[65] Ozsoz, 2003[72] Huang, 2002[73]	Murphy, 2003[61]
Total Population*	1369 + 371 + 368	132 + 2131 + 200 + 110 + 155	115 + 100 + 100	90 + 90 + NR	304
Concordance	99.5% - 100%	100%	100%	93% - 100%	99.3%
Source of Zygosity Discordance**	5 samples were typed WT by reference standard but heterozygous by Invader assay. With retyping, there was concordance, with all genotypes unequivocally WT. In Ledford, 2 samples were discordant. Retesting yielded results concordant with the original PCR-RFLP results. In one case, a heterozygous sample was called homozygous by the Invader assay; the discrepancy was due to a failure to add genomic DNA to the well. In the second case, a heterozygous sample was called WT.	NA	NA	In Ozkan et al., the source and nature of the 7% discordance were not explained. Ozsoz et al. reported neither reference standard nor quantitative results. Rather, it stated that there was "good agreement" and ongoing work to achieve 100% accuracy. Huang et al. did not report sample size or reference standard.	One heterozygous sample falsely identified as homozygous mutant by TGCE. One WT/WT falsely reported as WT/Leiden by TGCE.
Did Repeating Resolve the Discordance*?	Yes (technical/operator error)	NA	NA	Not reported	Yes for the heterozygous; did not resolve for the WT/WT (see footnote)

Table 2. Analytic validity of testing for the FVL mutation (continued)

Experimental Test	MALDI-TOF Mass Spectrometry	PCR-Single Strand Conformation Polymorphism	Simultaneous Allele-Specific Amplification Followed by RFLP	Fluorogenic Probe-Based Allelic Discrimination PCR Assay	Melting Analysis of Labeled Probes System	Lanthanide-Labeled Probe
Reference Standard	**PCR-RFLP**	**PCR-RFLP**	**PCR-RFLP**	**PCR-RFLP**	**PCR-RFLP or Conventional Dual-Hybridization Probe Genotyping**	**PCR-RFLP**
Studies	Hung, 2002[50] Humeny, 2001[55]	Simundic, 2003[74]	DelRio-LaFreniere, 2001[64]	Sanders, 2000[75]	El Housni, 2003[80] (dual-labeled probes); Vaughn, 2004[81] Crockett, 2001[82] (single-labeled fluorophore-based systems)	Potter, 2001[49]
Total Population*	27 + 70	150	49	120	100 + 100 + 100	379
Concordance	100%	100%	100%	100%	100%	99-100%
Source of Zygosity Discordance**	NA	NA	NA	NA	NA	Not reported, but at least 4 samples (1%) were discordant on the first test, and 1 (0.26%) remained discordant on repeat testing.
Did Repeating Resolve the Discordance?**	NA	NA	NA	NA	NA	Yes, but not all

Table 2. Analytic validity of testing for the FVL mutation (continued)

Experimental Test	Rolling Circle Amplification Assay Using Open Circle Probes Ligation	High-Resolution Melting Analysis of Small Amplicon	Pyro-sequencing	READIT SYSTEM (Pyrophosphoro-lysis)	ELISA	Reverse Allele-Specific Oligonucleotide (ASO) Hybridization Assay	First Nucleotide Change Technology
Reference Standard	**PCR-RFLP**	**Light Cycler**	**LightCycler or Direct Sequencing**	**PCR-RFLP or Not Specified**	**Direct Sequencing or PCR-RFLP**	**PCR-RFLP**	**PCR-RFLP**
Studies	Alsmadi, 2003[83]	Liew, 2004[76]	Verri, 2005[77]	Tsongalis, 2001[84] Rhodes, 2001[59]	Lopez, 2007[66] Carmi, 2004[85]	Kowalski, 2000[58] Leyte, 2000[86]	Pecheniuk, 2000[46]
Total Population*	216	104	100	280 + 510	264 + 284	256 + 99	500
Concordance	99.4%	100%	100%	99-100%	95-100%	100%	100%
Source of Zygosity Discordance**	Not reported	NA	NA	Unclear (in Tsongalis et al., one discordant result was due to an equivocal relative light unit, while the other was indeterminate); NA[59]	No explanation was given as to the source of the discrepancies in Lopez et al.; NA[85]	NA	NA
Did Repeating Resolve the Discordance?**	Yes (if tests were performed in triplicate).	NA	NA	Yes[84]; NA[59]	Yes	NA	NA

Table 2. Analytic validity of testing for the FVL mutation (continued)

Experimental Test	Linked Linear Amplification - ASO Capture	Nanochip Electronic Microarray	Photo-Cross-Linking Oligonucleotide Hybridization Assay	MGB-NFQ Probes (7700 SDS)	Single- and Dual-Labeled HyBeacon Probes
Reference Standard	**PCR-RFLP & PCR-ASO**	**PCR-RFLP, Light Cycler, Invader Monoplex Assay**	**PCR-Based Assay**	**FRET + Melting Curve Analysis (LightCycler)**	**RFLP or the Roche FVL Hybridization Probe Kit**
Studies	Reyes, 2001[78]	Schrijver, 2003[87] Erali, 2003[88] Evans, 2002[89]	French, 2004[90]	Castley, 2005[79]	French, 2008[91]
Total Population	111	197 for the comparison to RFLP and 195 + 224 + 758 for the comparison to LightCycler	1054	151	> 500
Concordance	100%	98-100%	98.6%	100%	100%
Source of Zygosity Discordance*	NA	NA[87 88]; In Evans et al., the reference test did not correctly identify 17 samples (details as in footnote).	2 false-negatives: For one, the reference indicated homozygous, but the experimental reported heterozygous. For the other, the reference indicated heterozygous, but the experimental test indicated WT. Repeat testing confirmed the results of the reference standard for both. False-positives: Reference indicatedWT, but the experimental test reported heterozygous initially. Repeat testing confirmed WT.	NA	n/a
Did Repeating Resolve the Discordance?*	NA	NA[87 88]; No[89]	Yes; 15 samples were discordant. All 15 samples were genotyped correctly (i.e., 100% concordance) upon retesting (reextraction of genomic DNA; resolution of the indeterminate, invalid, and discordant samples).	NA	n/a

* the order of the counts corresponds to the order of the studies in the row above;

**if <100% concordance ASO = allele-specific oligonucleotide; AS-PCR = allele-specific PCR; DNA = deoxyribonucleic acid; ELISA = enzyme-linked immunosorbent assay; FRET = fluorescence resonance energy transfer; FVL = Factor V Leiden; MALDI-TOF = matrix-assisted laser desorption/ionization – time of flight; MGB-NFQ = minor groove–binding non-fluorescent quencher; NA = not applicable; NR = not reported; PCR = polymerase chain reaction; READIT = reversed enzyme activity DNA interrogation test; RFLP = restriction fragment length polymorphism; SDS = sequence detection system; TGCE = temperature gradient capillary electrophoresis; WT = wild-type

Table 3. Details of studies on the analytic validity of testing for the FVL mutation

Study(s), Year(s)	Description
Hessner, 2000[43]	Seven samples (0.5%) were classified as equivocal; upon retesting, they were correctly typed as WT/WT (1691GG). Also, 16 samples (1.2%) generated invalid results because of unacceptable signal strength. These 16 samples were repeated from the same DNA preparations, and all were successfully typed as WT/WT.
Louis, 2004[62]	The aim of the study was to assess the cost-effectiveness and specificity of a high-throughput real-time PCR assay based on allele-specific fluorescent oligonucleotides that contain a 3' minor groove binding (MGB) probe.
Benson, 2001[47]	6295 adult subjects from a large managed-care population in Southern California were analyzed for FVL and the R2 allele; a discrepancy rate of 0% was found on duplicate testing for all results different from WT among the first 1000 samples; the first 100 heterozygous/homozygous samples were also analyzed by the traditional method involving restriction digestion followed by GeneScan analysis, with complete agreement between both analyses.
Murphy, 2003[61]	A low PCR product peak height on initial TGCE analysis was the source of the false homozygous result noted above. The heterozygosity was confirmed by repeat testing using both methodologies. The false WT/Leiden involved a G->A mutation 95 bases downstream of the Leiden site. Thus, this sample was WT/WT for Leiden allele but contained a new polymorphism in close proximity to Leiden position that caused the sample to be reported as heterozygous by TGCE.
Huang, 2002[73]	A method to identify SNP alterations by combining hairpin-forming DNA probes and electrochemical detection of sandwich DNA hybridization was reported. The results obtained using electrochemical detection of mismatches in nucleic acids (EDEMNA) agreed completely with the results determined independently at the UCLA Diagnostic Molecular Pathology Laboratory.
Hung, 2002[50]	A matrix-assisted laser desorption/ionization time-of-flight (MALDI/TOF) method was used. Blood samples were collected from 11 healthy controls and 16 patients with activated protein C resistance. The genotype of all 12 heterozygous and 4 homozygous FVL individuals was not known to the MALDI-TOF group before genotyping of all samples was completed.
Humeny, 2001[55]	Samples: Women from the Obstetrics and Gynecology outpatient clinic who presented with various endocrine problems.
Potter, 2001[49]	Detection of FVL w/lanthanide-labeled probe: an assay using oligonucleotide probes for normal and mutant sequences, labeled with europium and samarium, respectively, and measured by time-resolved fluorescence.
Verri, 2005[77]	Failure to make a genotype call at the first attempt was infrequent (about 5%) and was mostly attributable to insufficient signal-to-noise ratios caused by poor PCR amplification.
Tsongalis, 2001[84]	The Reversed Enzyme Activity DNA Interrogation Test (READIT) System was claimed to offer the following advantages: (1) rapid SNP analysis, (2) accuracy and precision, (3) cost effectiveness, (4) decreased turn-around times, (5) high throughput, and (6) excellent analysis software.
Rhodes, 2001[59]	Residual coded samples (n=510) from three independent laboratories were analyzed in a blinded format for mutations in the Factor V gene using the READIT Assay. A blinded retrospective analysis of the FVL mutation generated by independent laboratories was used to determine the concordance.

Table 3. Details of studies on the analytic validity of testing for the FVL mutation (continued)

Study(s), Year(s)	Description
Reyes, 2001[78]	A simple LLA model might not hold true for all situations (e.g., degradation of downstream primer extension products might not be complete at each cycle because of premature termination of primer extension or <100% efficiency of the polymerase 5'-3' exonuclease activity. Results of a simulated amplicon contamination experiment showed that LLA carryover amplification efficiency was substantially lower than that seen in PCR. In practical terms, this suggests that LLA is less susceptible than PCR to false-positive results that are attributable to amplicon contamination.
Schrijver, 2003[87]	Some of the samples failed to amplify with the NanoChip system. In some of these cases, the authors were unable to re-run the analysis because there was no sample remaining. The others failed re-amplification on the NanoChip System.
Evans, 2002[89]	Of the 635 samples classified by the Third Wave Assay (Invader Monoplex Assay) as FV WT, 10 were identified as heterozygous FVL by the NanoChip technique. Similarly, of the 114 putative heterozygous samples, 4 were identified as WT by the NanoChip technique. Of the 9 reported homozygous samples, 6 were homozygous, 2 were heterozygous, and 1 was FV WT by the NanoChip assay. All 17 results that were discordant with the Third Wave analysis were confirmed by DNA sequencing to be correctly classified by the NanoChip technology. The Nanochip system was 100% accurate in characterizing WT/WT, heterozygous, and homozygous samples, as compared to accuracies of 99.2%, 90.2%, and 100% for the comparable Third Wave analyses.
Castley, 2005[79]	MGB-NFQ: minor groove–binding nonfluorescent quencher; 7700 SDS: ABI PrismTM 7700 sequence detection system.
Mammo, 2006[69] El Housni, 2003[80] Verri, 2005[77] Pecheniuk, 2000[46]	Samples reported were FVL and prothrombin G20210A combined.

DNA = deoxyribonucleic acid; EDEMNA = electrochemical detection of mismatches in nucleic acids; FVL = Factor V Leiden; LLA = linked linear amplification; MALDI-TOF = matrix-assisted laser desorption/ionization – time of flight; MGB-NFQ = minor groove–binding non-fluorescent quencher; PCR = polymerase chain reaction; READIT = Reversed Enzyme Activity DNA Interrogation Test; SDS = sequence detection system; SNP = single nucleotide polymorphism; SSCP = single strand conformation polymorphism; TGCE = temperature gradient capillary electrophoresis; UCLA = University of California at Los Angeles; WT = wild-type.

Outliers were the results reported by Ozkan et al.[65] and by Lopez et al.,[66] which detected FVL using electrochemical and enzyme-linked immunosorbent assay (ELISA) methods, respectively. In many studies, the discordances resolved after the tests were repeated. We describe the studies in detail below.

Detection of FVL

Forty-one studies compared at least two methods for FVL detection (Tables 2 and 3; Appendix G, Evidence Table 2). Three studies compared flap endonuclease combined with fluorescence resonance energy transfer (FRET/invader assay) to the reference standard.[43 44 67] The concordance ranged from 99.5 percent to 100 percent. Five samples were typed as wild-type by the reference standard but were initially found to be heterozygous by the invader assay. Retyping of these samples by both methods was concordant, with all genotypes being unequivocally wild-type. In Ledford et al.,[44] two samples were discordant with the PCR-RFLP method. Repeating both the invader and PCR-RFLP testing yielded results concordant with the original PCR-RFLP results. In one case, in which a heterozygous sample was called homozygous mutant by the invader assay, the discrepancy was due to a failure to add genomic DNA to the well. In the second case, a heterozygous sample was called wild-type, and there was no obvious cause for the discrepancy. The source of discordance was reported to be technical or operator errors.

Five studies compared FRET combined with melting curve analysis (Light Cycler Assay) to the reference standard.[45 48 68-70] The concordance was 100 percent for all four studies.

Three studies compared Taqman real time polymerase chain reaction (PCR) to PCR-RFLP.[47 62 71] The concordance was again 100 percent in all these studies.

In three studies, electrochemical genosensors were used to detect the FVL mutation in a research, rather than a clinical setting.[65 72 73] The concordance ranged from 93 percent to 100 percent. In the study by Ozkan et al., the source and nature of the 7 percent discordance were not explained.[65] The study by Ozsos et al. reported neither the reference standard nor quantitative results.[72] Rather, the authors stated that there was "good agreement" between the experimental and reference methods and that efforts to achieve 100 percent accuracy were underway**.** Huang et al. did not report the sample size or specify the reference standard.[73]

Murphy et al., in a study with more than 300 samples, used temperature gradient capillary electrophoresis (TGCE) technology to detect FVL.[61] One heterozygous sample was falsely identified as being homozygous by TGCE but was corrected in repeat testing. A low PCR product peak height on the initial capillary electrophoresis analysis was identified as the source of the false homozygous result. One wild-type sample was falsely reported as being heterozygous by TGCE. Repeating the tests did not resolve this inconsistency. Further investigation revealed that the false heterozygous mutation involved a G →A mutation 95 base pairs downstream of the Leiden site. Thus, this sample was really wild-type for the Leiden allele but contained a new polymorphism in close enough proximity to the Leiden position that TGCE detected it as a heterozygous mutant.

Two small studies used mass spectrometry to detect FVL and reported 100 percent concordance.[50 55] There are several single studies, each of which compared a research-based experimental method to the reference standard and reported a concordance of 100 percent.[46 64 74-79] Three studies used a fluorophore probe to detect FVL,[80-82] and all three reported 100 percent concordance. Potter et al. tested a lanthanide-labeled probe for FVL detection.[49] There was a 1

percent discordance on the first test, and 0.26 percent remained discordant after repeating the test. Alsmadi et al. tested the rolling circle amplification assay using open circle probes ligation and reported a 99.4 percent concordance rate with PCR-RFLP.[83]

Taking advantage of pyrophosphorolysis chemistry, Tsongalis[84] and Rhodes[59] used the Reversed Enzyme Activity DNA Interrogation Test (READIT) System for FVL detection.[59 84] The concordance rate was greater than 99 percent when compared to the reference standard. Tsongalis et al. reported one discordant result that was due to an equivocal relative light unit and another that was indeterminate.[84]

Two studies used ELISA to detect FVL:[66 85] Carmi et al.[85] reported 100 percent concordance with the reference method. The initial concordance in the other study was just 95 percent.[66] No explanation was given regarding the source or nature of the discrepancies. Upon repeat testing, the discordance resolved.

Three studies compared nanochip electronic microarray technology to various reference standards for the detection of FVL.[87-89] Two reported 100 percent concordance rates.[87 88] Interestingly, in the study by Evans et al., of the 635 samples classified by the Third Wave Assay (Invader Monoplex Assay) as wild-type, 10 were identified as heterozygous FVL by the NanoChip technique.[89] Similarly, of the 114 putative heterozygous samples, 4 were identified as wild-type by the NanoChip technique. Of the 9 reported homozygous samples, 6 were read as homozygous, 2 as heterozygous, and 1 as wild-type by the NanoChip assay. All 17 results that were discordant were confirmed by DNA sequencing to have been correctly classified by the NanoChip technology. Compared to DNA sequencing, the NanoChip system was 100 percent accurate in characterizing wild-type, heterozygous, and homozygous samples, as compared to accuracies of 99.2, 90.2, and 100 percent for the comparable Third Wave analyses.

Detection of Prothrombin G20210A

The concordance rates between the experimental methods and the reference standards for the detection of prothrombin G20210A were 100 percent in nearly all of the 23 studies (Tables 4 and 5, Appendix G, Evidence Table 3). Three studies had some initial discordance between ELISA and direct sequencing or PCR-RFLP (0 to 2 percent), which upon repetition completely resolved.[54 66 85] Alsmadi et al. and Pecheniuk et al. reported concordance rates of 99.5 and 99 percent, respectively.[46 83]

Simultaneous Detection of FVL and Prothrombin G20210A Mutations

All 12 studies that employed multiplex technologies for the simultaneous detection of the two mutations reported 100 percent concordance between these technologies and the matched reference standard (Tables 6 and 7; Appendix G, Evidence Table 4).[51 56 86 92-100]

External Quality Assurance

We identified three studies that addressed external quality assurance or laboratory performance relative to the gold standards.[63 101 102] Studies assessing quality assurance that were published before the year 2000 were excluded by our search strategy, including that by Lutz et al.[103]

Table 4. Analytic validity of testing for prothrombin G20210A

Experimental Test	PCR-RFLP	Flap Endonuclease + FRET (Invader Assay)	FRET + Melting Curve Analysis (Light Cycler)	Taqman Real-Time PCR Assay	Electro-chemical Detection	Single Strand Conformation Polymorphism (SSCP) and Denaturing Gradient Gel Electrophoresis (DGGE)	MALDI-TOF Mass Spectrometry
Reference Standard	**PCR-RFLP**	**PCR-RFLP or AS-PCR**	**PCR or PCR-RFLP**	**PCR-RFLP**	**PCR-RFLP**	**Direct DNA Sequencing**	**Sequencing**
Studies (ref. no.)	Bravo-Osorio, 2000[104]	Hessner, 2004[105] Patnaik, 2004[67]	Schroell-Metzger, 2003[68] Cooper, 2003[70] Nauck, 2000[45] Parks, 2001[48]	Louis, 2004[62] Happich, 2000[106] Behrens, 2004[71]	Gellings, 2001[53]	Meyer, 2000[52]	Humeny, 2001[55]
Total Population*	98	522 + 377	132 + 200 + 110 + 157	122 + 233 + 100	12	527	11
Concordance	100%	100%	100%	100%	100%	100% (based on 8 samples)	100%
Source of Zygosity Discordance**	NA	NA	NA	NA	NA	NA	NA
Did Repeating Resolve the Discordance?**	NA	NA	NA	NA	NA	NA	NA

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Table 4. Analytic validity of testing for prothrombin G20210A (continued)

Experimental Test	SASA-PCR Followed by RFLP	ELISA	Rolling Circle Amplification Assay Using Open Circle Probes Ligation	First Nucleotide Change Technology	Nanochip Electronic Microarray	High-Resolution Melting Analysis of Small Amplicon	MGB-NFQ Probes (7700 SDS)
Reference Standard	**PCR-RFLP**	**Direct Sequencing or PCR-RFLP**	**PCR-RFLP**	**PCR-RFLP**	**PCR-RFLP & Light Cycler**	**Light Cycler**	**FRET + Melting Curve Analysis (LightCycler)**
Studies	DelRio-LaFreniere, 2001[64]	Lopez, 2007[66] Gilchrist, 2001[54] Carmi, 2004[85]	Alsmadi, 2003[83]	Pecheniuk, 2000[46]	Schrijver, 2003[87] Erali, 2003[88]	Liew, 2004[76]	Castley, 2005[79]
Total Population*	50	122 + 459 + 283	298	500	199 for the comparison to RFLP and 198 + 224 for the comparison to LightCycler	22	310
Concordance	100%	98-100%	99.5%	99%	100%	100%	100%
Source of Zygosity Discordance**	NA	No explanation of discrepancies in Lopez.[54] Sensitivity was 100% with no false negatives, specificity was 99.2% with 2 false positives; 2 samples corrected on second attempt.	Not reported	No explanation was given on the source of discrepancies.	NA		NA
Did Repeating Resolve the Discordance?**	NA	Yes[66 85], NA[54]	Yes (If tests were performed in triplicate).	Unclear: 1% misclassification was in accordance with 2.2% error noted when establishing the cut-off criteria.	NA		NA

* the order of the counts corresponds to the order of studies in the row above; **if <100% concordance

AS-PCR = allele-specific PCR; DGGE = denaturing gradient gel electrophoresis; DNA = deoxyribonucleic acid; ELISA = enzyme-linked immunosorbent assay; FRET = fluorescence resonance energy transfer; MALDI-TOF = matrix-assisted laser desorption/ionization – time of flight; MGB-NFQ = minor groove–binding non-fluorescent quencher; NA = not applicable; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; SASA-PCR = simultaneous allele-specific amplification PCR; SDS = sequence detection system; SSCP = single strand conformation polymorphism

Table 5. Details of studies on analytic validity of testing for prothrombin G20210A

Study(s), Year(s)	Description
Bravo-Osorio, 2000[104]	Since control of digestion is of importance to avoid false results in RFLP, this study described a method for detecting prothrombin G20210A and methylenetetrahydrofolate reductase (MTHFR) mutations, in which a second restriction site was introduced in order to control the enzyme digestion of the respective PCR products. There were 49 individuals with either a prothrombinG20210A or MTHFR mutation, and 49 individuals with neither mutation as negative controls. Those performing the tests were aware of the results.
Patnaik, 2004[67]	AS-PCR reference standard mistyped 14 samples that the invader experimental assay typed correctly.
Louis, 2004[62]	The aim of the study was to assess the cost-effectiveness and specificity of a high-throughput real-time PCR assay based on allele-specific fluorescent oligonucleotides that contain a 3' minor groove binding (MGB) probe.
Happich, 2000[106]	Samples of 233 individuals previously genotyped for PT-G20210A and MTHFR by restriction fragment analysis were re-analyzed by the 5'nuclease fluorescence assay (Taqman).
Gellings, 2001[53]	Genotype was determined based on the electrochemiluminescence signal generated by the ruthenium complex from the 5' primer.
Meyer, 2000[52]	A series of 383 patients with thrombosis and 144 controls were tested with SSCP and DGGE. Eight of the normal samples, the heterozygous samples, and the homozygous samples were tested with direct DNA sequencing, and the results were confirmed. The prevalence of prothrombin mutation in thrombotic and healthy patients was reported.
Humeny, 2001[55]	Sample: women from the Obstetrics and Gynecology outpatient clinic who presented with various endocrine problems.
Gilchrist, 2001[54]	Only 100 patients were tested with RFLP, although 459 patients were tested with microparticle enzyme immunoassay (MEIA).
Pecheniuk, 2000[46]	Samples reported FVL and prothrombin G20210A combined.
Erali, 2003[88]	Two prothrombin samples were identified as containing mutations at sites other than position 20210. Both were WT at 20210, but one was heterozygous at A20218G and the other at C20209T. Both were correctly identified as WT G20210 in the NanoChip system, but the fluorescence signal for the WT probe was approximately one-half of the signal for the WT control sample. The most likely explanation for the lower signal is that the WT probe was binding only to the exact WT strand and did not bind to the strand with the A20218G mutation. The mutant probe did not bind to either strand.
Castley, 2005[79]	The goal of the study was to show that whole-blood PCR after formamide addition is as good as DNA-extracted PCR (DNA EXTRACTION). There were two comparisons: (1) whole blood vs. DNA-extracted real-time detection (RTD) PCR comparison (concordance results in Tables 2 and 3 of online Data Supplement [not available in this program]). (2) Whole-blood comparison between RTD-PCR and SDS. MGB-NFQ: minor groove–binding nonfluorescent quencher, 7700 SDS: ABI PrismTM 7700 sequence detection system.

AS-PCR = allele-specific PCR; DGGE = denaturing gradient gel electrophoresis; DNA = deoxyribonucleic acid; FVL = Factor V Leiden; MEIA = microparticle enzyme immunoassay; MGB-NFQ = minor groove–binding non-fluorescent quencher; MTHFR = methylenetetrahydrofolate reductase; PCR = polymerase chain reaction; PT-G20210A = prothrombin G20210A mutation; RFLP = restriction fragment length polymorphism; RTD = real-time detection; ; SDS = sequence detection system; SSCP = single strand conformation polymorphism; WT = wild-type.

42

Table 6. Analytic validity of simultaneous detection (multiplex) of FVL and prothrombin G20210A mutations

Experimental Test	FRET + melting Curve Analysis (Light Cycler)	Mutagenically Separated PCR Followed by Gel Electrophoresis	Multiplex PCR-RFLP	Multiplex PCR – Reverse Hybridization Line Probe Assay	Multiplex AS-PCR - Ion-Pair Reversed Phase HPLC	Multiplex PCR with ASO Hybridization Using the 5' Nuclease Assay
Reference Standard	**PCR-RFLP**	**PCR-RFLP**	**PCR-RFLP or Light Cycler**	**PCR-RFLP**	**PCR-RFLP**	**PCR-RFLP**
Studies (ref. no.)	van den Bergh, 2000[92] Hobson-Peters, 2005[93] Ameziane, 2003[94]	Endler, 2001[56]	Baris, 2004[95] Angeline, 2005[51] Huber, 2000[96] Lucotte, 2003[97] Koksal, 2007[57]	Leyte, 2000[86]	Nietzel, 2003[99]	Ugozzoli, 2004[100]
Total Population*	47 + 43 + 200	153	408 + 72 + 205 + 175 + 104	99	> 100	52
Concordance	100%	100%	100%	100%	100%	100%
Source of Zygosity Discordance**	NA	NA	NA	NA	NA	NA
Did Repeating Resolve the Discordance?**	NA	NA	NA	NA	NA	NA

* The order of the counts corresponds to the order of studies in the row above; **if <100% concordance

ASO = allele-specific oligonucleotide; AS-PCR = allele-specific PCR; FRET = fluorescence resonance energy transfer; HPLC = high performance (pressure) liquid chromatography; NA = not applicable; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism

Table 7. Details of studies on the analytic validity of simultaneous detection (multiplex) of FVL and prothrombin G20210A mutations

Study(s), Year(s)	Description
Hobson-Peters, 2005[93]	Used FRET with a dual-probe quenching system that is similar to the Light Cycler assay.
Endler, 2001[56]	70 known patients with recurrent venous thrombosis and 83 selected healthy controls from the Austrian Study of Recurrent VTE for whom previous determination of the genotypes for the FV:R506Q (G1691A), the FII:G20210A, and MTHFR:A223V (C677T) variants had been established by PCR followed by RFLP
Baris, 2004[95]	No reference method was reported. For concordance, known patients with recurrent venous thrombosis with "previously determined" genotypes for FVL and prothrombin G20210A were used. FVL was amplified significantly less than prothrombinG20210A, with an initial primer concentration of 2:1. The effects of various factors, including magnesium cation concentration, primer concentration, and PCR cycling conditions, on PCR specificity and efficiency were determined and optimized in this study.
Huber, 2000[96]	Concordance was only tested on FVL. For prothrombin, the multiplex PCR-RFLP identified 9 heterozygous and 196 wild-type samples. To independently verify prothrombin results, DNA sequencing confirmed 9 heterozygous mutant and 11 wild-type samples (10% of the sample). The study also genotyped 123 samples with multiplex PCR-RFLP and the invader assay for Factor II, with 100% concordance.
Lucotte, 2003[97]	Duplex PCR-RFLP for simultaneous detection of FVL and prothrombin G20210A was used.
Koksal, 2007[57]	Significantly less amplification was noted for FV relative to prothrombin; the FV primer was therefore 2x concentrated relative to prothrombin.

DNA = deoxyribonucleic acid; FII = Factor II; FV = Factor V; FRET = fluorescence resonance energy transfer; FVL = Factor V Leiden; MTHFR = methylenetetrahydrofolate reductase; PCR = polymerase chain reaction; RFLP = restriction fragment length polymorphism; VTE = venous thromboembolism

Jennings et al have described the results of the United Kingdom National External Quality Assessment Scheme (UK NEQAS) Thrombophilia Screening Program.[101] Two hundred eighty centers participated in the thrombophilia screening exercises. This group reported the details of their first three evaluation exercises in an earlier publication by Preston et al.[107] They described an important error rate in 6 years of UK NEQAS experience.[107] Among centers performing genetic analysis for FVL and the prothrombin mutation, an error rate of 3 to 6 percent was identified, with both transcription and analytical errors being observed. For example, a staff member transcribing the wrong genotype in preparing a report of the results was considered a transcription error; misinterpretation of the results of the testing procedure was an analytic error.

Hertzberg et.al. have reported the results of The Royal College of Pathologists of Australasia's external quality assurance program.[63] This program sent 133 DNA samples with known mutations to laboratories in 10 separate surveys. For the 3,799 responses received, the overall successful identification rate was 98.63 percent; the poorest individual sample result was a 15 percent incorrect identification of a homozygous FVL sample. Success rates in identifying specific mutations were 98.13 percent for FVL and 98.84 percent for prothrombin G20210A. Of the 39 responding laboratories, 20 (51 percent) made at least one error. Importantly, 3 of the 39 laboratories were responsible for 46 percent of all errors.

Finally, Tripodi et al.[102] confirmed the findings of Preston et al.[107] described above. Their survey was organized by the Subcommittee on Hemostasis of the Italian Committee for Standardization of Laboratory Tests (CISMEL). They sent four samples with known genotypes to 52 participating laboratories and received 41 responses. One laboratory misidentified a heterozygous FVL as a wild-type specimen, one misidentified a homozygote as a heterozygote, and one misidentified a heterozygote as a homozygote. Eight samples were given no interpretation. Similarly, one wild-type prothrombin G21210A was called a heterozygote, four heterozygotes were misclassified, and one homozygote was called heterozygote, with 10 being uninterpreted. The authors concluded that regular quality control programs aimed at identifying causes of failure are warranted.

Summary of the Evidence for Key Question 2

- There was high-grade evidence that tests for the detection of FVL have excellent analytic validity.
- There was high-grade evidence that tests for the detection of prothrombin G20210A have excellent analytic validity.
- There was high-grade evidence that most, but not all, clinical laboratories can test for FVL and prothrombin G20210A very accurately. There may be some laboratories that, for technical or administrative reasons, report inaccurate results.

Key Question 3:
Clinical Validity of Testing for FVL and/or Prothrombin G202010A

The next section of evidence describes the clinical validity of testing for these mutations. While Key Question 2 asked whether the mutations *could be* detected, Key Question 3 asked whether the detection of the mutations could predict patient-relevant outcomes (i.e., thrombosis).

FVL and Recurrent Venous Thrombosis in Probands

We identified 22 articles that examined the rates of recurrent VTE in individuals with a history of thrombosis (the probands) and the FVL mutation (Table 8 and Appendix G, Evidence Tables 5-12). Eighteen were conducted in Europe[14 19 108-123] and four in North America.[124-127] As we required, all studies followed patients prospectively for the occurrence of VTE and objectively diagnosed venous thrombosis with radiographic testing. Most were prospective cohort studies, but six were cohorts nested within randomized controlled trials. Of these, five tested anticoagulation management strategies[116 119 123-125] and one tested the effect of hormone replacement on acquired activated protein C resistance.[114] Two articles were derived from the same cohort (the Physicians' Health Study).[126 127] Two other studies came from a cohort of Austrian patients.[14 112]Total sample sizes for the studies ranged from 30 to 953 genotyped probands. Ages were variable; one study targeted patients under 40 years old.[117] Follow-up ranged from a median of 0.5 years to over 8 years.

Given our selective criteria for article inclusion, the study quality was generally high (Appendix G, Evidence Table 5). Some studies did not have a primary objective of quantifying the rates of thrombosis recurrence according to mutation status. Specifically, nine articles had different objectives, including evaluation of the prognostic value of other variables (such as D-dimer levels, positive family history of thrombosis, or sex),[14 19 115 118] assessing anticoagulation strategies,[123 124] examining the natural history of treated upper-extremity thrombosis,[109] evaluating the relationship between mutation status and non-clinical hematologic variables,[121] or assessing the impact of post-menopausal hormone replacement on resistance to activated protein C.[114] Most studies described the outcomes separately for individuals who were homozygous and those who were heterozygous for FVL, but some combined homozygotes with heterozygotes when quantifying the number of recurrent events.[14 109-111 115 117 119 121 122] Two studies did not include an FVL mutation-free comparison group.[108 122]

The FVL mutation-free comparison groups varied in terms of whether other mutations or thrombophilic defects were included or excluded, as shown in Appendix G, Evidence Table 5. However, those studies that included individuals with other thrombotic defects in the control group generally also included them in the group of FVL-positive individuals. Many studies did not present descriptive data separately for individuals with and without FVL mutations. It was therefore not possible to confirm that those with and without the mutation were broadly similar. No study described interactions between FVL and other mutations (besides prothrombin G20210A). We saw no evidence of funding support that would have been likely to bias the results.

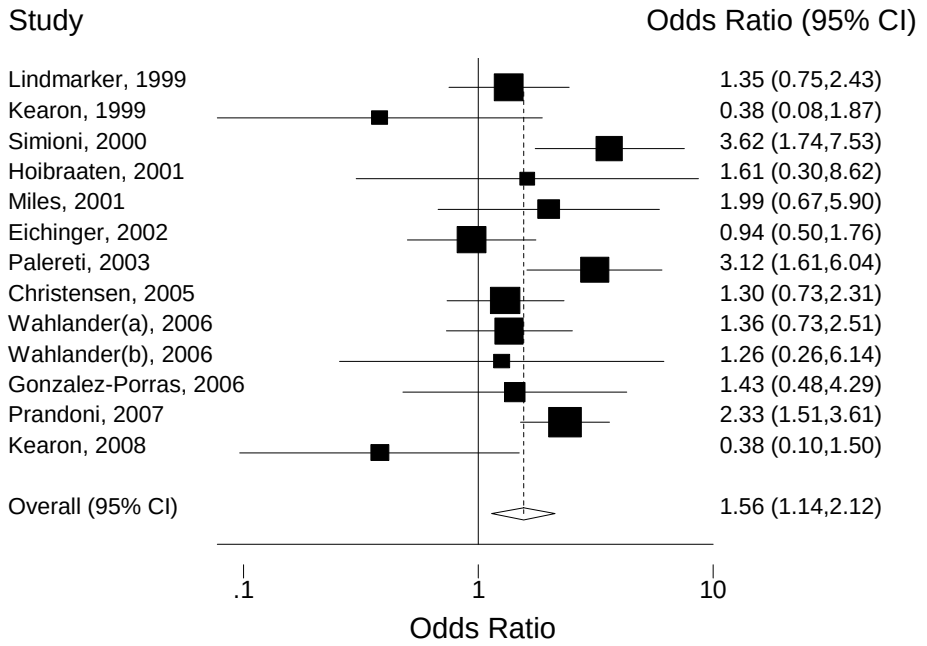
Table 8 Pooled results describing the risk of recurrent VTE for probands with VTE compared to probands without mutations

Mutation	Studies, N	Studies With Data Appropriate for the Pooled Odds Ratio, n	Individuals Contributing to the Pooled Odds Ratio*, n	Pooled Odds Ratio† (95% CI)
Heterozygous FVL	14	13‡	4730	1.56 (1.14-2.12)
Homozygous FVL	9‡	8‡	2382	2.65 (1.18-5.97)
Unspecified FVL§	8	3	362	1.56 (0.75-3.25)
Double Heterozygous FVL and PT 20210A	4	3	843	4.81 (0.50-46)
Heterozygous PT 20210A	11‡	10‡	3636	1.45 (0.96-2.21)
Mixed PT 20210A	7	4	1143	0.73 (0.37-1.44)
Homozygous PT 20210A	2	NA	NA	NA

*studies without comparison groups or having zero events in both arms are not included
†unadjusted odds ratios, relative to probands without mutations
‡the count includes both arms of a study that reported results separately for drug- versus placebo-treated patients
§unspecified means that heterozygous and homozygous individuals were not distinguished in the study
CI = confidence interval; FVL = Factor V Leiden; NA = not applicable; PT 20210A = prothrombin G20210A; VTE = venous thromboembolism

Heterozygous FVL in probands. Thirteen studies described event rates in probands who were heterozygous for FVL as well as probands without mutations (Table 9; Appendix G, Evidence Table 6).[19 111-114 116 118 120 123-127] Since two studies were derived from the same cohort,[126 127] we included only the more recent and larger of the two in our pooled analysis, so as to not count patients twice.[126] One of the studies presented data separately for two treatment arms (ximelagatran extended prophylaxis versus placebo) and presented adequate data to allow us to analyze these two arms separately; therefore, we counted these arms as two different studies.[123] In one article, the number of mutation-free patients was not given but could be calculated from percentages presented in the article; this calculation may have been subject to a small degree of rounding error.[118] The number of probands heterozygous for FVL in these studies ranged from 19 to 161 (median = 83). The number of probands without this mutation ranged from 55 to 724 (median = 204). There were a total of 979 heterozygous individuals who suffered a total of 161 recurrent thrombotic events, and a total of 3751 mutation-free individuals who suffered a total of 473 thrombotic events. The pooled odds ratio for recurrent thrombosis was 1.56 (95 percent CI, 1.14-2.12) for FVL heterozygous individuals when compared to patients without the mutation (Figure 3). There was a fair amount of heterogeneity (I^2 = 48 percent), but no significant evidence of publication bias. In our sensitivity analysis, in which we removed each study sequentially, the odds ratios were little changed. Annualized event rates in heterozygous individuals were reported in three studies and ranged from 3.1 to 7.5 percent.[108 113 127]

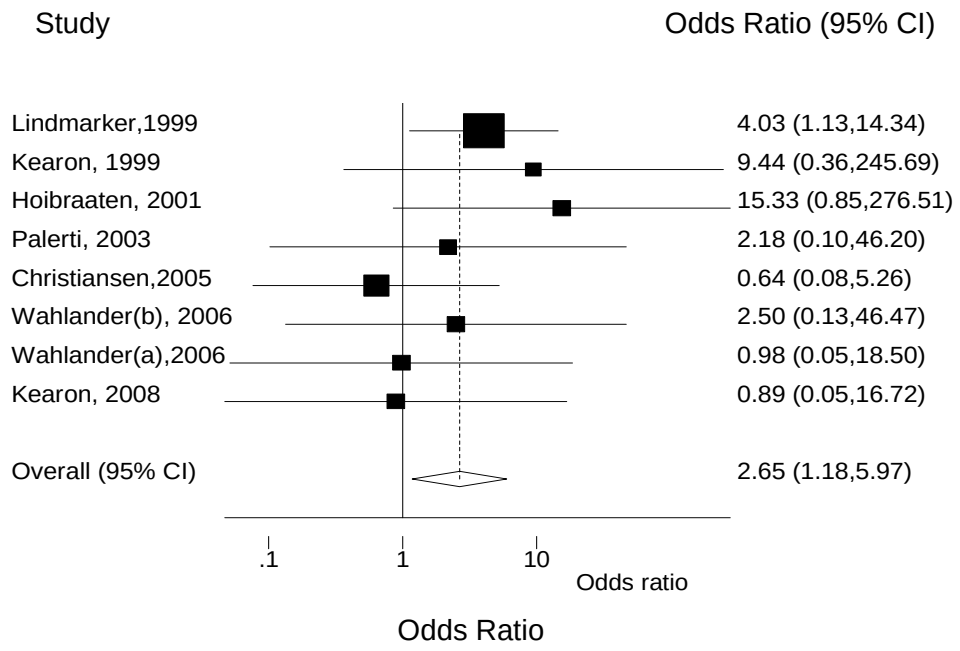
Figure 3. Odds ratios for VTE for probands with heterozygous FVL mutation, as compared to probands without the mutation*



*the size of the box is proportional to the study sample size; the confidence intervals (CI) for the pooled results are derived from a random effects model

Homozygous FVL in probands. Seven studies described event rates in probands homozygous for FVL and also in those without mutations (Table 5; Appendix G, Evidence Table 7).[19 111 114 116 123-125] One of the studies presented data separately for two treatment arms within one study (ximelagatran extended prophylaxis versus placebo), and we analyzed these arms separately as two different studies.[123] The number of FVL-homozygous probands in these studies ranged from 1 to 11 (median = 6). The number of FVL-negative probands ranged from 55 to 469 (median = 360). There were a total of 49 homozygotes who suffered a total of 7 recurrent thrombotic events, and a total of 2333 mutation-free controls who suffered a total of 225 thrombotic events. The pooled odds ratio was 2.65 (95 percent CI, 1.18-5.97) (Figure 4). There was no evidence of significant heterogeneity (I^2 = 0 percent). In our sensitivity analysis, in which we removed each study sequentially, the odds ratios changed little. One study specifically sought to determine whether FVL homozygosity carried a higher rate of recurrence than did FVL heterozygosity.[108] This study did not include a control group without FVL. In this study, 5 of 32 individuals homozygous for the mutation (16 percent) developed recurrent thrombosis, and 12 of 108 individuals with heterozygosity developed recurrence (11 percent). This difference was not

Figure 4. Odds ratios for VTE for probands with homozygous FVL mutation, as compared to probands without the mutation*



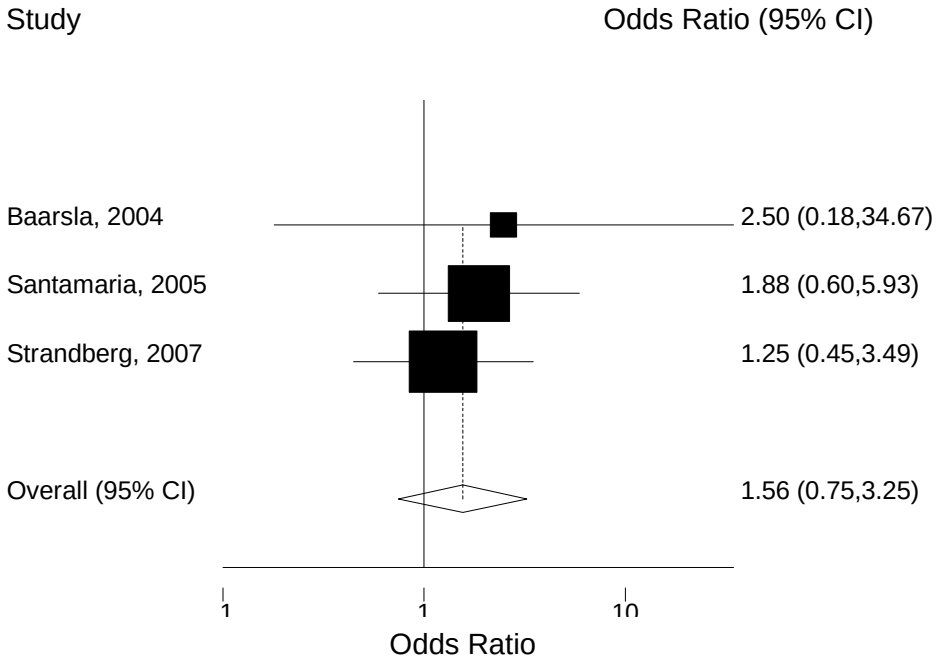
*the size of the box is proportional to the study sample size; the confidence intervals (CI) for the pooled results were derived from a random effects model

statistically significant, but the relative risk for recurrence was marginally significant after excluding from both groups those patients who also carried the prothrombin G20210A mutation (relative risk, 1.8; 95 percent CI, 1.0-6.2). The annualized event rate was reported for homozygotes in only one study, as 5.8 percent (95 percent CI, 4.9-7.4).[108]

Unspecified FVL in probands. The authors of eight articles combined the individuals who were heterozygous and those who were homozygous for FVL in their analyses,[14 109 110 115 117 119 121 122] without providing adequate detail in the text or tables to allow us to report the results separately for these individuals (Table 8; Appendix G, Evidence Table 8). Only three of these reported the number of events in the group with mutations as well as a group of probands without mutations, allowing for pooling.[109 119 121] The number of probands with FVL in these studies ranged from 3 to 50, and the number of mutation-free individuals ranged from 24 to 138. There were 68 individuals with FVL who suffered a total of 12 events. Among 294 control patients, there were 46 events. The pooled odds ratio was very similar to that for heterozygotes, at 1.56 (95 percent CI, 0.75-3.25) (Figure 5). There was no evidence of heterogeneity (I^2 = 0 percent). Among the studies that did not report the actual number of events, and therefore could not be included in our pooled analysis, four did test whether the FVL mutation was associated with recurrent thrombosis.[14 110 115 117] None of these individual analyses demonstrated that the mutation was a significant predictor of recurrent events. The reported hazard ratio (HR) or relative risk values ranged from 1.2-1.35. One study simply reported the results as non-significant without further quantification.[115]

We pooled these studies of unspecified zygosity with the studies of heterogeneous FVL, since we presumed that most of the unspecified individuals were indeed heterogeneous for the mutation. The pooled estimate from combining these 17 studies was an odds ratio of 1.61 (95 percent CI, 1.24 – 2.10), which is just slightly above the estimate for heterogenous FVL alone.

Figure 5. Odds ratios for VTE for probands with unspecified FVL mutations, as compared to probands without the mutation*



*the size of the box is proportional to the study sample size; the confidence intervals (CI) for the pooled results were derived from a random effects model

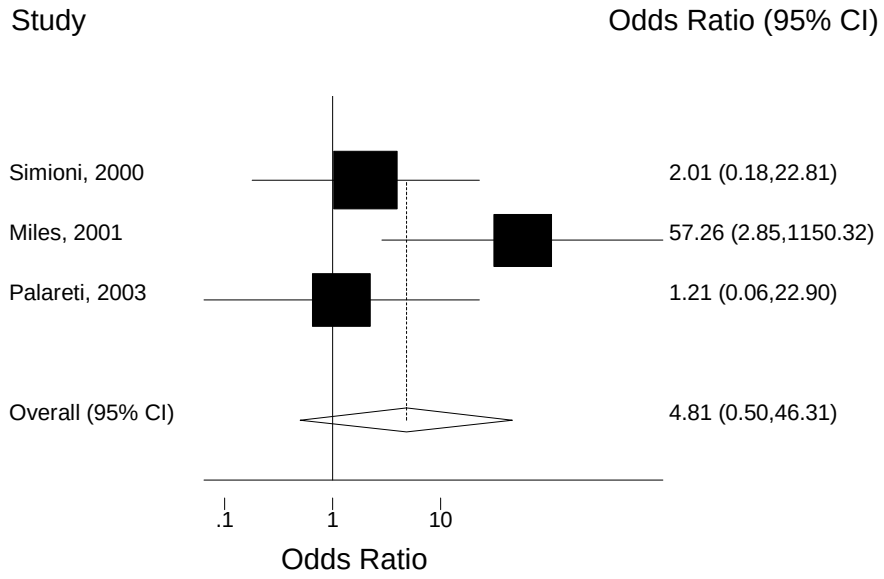
Individuals with an idiopathic index event. Patients with idiopathic events (unprovoked VTE) are often advised to take life-long anticoagulation. There is a great deal of interest in whether this patient population can be better stratified according to risk of recurrence. Among the studies that included only patients with idiopathic index thromboses[112 124 125 127] and the studies in which results were reported separately for individuals with idiopathic index events,[119 120] the pooled odds ratio for recurrence was 1.17 (95 percent CI, 0.63 - 2.18.) for individuals with FVL, as compared to individuals without. Only one of these studies was not exclusively composed of probands with heterogeneous FVL (the zygosity was unspecified).[119] A single study described recurrence rates for individuals with FVL who had a clearly provoked VTE (non-idiopathic VTE), with an odds ratio of 6.5 (95 percent CI, 2.5 –18).[120]

Double (or compound) heterozygous (FVL plus the prothrombin G20210A mutation) probands. Three articles presented event rates in individuals having a FVL mutation as well as a prothrombin G20210A mutation and in mutation-free control patients (Table 8; Appendix G, Evidence Table 9).[19 120 126] There were 10 doubly heterozygous individuals in all, 4 of whom suffered recurrent events. Among the mutation-free controls, there were 95 recurrent thromboses in 833 patients. The pooled odds ratio was 4.81 (95 percent CI, 0.50-46.3) (Figure 6). In one study, all three double heterozygotes developed recurrent thrombosis,[126] requiring a continuity

correction for calculating pooled odds ratios. The odds ratio was lower when this study was excluded during sensitivity testing analysis (odds ratio, 1.64; 95 percent CI, 0.25-11). Annual incidence rates were not reported in these studies.

Prothrombin G20210A and recurrent venous thrombosis in probands. We identified 18 articles that examined the rates of recurrent VTE in probands with the prothrombin G20210A mutation (Table 8; Appendix G, Evidence Table 10).[14 19 109-112 115-120 122-126 128] All of these studies were included in the 22 studies examining FVL (above), and therefore they generally shared common control groups with the FVL analyses. They also suffered from similar limitations, as discussed above in the section *FVL and recurrent venous thrombosis in probands*. These limitations included: a limited description of covariates, such as age and sex, in those with and without the mutation; combining individuals with

Figure 6. Odds ratios for VTE for probands who were doubly heterozygous for the FVL and prothrombin G20210A mutations, as compared to probands without mutations*



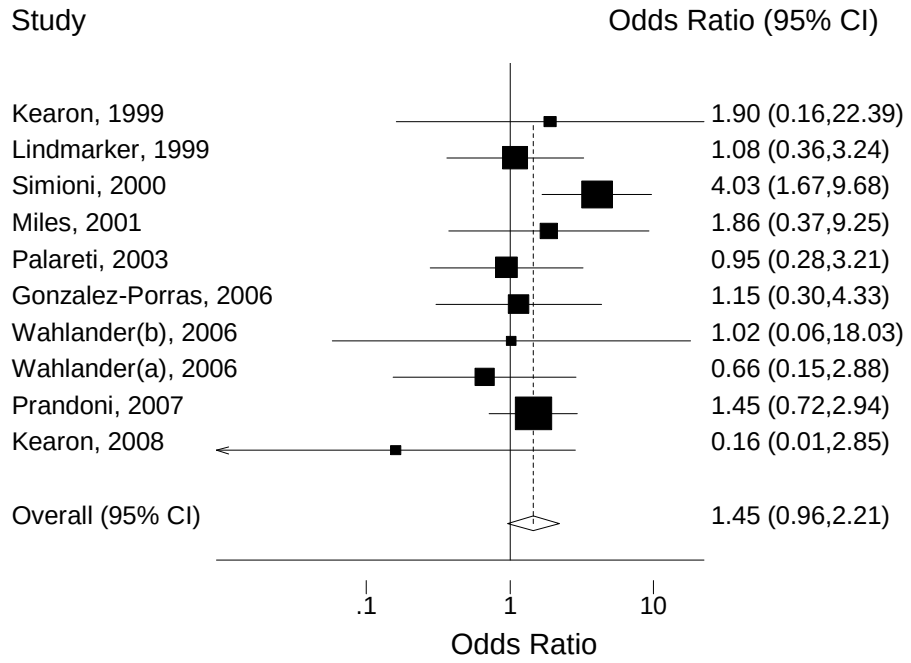
*the size of the box is proportional to the study sample size; the confidence intervals (CI) for the pooled results are derived from a random effects model

homozygous and heterozygous mutations when reporting results; and, in some studies, not specifying the exact number of thrombotic events in each arm in the case of those with and without the mutation.

Heterozyous prothrombin G20210A in probands. Nine articles quantified event rates in the probands who were heterozygous for prothrombin G20210A and also in mutation-free probands (Table 8; Appendix G, Evidence Table 11).[19 113 116 118 120 123-126] One of the studies presented data separately for two treatment arms (ximelagatran extended prophylaxis versus placebo), and we analyzed these two arms separately.[123] The number of probands with a heterozygous prothrombin G20210A mutation in these studies ranged from 3 to 58 (median = 26.5). The number of comparison individuals without the mutation ranged from 72 to 724 (median = 307). There were a total of 281 heterozygous individuals who suffered 38 recurrent thrombotic events,

and a total of 3355 mutation-free individuals who suffered 385 thrombotic events. The pooled odds ratio was 1.45 (95 percent CI, 0.96-2.21) (Figure 7). There was little evidence of heterogeneity (I^2 =8 percent), and no evidence of publication bias. In our sensitivity analysis, one study did have a major impact on the pooled odds ratio: This was the study that had an odds ratio of 4.0.[120] Removal of this study from the meta-analysis decreased the pooled odds ratio to 1.30. Annual recurrence rates were reported in two studies and were 0 and 2.9 percent.[113 125]

Figure 7. Odds ratios for VTE for probands who were heterozygous for prothrombin G20210A, as compared to probands without the mutation*



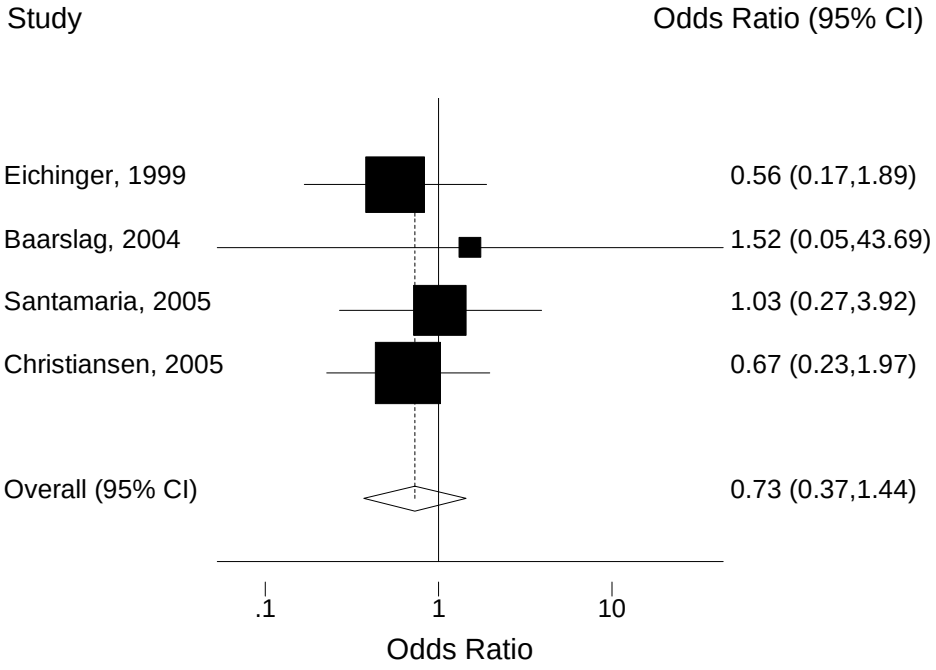
*the size of the box is proportional to the study sample size; the confidence intervals (CI) for the pooled results are derived from a random effects model

Unspecified prothrombin G20210A in probands. The authors of seven articles combined individuals who were heterozygous and those who were homozygous for prothrombin G20210A[14 109-111 115 119 128] (Table 8; Appendix G, Evidence Table X). Four of these articles described event rates in this combined prothrombin G20210A group as well as in a group of mutation-free probands.[109 111 119 128] In our pooled analysis, there were a total of 86 individuals with the prothrombin G20210A mutation who had 10 recurrent events, and 1057 patients without the mutation who suffered 158 recurrent events. The pooled odds ratio was 0.73 (95 percent CI, 0.37-1.44) (Figure 8). There was no evidence of significant heterogeneity (I^2 = 0 percent). Relative risk or HR values were reported in five of the studies[14 110 111 115 128]and ranged from 0.7 to 2.1. Of note, three of the studies that presented relative risk or HR were not included in the pooled analysis, since the specific number of events was not reported.[14 110 115] Two of these studies reported adjusted relative risks that were significantly different from 1.0, with adjusted

relative risk estimates of 2.1 in both cases.[14 115] The annual incident rate of recurrent thrombosis was reported in only one study, at 1.9 percent.[111]

Since probands with unspecified prothrombin G20210A mutations are almost certainly heterozygous for the mutation, given the rarity of homozygous prothrombin G202010A, we pooled these studies with the studies of probands known to be heterozygous. The combined odds ratio was 1.23 (95 percent CI, 0.87-1.7).

Figure 8. Odds ratios for VTE for probands with unspecified prothrombin G20210A mutation, as compared to probands without the mutation*



*The size of the box is proportional to the study sample size; the confidence intervals (CI) for the pooled results are derived from a random effects model

Homozygous prothrombin G20210A in probands. Only two articles quantified event rates in individuals homozygous for prothrombin G20210A and in mutation-free probands.[123 125] There were a total of only three homozygous individuals in these studies, and none of them developed recurrent thrombosis.

Summary of the Evidence for Key Question 3 (Probands)

Based upon our review of the published literature, we can summarize the evidence regarding the clinical validity of testing for FVL and prothrombin G20210A as follows:

- There was moderate-grade evidence that homozygosity for FVL in probands is predictive of recurrent VTE.

- There was moderate-grade evidence that heterozygosity for FVL in probands is predictive of recurrent VTE.

- Homozygosity for prothrombin G20210A is a rare genotype, and its association with recurrent venous thrombosis in probands is currently unknown, because of a lack of sufficient evidence.

- There was only low-grade evidence that heterozygosity for prothrombin G20201A in probands is not predictive of recurrent VTE.

- There is insufficient evidence to allow us to draw any conclusions about double heterozygosity (FVL and prothrombin G20210A) in probands.

FVL and Prothrombin G20210A and Venous Thrombosis in Family Members

We identified 18 articles that evaluated venous thrombosis in family members of probands. All were conducted in Europe, with the majority in the Netherlands or France (Table 9, Appendix G, Evidence Tables 13-17). Five studies were prospective or included a prospective component,[129-133] and the rest were retrospective cohort studies. One was a letter to the editor.[133] The smallest study enrolled 13 people from a single family;[134] the largest enrolled 1,093 family members.[26] Inclusion criteria were not always stated, except for requiring that subjects be family members of probands with VTE. Three of the five prospective studies had scheduled clinical surveillance for VTE events, either annually or biannually.[129 130 132] The individuals in the mutation-free comparison groups described below were generally the same in each of the comparisons within a given study. Only two studies explicitly excluded family members with prothrombin G20210A in their calculation of the odds ratio associated with FVL positivity.[26 130]

Table 9. Pooled results describing the risk of VTE for family members of probands with VTE, comparing those with a mutation to those without a mutation

Mutation	Studies, N	Studies With Data Appropriate for the Pooled Odds Ratio, n	Individuals Contributing to the Pooled Odds Ratio*, n	Pooled Odds Ratio† [95% CI]
Heterozygous FVL	9	6	2,091	3.5 [2.5 to 5.0]
Homozygous FVL	6	5	896	18 [7.8 to 40]
Mixed FVL	6	6	3831	2.9 [1.8 to 4.8]
Double Heterozygous FVL and PT 20210A	4	3	727	6.7 [2.9 to 15]
Mixed PT 20210A	1	NA	NA	NA
Homozygous PT 20210A	1	NA	NA	NA
Heterozygous PT 20210A	3	3	291	1.9 [0.35 to 10]

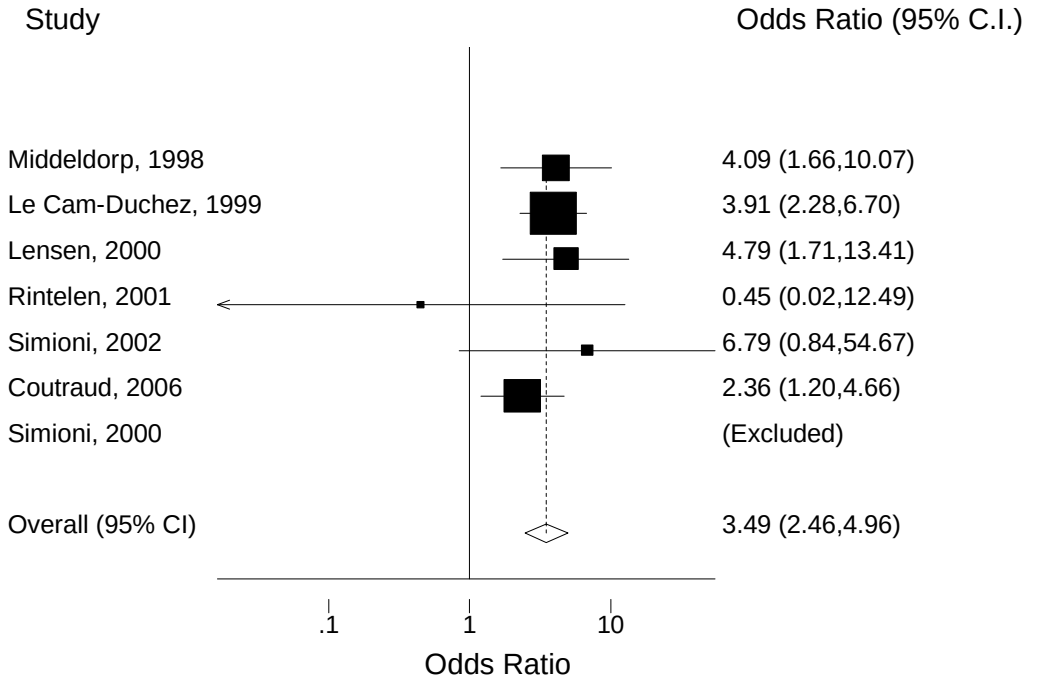
*Studies without comparison groups or having zero events in both arms not included
†Unadjusted odds ratios, relative to family members without mutations

CI = confidence interval; FVL = Factor V Leiden; NA = not applicable; PT 20210A = prothrombin G20210A; VTE = venous thromboembolism

Study quality was mixed (see Appendix G, Evidence Table 13). To some extent, it was challenging to assess the quality of these studies, since they were not necessarily designed to answer our question (e.g., the clinical validity question was secondary to their primary aims). In our quality assessment, we focused mostly on biases that might result from the sampling scheme. For the prospective studies, we also were attentive to the description of losses to follow-up and the percentage of participants lost; this was only relevant to five of these studies. Three described the number lost, and it was low;[129-131] the others provided no description.[132 133] Studies varied greatly in terms of the degree to which they described the population from which the participants came. Since these were family members of probands, studies with adequate description generally described the source of the probands, and then provided a description of the recruitment process used to enroll family members. Several of the studies had very little description of the inclusion and exclusion criteria for the family members.[133 135-137] Even among those that had explicit inclusion criteria, not all uniformly provided a detailed description of the enrolled participants. Even age and sex were inconsistently described. The majority of these studies were published before journals began requiring disclosure of funding sources and potential conflicts of interest. More studies than not, however, did report on the source of funding; we considered these sources of funding to be unlikely to bias the results, since most of the reported funding was from governmental grant support.

Heterozygous FVL in family members. Nine studies described results for people heterozygous for FVL (Table 9, Appendix G, Evidence Tables 14 and 15).[129 130 132 134 137-141], although one of these included a single person homozygous for the mutation among the 91 studied.[138] Two of the studies did not report on a comparison group without mutation.[129 141] The seven studies with comparison groups enrolled between 5 and 299 affected individuals, for a total of 1,066 heterozygous family members. We show the rates of events among the affected family members, as compared to the rates of events among 940 unaffected family members (Appendix G, Evidence Table 15). The odds ratios across these studies were quite comparable except for the one small study by Simioni, in 2000, in which there were no events among the eight heterozygous individuals.[137]

Figure 9. Odds ratios for VTE for family members with a heterozygous FVL mutation, as compared to family members without the mutation*



*the size of the box is proportional to the study sample size; the confidence intervals (CI) for the pooled results are derived from a random effects model

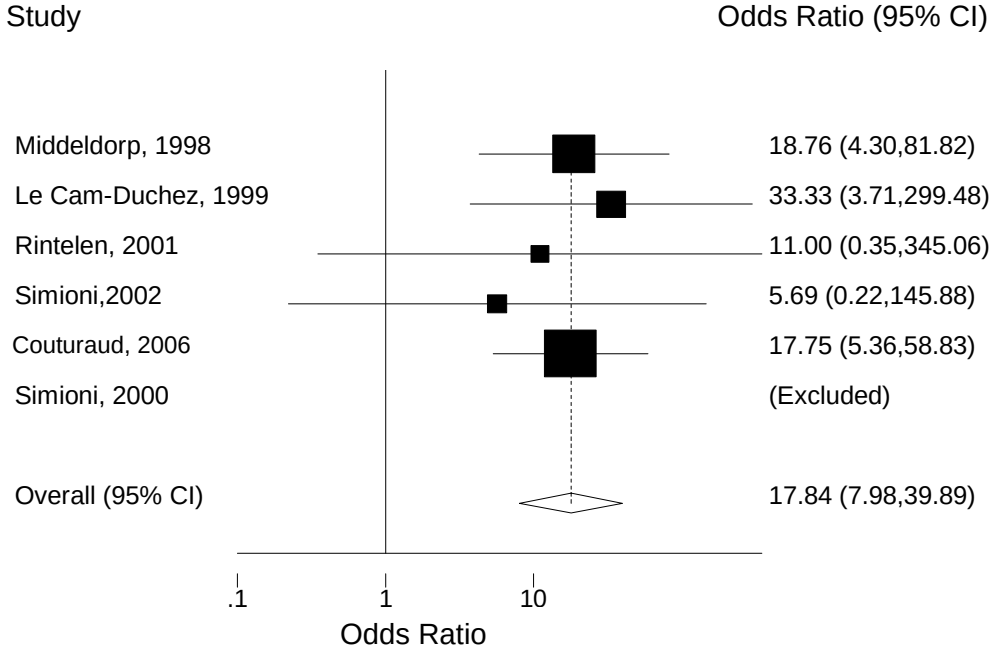
The pooled odds ratio was 3.49 (95 percent CI, 2.46 to 4.96), with very little heterogeneity between studies (I^2= 4 percent) (Figure 9). The small study by Rintelen contributed little to the pooled estimate.

Three of these studies reported person-years of observation to allow calculation of the annual rate of VTE events.[129 130 132] Our sensitivity analysis, in which we removed each study sequentially, demonstrated that removal of the study by Couturaud et al.[132] raised the pooled odds ratio to close to 4.0, although the CI values overlapped the pooled odds ratio when that study was included. The annualized event rate among heterozygous individuals in this study was 0.36 percent (95 percent CI, 0.24 to 0.49), which is lower than those in the other two studies in which rates were reported.[129 130] It is not apparent from either the inclusion criteria or the patient characteristics why the rates were lower in this study, which was of adequate quality. The event rates in the mutation-free groups were fairly comparable across the three studies. There was no evidence of important publication bias.

Homozygous FVL in family members. Six studies described results for 48 people homozygous for FVL[130 132 134 137 139 140]. The annualized rates were given in only three studies and are shown in Table 9, Appendix G, Evidence Tables 14 and 15.[129 130 132] The odds of events were compared to the odds for 850 family members without mutations (Figure 9). The pooled odds ratio was 18 (95 percent CI, 7.8 to 40), with very little heterogeneity between studies (I^2=0 percent) (Figure 10). One small study was excluded from pooling because it had no events in either arm and only a single individual in each arm.[134] One study, a French study by Couturaud et al.,[139] was

particularly influential in raising the odds ratio. Removal of that study in the sensitivity analysis resulted in a pooled odds ratio of roughly 16, with an upper limit of the CI that did not reach 18. It is not apparent why the event rate was so high in this French study (five of the six individuals with homozygosity had VTE events). The annualized rate was not described for their mutation-free comparison group. There was no evidence of important publication bias for this comparison.

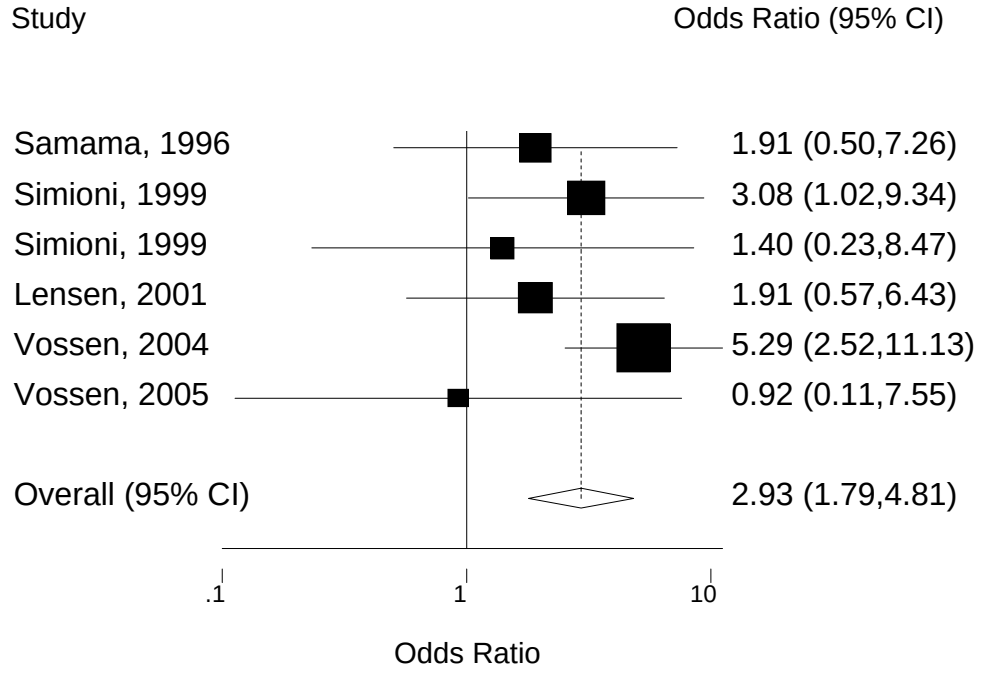
Figure 10. Odds ratios for VTE for family members with homozygous FVL mutation, as compared to family members without the mutation*



*the size of the box is proportional to the study sample size; the confidence intervals (CI) for the pooled results are derived from a random effects model

Mixed or unspecified FVL in family members. Nine studies reported the results from an unspecified group of homozygous and heterozygous individuals (Table 9, Appendix G, Evidence Tables 14 and 15).[26 131-133 135 136 140 142 143] Three of these studies also described the results separately for these groups, so we will not discuss these composite groups here.[26 132 140] The odds ratios for each study as well as the pooled odds ratio are given in Figure 11. The pooled odds ratio was 2.9 (95 percent CI, 1.8 to 4.8), with little heterogeneity between studies (I^2=4 percent). One large study was particularly influential.[142] The odds ratio of this study was 5.2; when it was removed from pooling, the aggregated odds ratio dropped to close to 2.0, with the upper limit of the CI extending only to 2.8. This influential study involved 1,437 family members from nine European countries and was part of the European Prospective Cohort on Thrombophilia (EPCOT) study of familial thrombophilia. Thirteen of the 225 family members with FVL were homozygous for the mutation. The event rate in members without mutations was quite low, at 0.03 percent (95 percent CI, 0.02 to 0.05). The prospective portion of the EPCOT study described very few VTE events among the family members with FVL, with an odds ratio of only 0.92 and wide confidence intervals (95 percent CI, 0.11 to 7.5).[131]

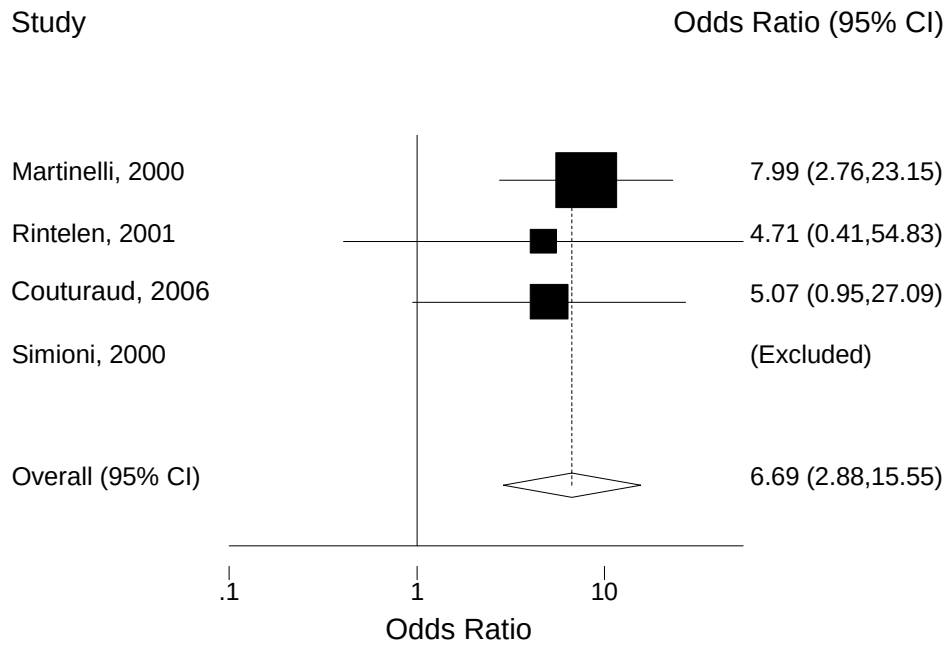
Figure 11. Odds ratios for VTE for family members with an unspecified FVL mutation, as compared to family members without the mutation*



*The size of the box is proportional to the study sample size; the confidence intervals (CI) for the pooled results are derived from a random effects model

Family members doubly heterozygous for FVL and prothrombin G20201A. Four studies described VTE events in a total of 59 doubly heterozygous individuals (Table 9, Appendix G, Evidence Tables 14 and 15).[26 132 134 137] These individuals were compared to a total of 674 individuals without mutations. The very small study[134] observed no events in either arm. The odds ratios for each study as well as the pooled odds ratio are given in Figure 12. The pooled odds ratio was 6.7 (95 percent CI, 2.9 to 16), with very little heterogeneity between studies (I^2=0 percent). The odds ratio associated with double heterozygosity in the large study by Martinelli et al. was higher than in the other two studies (odds ratio, 8.0; 95 percent CI, 2.8 to 23). This difference may be partially explained by the low rate of events in their study among the family members without mutations. The rate of events in the mutation-free group was 0.06 percent per year, whereas in most studies it was closer to 0.1 percent per year. In the case of this comparison, there may have been some degree of publication bias, given the paucity of small studies reporting large effect sizes (high odds ratios).

Figure 12. Odds ratios for VTE for family members with double heterozygous mutations, as compared to family members without mutations*



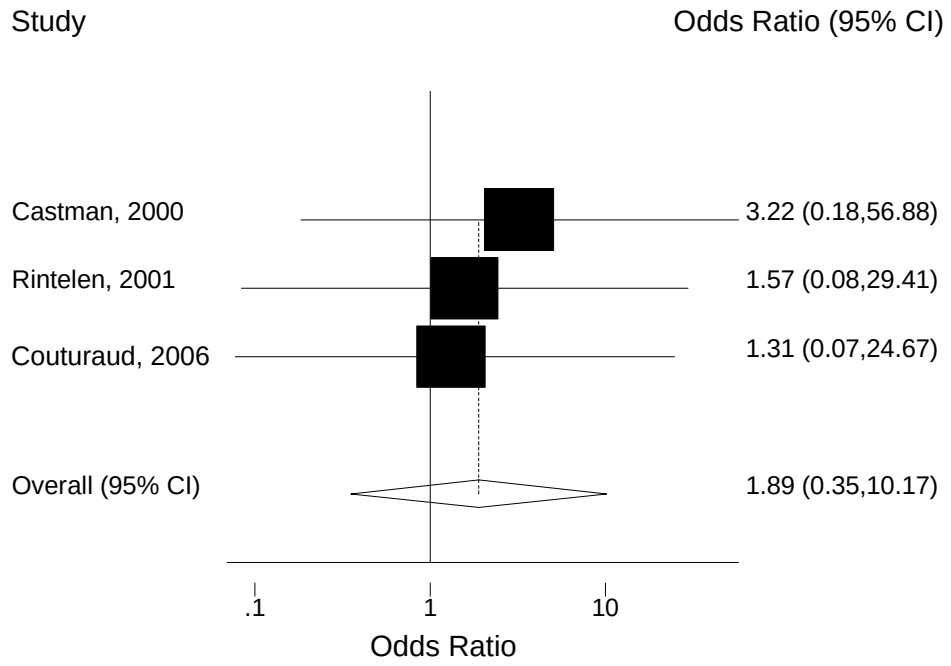
*The size of the box is proportional to the study sample size; the confidence intervals (CI) for the pooled results are derived from a random effects model

Prothrombin G20210A and venous thrombosis in family members. Five studies evaluated prothrombin G20210A and venous thrombosis in family members of probands.[26 132 134 137 144] As mentioned above, all five studies were conducted in Europe. Only one of these studies was not described above in the discussion of FVL.[144] This was a small study of adequate quality conducted in Italy that exclusively investigated outcomes from heterozygosity for prothrombin G20210A. This earliest study, by Castaman, had rigorous inclusion criteria and excluded women on oral contraceptive or hormonal therapies.[144] Similarly, Martinelli et al. excluded people with malignancies or known autoimmune disease.[26]

Homozygous prothrombin G20210A in family members. Only a single study described a single patient who was homozygous for this mutation (Table 9, Appendix G, Evidence Tables 16 and 17).[137] This patient was identified among 44 family members from four families and had no reported VTE events.

Heterozygous prothrombin G20210A in family members. Family members who were heterozygous for this mutation were compared to family members without the mutation in three studies (Table 9, Appendix G, Evidence Tables 16 and 17).[132 137 144] In one study, an age-adjusted relative rate of events of 3.4 (95 percent CI, 0.2-56) was reported, when compared to family members without mutation.[144] The other studies were very small, with one event in eight patients in one study[137] and no events in six people in the other.[132] Pooling these small studies yielded an odds ratio for VTE events of 1.89 (95 percent CI, 0.35-10), with an I^2 of 0 percent, suggesting that family members who are heterozygous for this mutation do not have an elevated risk of thrombosis (Figure 13).

Figure 13. Odds ratios for VTE for family members with heterozygous prothrombin G20210A, as compared to family members without the mutation*



*The size of the box is proportional to the study sample size; the confidence intervals (CI) for the pooled results are derived from a random effects model

The doubly heterozygous individuals were described above in the discussion of FVL. The risk of VTE events was notably high for doubly heterozygous individuals.

Association of FVL and Prothrombin G20210A With Venous Thrombosis During Pregnancy and the Postpartum Period

Testing asymptomatic family members for the FVL and prothrombin G20210A mutations would be important if the mutations raise the risk of venous thrombosis *and* if other risk factors can be modified or prophylactic interventions can be employed to lower the risk. A particularly important time with regard to risk is pregnancy and the postpartum period. These are high-risk times for VTE because hormonal and mechanical changes shift the balance in favor of hypercoaguability.[145]

Several of our included articles described the experience of family members of probands during their pregnancies. Here we report the results of studies that quantified the risk of VTE associated with FVL and prothrombin G20210A specifically during pregnancy and the postpartum period for these family members of patients with VTE. Occasionally these studies included probands and evaluation of their subsequent pregnancies; this situation was rare, and we excluded these probands from our analyses.

We identified four articles that specifically addressed the risk of venous thrombosis attributable to FVL and prothrombin G20210A during the pregnancies of family members,[146-149] and one additional study that included useable pregnancy data.[132] All were retrospective cohort

studies conducted in Europe; one included a prospective component[132] (Appendix G, Evidence Tables 18 and 19). Two explicitly excluded women who had had a venous thrombosis prior to pregnancy.[147 149]

Although not explicitly stated, we believe that the two studies by Martinelli et al. include some of the same women, so we did not extract data about the women who were homozygous for FVL from the more recent of the two studies.[146 149] The mutation-free groups described in these two studies may also include many of the same women. We show the results in Appendix G, Evidence Tables 18 and 19. The odds ratios compare the odds of a VTE event per pregnancy across two groups, with the reference group being women with neither FVL nor the prothrombin G20210A mutation.

Two studies evaluated the risk associated with homozygous FVL.[146,147] The number of pregnant women with homozygous FVL was small, with only nine in one study[146] and six in the other,[147] and a total of 33 pregnancies among them. The odds ratios for venous thrombosis were very high, although with wide CIs, suggesting that homozygosity for FVL is associated with venous thrombosis in the pregnancy or post-partum periods.

The odds ratios associated with heterozygous FVL were close to 1.0 and quite similar in the two studies reporting this outcome. The rates of events were low, at 2.5 percent per pregnancy (95 percent CI, 0.9-5.4)[147] and 1.5 percent per pregnancy (95 percent CI, 0.5-4.3).[149] Two studies evaluated the risk associated with heterozygosity for the prothrombin G20210A mutation. A total of 247 women with this mutation were studied. The rates of VTE were low, at 0.3 percent per pregnancy (95 percent CI, 0.1 to 1.6)[148] and 1 percent per pregnancy (95 percent CI, 0.2-3.6).[149] These odds ratios were also close to 1.0.

Individuals who were doubly heterozygous were evaluated in two studies.[147,149] The larger of the two studies reported an odds ratio that was quite similar to the odds ratios associated with heterozygosity of either of the two mutations.[149] The small study (five doubly heterozygous women with pregnancies) reported an odds ratio comparable to that seen in the women with homozygosity for FVL.[147] The study with a prospective component only reported the results for a group of women with unspecified FVL that may have included some women who were homozygous for the mutation. The number of pregnancies was not described, only the number of women with pregnancies. The risk ratio for venous thrombosis in women with FVL as compared to those without was 2.9 (95 percent CI, 0.96-8.4).

Age and VTE Among Family Members With Mutations

We sought to examine whether the risk attributable to the mutations varied with age. Few studies reported results stratified by age. None of the studies of prothrombin G20210A described the results according to age. Three studies examining the risk from the FVL mutation described age-specific rates of events.[138,140,143] One additional study described the age of the individuals at the time of their first thromboses in the family members with and without mutations.[139] The mean age at thrombosis was 40 years in the family members without a mutation, 34 years in people who were heterozygous for FVL, and 21 years in homozygous individuals. The difference was not statistically significant when heterozygous and mutation-free individuals were compared (p=0.07), but it was significant when homozygous and mutation-free individuals were compared (p=0.009).

Middledorp et al. described age-specific relative rates of events for a group (homozygous, heterozygous, double heterozygous) with FVL mutation, as compared to family members

without mutations (Appendix G, Evidence Tables 14 and 15). The relative risk posed by the mutation was highest in the youngest patients, aged 15 to 30 (relative rate of approximately 15) and was lower and more comparable across the older age strata.[140] Similarly, Simioni et al. described age-specific rates in a group of family members with unspecified FVL mutations.[143] The relative rate in the youngest age group was much lower than that reported in the Middeldorp study. Similarly, Lensen et al. described age-specific rates among heterozygous individuals that were not importantly different across age strata.[138]

The study by Lensen et al. presented a relative hazard value from a Cox model across the lifetime of these heterozygous relatives, as compared to the mutation-free relatives. The relative hazard was 3.4 (95 percent CI, 1.3-9.2), which is remarkably similar to the odds ratio we calculated for the pooled studies of heterozygous individuals, 3.42 (95 percent CI, 2.40 to 4.89). Another study reported an HR (adjusted for age, sex and other factor deficiencies).[26] The relative hazard for the FVL heterozygous individuals was 2.7 (95 percent CI, 1.4-18) and 6.5 (95 percent CI, 2.4-18) for the doubly heterozygous family members. This study also reported an HR for individuals with an isolated prothrombin G20210A mutation (Appendix G, Evidence Tables 16 and 17).

Other High-risk Periods

In addition to analyzing the pregnancy and postpartum periods, we sought information on whether FVL or prothrombin G20210A additively or multiplicatively raised the risk of VTE during other high-risk periods, such as following surgery or during the use of hormonal therapy. Very few studies reported event rates stratified by high-risk periods,[135 143] and we were unable to draw any conclusions regarding such periods.

Summary of the Evidence for Key Question 3 (Family Members)

The literature supported the following conclusions about the clinical validity of testing for FVL and prothrombin G20210A:

- There was high-grade evidence that homozygosity for FVL in family members is predictive of VTE.
- There was moderate-grade evidence that heterozygosity for FVL in family members is predictive of VTE.
- The evidence was insufficient to allow us to draw any conclusions about the predictive value of heterozygosity for prothrombin G20201A in family members.
- There was low-grade evidence that double heterozygosity (for FVL and prothrombin G20210A) in family members is predictive of VTE.
- There was low-grade evidence that homozygosity for FVL in pregnant family members is predictive of VTE.
- The evidence was insufficient to allow us to draw any conclusions about the predictive value of heterozygosity for FVL in pregnant family members.
- Homozygosity for prothrombin G20210A is a rare genotype, and its association with recurrent VTE in family members is unknown, because of the lack of sufficient evidence.

- The evidence was insufficient to allow us to draw any conclusions about the predictive value of heterozygosity for prothrombin G20210A in pregnant family members.
- The evidence was insufficient to allow us to draw any conclusions about the predictive value of double heterozygosity for FLV and prothrombin G20210A in pregnant family members.

Key Question 4
Clinical Utility of Testing for FVL and/or Prothrombin G202010A

We identified 10 studies addressing the clinical utility of testing for these mutations. The studies varied substantially in their methodology and design, sources of the participants, geographical locations, and outcomes. We summarize our findings, including our assessment of the quality of these studies, according to the four main sub-questions of KQ4 (Tables 10-12).

Effect of Testing on Clinicians' Management (KQ 4a)

We found a single study addressing how physicians' management decisions are affected by FVL testing (Appendix G, Evidence Table 21).[150] Rodger et al surveyed 662 Canadian obstetrical care providers (65 percent of whom were obstetricians, 28 percent family practitioners, and 5 percent perinatologists), about their management recommendations in response to four clinical scenarios involving pregnant women with a history of FVL. The survey asked whether the practitioners would recommend peripartum thromboprophylaxis and whether they would refer the patient to a hematologist or to another specialist. The survey response rate was 46 percent (1,448 members of the Society of Obstetricians and Gynecologists of Canada had been invited to participate); 57 percent of the respondents were male. For scenarios involving asymptomatic women, the recommendation for thromboprophylaxis almost doubled, from 26-34 percent to 58 percent, if the patient was described as having a family history of VTE. For scenarios involving symptomatic women, 60-68 percent of clinicians recommended prophylaxis for women with recurrent fetal loss, and 83-84 percent of clinicians recommended it for women with a personal history of VTE. Uncertainty was prevalent regarding the use of prophylaxis: 14 percent of clinicians were unsure as to whether or not to recommend prophylaxis when the patient had a history of prior VTE, and 29 percent were unsure in cases in which the patient had a family history of VTE or recurrent fetal loss. More than 80 percent of clinicians would refer a pregnant woman with a history of FVL to a specialist. This study suggests a high degree of uncertainty regarding the management of pregnant patients with a history of FVL, with clinicians reporting a preference to recommend prophylaxis and referral, particularly in cases of symptomatic women.

Effect of Testing and Resultant Mnagement on VTE-related Otcomes (KQ 4b)

There were four studies yielding VTE recurrence rates among probands with FVL or prothrombin G20210A during anticoagulation therapy (Table 10, Appendix G, Evidence Tables

22-24).[122,123,125,151] Each was a subgroup analysis; three involved individuals participating in randomized controlled trials,[123,125,151] while the fourth was nested within a prospective cohort study.[122] Three of the four studies included participants with FVL or prothrombin G20210A,[123, 125,151] while the fourth addressed only FVL.[122] Two studies investigated the effect of warfarin on recurrence rates,[125,151] one the effect of ximelagatran,[123] and one did not specify the treatment that patients received.[122] None of these studies was designed to examine how testing effects changes in management or whether choosing treatment based on test results alters outcomes. As required for inclusion in this report, all index events and recurrences were objectively documented. There were quality flaws across the studies, including inadequate description of how patients were recruited, raising the possibility of selection bias (Appendix G, Evidence Table 20). Only one of the studies described the number of patients lost to follow-up.[122] Although some of the studies had stringent inclusion and exclusion criteria, for the most part these were the criteria for safely using these drugs. Ridker et al assessed thromboembolism recurrence rates among individuals with FVL or prothrombin G20210A who received either a low-intensity warfarin regimen (international normalized ratio [INR] target between 1.5 and 2.0) or placebo[151] (Table 10). Each participant had experienced a prior unprovoked VTE and had completed at least 3 months of uninterrupted treatment with warfarin. The patients randomized to the placebo and treatment groups had similar demographic and clinical characteristics. Of 77 patients with FVL or prothrombin G20210A in the placebo group, 14 had recurrences (8.6 events per 100 person-years), as compared to 3 of 66 such patients assigned to the low-intensity warfarin group (2.2 events per 100 person-years). Low-intensity warfarin reduced the rate of recurrence among thrombophilic patients by 75 percent (HR, 0.25; 95 percent CI, 0.07 to 0.85). This risk reduction, however, was not significantly different than the 58 percent reduction seen in patients without either mutation (HR, 0.42; 95 percent CI, 0.20 to 0.86; p-value for the interaction, 0.51). These results suggest that low-intensity warfarin significantly decreases the rate of recurrence of VTE in patients with FVL or prothrombin G20210A; however, this protective effect was not different than that observed for individuals without the mutations.

Kearon et al asked whether VTE recurrence rates differed among patients with and without FVL or prothrombin G20210A who were receiving either low-intensity (INR goal, 1.5-2.0) or conventional (INR goal, 2.0-3.0) warfarin therapy.[125] All patients were participants in the Extended Low-intensity Anticoagulation for Unprovoked Thromboembolism (ELATE) trial and were separately invited to participate in this study. Among 171 patients with FVL, three had recurrences. No recurrences were seen in the 60 patients with prothrombin G20210A. The recurrence rate for patients with FVL while receiving low- *or* conventional-intensity warfarin therapy was 0.8 percent per year (95 percent CI, 0.2 to 2.2). This rate was not statistically different than the rate among those participants without FVL (HR, 0.7; 95 percent CI, 0.2 to 2.6). The HR for recurrent VTE in the low-intensity warfarin group as compared to the conventional-intensity group was 2.7 among those with thrombophilia (not just FVL or prothrombin G20210A; 95 percent CI, 0.5 to 14), and 5.6 in those without thrombophilia (95 percent CI, 0.7 to 47; p-value for interaction, 0.6). These results were not reported separately according to mutation.

Table 10. Four studies examining the effect on VTE-related outcomes of changes in therapy based on testing for FVL or prothrombin G20210A

Author, year	Type of Study	Population	Sample Size	Outcome of Interest	Key Conclusions
Ridker, 2003[151]	Randomized controlled trial	• FVL or PT 20210A (pooled) • Prior unprovoked VTE • Had 3 months of uninterrupted warfarin • United States-based study • Age range 46-65 • 53% males • Mean follow-up 2.1 years (maximum 4.3)	508 participants: 253 assigned to placebo (26.6% FVL carriers, 4.8% PT G20210A carriers), 255 assigned to low-intensity warfarin (22.0% FVL carriers, 4.7% PT G20210A carriers)	• VTE recurrence while treated with low-intensity warfarin • VTE recurrence rate among carriers	Low-intensity warfarin reduces recurrence in non-carriers by 58% (HR, 0.42; 95% CI, 0.20-0.86) and it reduces recurrence in carriers by 75% (HR, 0.25; 95% CI, 0.07-0.87).
Kearon, 2008[125]	Cohort analysis nested within randomized controlled trial	• FVL or PT 20210A • Prior VTE • Had 3 months of uninterrupted warfarin • Canada-based study • Mean age, 57 (SD, 15) • 54-58% males • Mean follow-up 2.3 years	661 participants (pooled): 337 assigned to low-intensity warfarin (91 FVL, 32 PT G20210A), 324 assigned to conventional-intensity warfarin (80 FVL, 28 PT 20210A)	• VTE recurrence while treated with either low-intensity or conventional-intensity warfarin • VTE recurrence rate among carriers, as compared to non-carriers	Conventional-intensity warfarin reduces recurrence when compared to low-intensity warfarin. The rate of recurrence was 1.5% per patient-year among the patients allocated to low-intensity therapy and 0.4% per patient-year among the patients allocated to conventional-intensity therapy (HR, 3.7; 95% CI, 1.03 to 13.2). The absolute rate of recurrence for FVL carriers in combined groups was 0.8% per patient-year (0.2, 2.2) and was less than in those without a defect (HR 0.7; 95% CI, 0.2-2.6).

65

Table 10. Four studies examining the effect on VTE-related outcomes of changes in therapy based on testing for FVL or prothrombin G20210A (continued)

Author, year	Type of Study	Population	Sample Size	Outcome of Interest	Key Conclusions
Wahlander, 2006[123]	Cohort analysis nested within randomized controlled trial	• FVL or PT 20210A • Prior VTE • Had completed 6 months of uninterrupted warfarin • European-based study • 18-month study period	1223 participants: 612 oral ximelagatran (111 FVL, 27 PT 20210A), 611 placebo (125 FVL, 27 PT G20210A)	• VTE recurrence while treated with ximelagatran • VTE recurrence rate among carriers	Oral ximelagatran reduces recurrence in non-carriers as well as FVL carriers (Figure 2, HR not presented).
Vossen, 2005[122]	Prospective cohort	• FVL or combined FVL-PT 20210A • Prior VTE • European-based study • Age range 14-78 • 35-48% males • Followed for 1710 person-years (mean, 5.6; range, 1-7)	304 participants: 124 on long-term anticoagulation (13 FVL, 1 combined FVL-PT 20210A), 180 not on long-term anticoagulation (79 FVL, 7 combined FVL-PT G2021A)	• VTE recurrence while treated with long-term anticoagulation • VTE recurrence rate among carriers	Long-term anticoagulation reduces recurrence in non-carriers. The rate of recurrence was 1.1% per year among the patients on long-term anticoagulation and 5.0% per year among the patients not on long-term anticoagulation. The rate of recurrence for FVL carriers not on long-term anticoagulation was 3.5%. There were no events in the group on long-term anticoagulation.

CI = confidence interval; FVL = Factor V Leiden; HR = hazard ratio; PT G20210A = prothrombin G20210A; SD = standard deviation; VTE = venous thromboembolism

Wahlander et al. assessed the risk of VTE recurrence in relation to thrombophilic factors in probands receiving ximelagatran, an oral direct thrombin inhibitor (not presently available), or placebo.[123] This study involved a group of participants recruited from the Thrombin Inhibitor in VTE (THRIVE III) trial.[152] Among 111 FVL carriers in the treatment group, two had recurrences of VTE, as compared to 16 in the 125 with mutations assigned to placebo; this difference in recurrence rates was described as statistically significant (HR under 0.25; p-value not reported). The recurrence rate with treatment was very similar for individuals without FVL, suggesting that there was no interaction between FVL and the effect of ximelagatran in preventing recurrent VTE (p-value for the interaction, 0.92). Among 27 prothrombin G20210A carriers in the treatment group, none experienced a recurrence, while 2 of 27 carriers in the placebo group had a recurrence (HR not calculable). Recurrence rates with treatment were very similar for individuals without FVL or prothrombin G20210A, suggesting that there was no interaction between FVL or prothrombin G20210A and the effect of ximelagatran in preventing recurrent VTE (p-value for the interaction, 0.92 for FVL and 0.98 for prothrombin G20210A). There were no significant differences in the risk of bleeding in carriers of FVL or prothrombin G20210A when compared to non-carriers (p-value for the interaction, 0.60 for FVL and 0.16 for prothrombin G20210A).

Vossen et al studied the rate of recurrence of VTE in a cohort of patients belonging to thrombophilic families, classified according to whether they were receiving long-term anticoagulation or not.[122] They focused on probands with a family history of VTE because of the potentially higher rates of recurrence among these individuals. All patients were recruited as part of the EPCOT trial. The mean follow-up was 5.6 years (range 1 to 7 years). Of 304 patients, 124 were on long-term anticoagulation, and 180 were not. The details of the anticoagulation regimens were not reported. The proportion of women was higher in the anticoagulation group (48 versus 35 percent). There were fewer FVL carriers in the long-term anticoagulation group than in the comparison group (13 and 44 percent, respectively). Of 79 patients with FVL who did not receive long-term anticoagulation, 13 had recurrences during 366 person-years (incidence rate, 3.5 percent per year; 95 percent CI, 1.9 to 6.1). Among the 13 FVL carriers who received anticoagulation, none had any recurrence during 43 person-years. This study showed that long-term anticoagulation decreased the rate of VTE recurrence among probands belonging to thrombophilic families. However a quantitative estimate was not calculable because there were no events in the group of patients receiving treatment. Confidence in the study's results is low because this study was observational, and there were important differences in the baseline characteristics and duration of follow-up in the two groups. Also, there was a paucity of detail about the anticoagulation regimens.

Effect of testing and results on other outcomes (KQ 4c)

Four studies addressed how probands' and family members' knowledge, behaviors, and healthcare experiences were affected by their being tested for FVL or prothrombin G20210A;[153-157] only one study evaluated individuals with prothrombin G20210A[153] (Table 11 and Appendix G, Evidence Table 21). Two publications by Saukko et al. investigated the same population of patients but provided complementary information and are considered as one study here.[155,156] Three studies were conducted in Europe[154-157] and one in Canada.[153]

Two studies employed cross-sectional surveys of convenience samples of probands and family members to assess risk perception and behavioral effects following genetic testing.[153,154] The other two studies used qualitative, structured interviews of probands and relatives to

Table 11. Studies examining the effect of FVL and prothrombin G20210A testing on non-VTE-related outcomes

Author, year	Type of Study	Population	Sample Size	Outcome of Interest	Key Conclusions
Bank, 2004[157]	Qualitative, structured interview	• Asymptomatic first-degree relatives of probands • Tested for FVL • In the Netherlands • Age, 26-56 years • 53% males	17 participants (65% response rate)	Overall experience with testing; effects of testing on daily life	• For most, testing not stressful (all received written information about FVL testing) • More concern about relatives' health than their own • No changes in daily life for the majority of participants
Teutsch, 2008[156] and Saukko, 2007[155]	Qualitative, structured interview	• Probands or relatives referred for FVL testing • In the United Kingdom • 12% males	42 participants (43% response rate)	Understanding of testing process and implications of results on daily life	• Most did not consider FVL testing to be different than other tests ordered by clinician • Less understanding among those of lower socio-economic status • Most did not change daily routine as a result of testing
Kaptien, 2008[154]	Cross-sectional survey	• Probands and relatives recruited from EPCOT study • Tested for FVL, AT, PC, or PS deficiency • In Europe (multiple countries) • Age, 26-87 years • 46% males	174 participants (78% response rate)	VTE risk perception and disease-related worry	• Increased risk perception and worry among those with prior VTE • Level of worry among those with FVL lower than in those with AT and similar to those with PS, PC
Heshka, 2008[153]	Cross-sectional survey	• Asymptomatic first-degree relatives of probands, not on long-term anticoagulation • Tested for FVL or PT 20210A • In Canada • Age, 21-78 years • 38% males	70 participants: 44 carriers (86% response rate) 26 non-carriers (55% response rate)	Perception of VTE risk and changes in behavior following FVL or PT 20210A testing	• No significant differences in recognition of VTE risk factors between carriers and non-carriers, despite counseling • Behavior changes in response to testing were uncommon in both groups

AT = antithrombin deficiency; EPCOT = European Prospective Cohort on Thrombophilia; FVL = Factor V Leiden; PC = protein C deficiency; PS = protein S deficiency; PT 20210A = prothrombin G20210A; VTE = venous thromboembolism

describe their experience during the process of testing as well as their interpretation of the results.[155-157] In all studies, except that of Saukko et al,[155,156] subjects were recruited from cohorts already enrolled in larger trials investigating venous thromboembolic outcomes in probands or relatives.

The quality of these studies was generally high (Appendix G, Evidence Table 20). Although all four studies used a cross-sectional design and looked at convenience samples of probands or relatives, the methods employed were appropriate to answer the research questions. The surveys used instruments that had been previously tested in patients with genetic mutations or that had been validated to measure specific constructs such as worry or risk perception.[153,154] Both of the qualitative studies used appropriate data collection methods, including the exploration and exhaustion of predetermined themes, the use of trained interviewers, and the use of quotes to represent participants' voices.[155-157]

Heshka et al surveyed the perception of VTE risk and changes in behavior following testing for FVL or prothrombin G20210A among first-degree relatives of probands.[153] The survey response rate was 86 percent for relatives who carried the FVL mutation and 55 percent for non-carriers; otherwise, the 44 carriers and 26 non-carriers were similar in age, education, and income. More mutation carriers recognized trauma as a risk factor for VTE than did non-carriers, but otherwise there were no statistically significant differences between the two groups regarding their recognition of risk factors for VTE, despite the carriers having received counseling after testing. Behavior changes following testing were uncommon in both groups, but carriers were slightly more likely to have tried to avoid long trips since testing, to have stopped using oral contraceptive pills or hormone replacement therapy, to have discussed the results of the test with their surgeons or gynecologists, or to have been placed on anticoagulants during surgery or during or after childbirth. The results of this study suggest that testing for FVL and prothrombin G20210A and sharing the results with patients do not lead to a substantial change in risk perception or an important increase in preventive health behaviors.

Kaptein et al investigated whether the type of thrombophilic mutation and history of VTE affected the perception of risk and level of worry among probands or their relatives with FVL, when compared to other thrombophilic mutations.[154] The core of the survey tool was a version of the Illness Perception Questionnaire adapted for patients with thrombophilia. Of the 251 individuals invited, 196 (78 percent) responded. Of these, 62 individuals had only FVL (no protein C, protein S, or antithrombin deficiency). This group included both relatives and probands, with and without a history of VTE. Overall, patients with a history of VTE had an increased perception of risk and worried more about VTE than did individuals without prior VTE. After controlling for age and prior VTE, patients with FVL had a significantly lower perception of risk of VTE than did individuals with antithrombin deficiency but a similar risk perception when compared to patients with protein C or S deficiency. Level of worry about thrombosis was similar across all thrombophilia groups. This study suggests that, among carriers of FVL, the level of worry is similar to that of carriers of other thrombophilic mutations, although individuals with antithrombin deficiency (which has the highest VTE risk of the four measured thrombophilias) had a higher perception of risk than did patients with the FVL mutation. However, worry and risk perception were not measured in non-carriers; therefore, although it appears that having the FVL mutation does not increase risk perception and worry beyond the levels seen with other relatively common thrombophilic mutations, this study did not specifically address how the testing process and knowledge of results affects individuals tested for FVL.

Bank et al conducted a qualitative study of asymptomatic relatives of probands with FVL to assess their overall experience with the testing process and how the results affected their daily lives.[157] Of 26 relatives invited, 17 (65 percent) agreed to be interviewed. The topics covered during the open-ended interviews included stigmatization, perception of risk, and preventive behaviors. Among the participants, most found that the testing process itself was not stressful; all had received written information about the test prior to testing. Most exhibited knowledge of the most common risk factors for VTE (e.g., pregnancy, surgery), citing as the source of this knowledge the information provided during testing and the information obtained from providers after receiving the test results. Some participants related an increased level of worry about developing VTE, while some felt stigmatized by friends, family, or even their providers, who saw them as having a health condition. One person in the Netherlands who disclosed his test results to his employer was discriminated against in his disability insurance policy. Although the majority of participants indicated that testing had not altered their daily lives, many wanted to screen their children to decrease their risk of VTE from pregnancy or oral contraceptive use. This study suggests that although, overall, no drastic changes in mood or behavior were observed among individuals tested for FVL, testing generated concern about the individuals' own health and the health of their children. The authors argued that the information provided on the day of testing and after the results were given played a crucial role in lessening patients' concerns and improved their testing experience.

Saukko et al. assessed the level of understanding of the testing process and the implications of the results among probands and relatives referred for FVL testing by their primary care doctor or specialist.[155,156] The study, which was carried out in the United Kingdom, involved structured qualitative interviews of 42 individuals (43 percent of the 97 invited to participate). The themes explored during the interviews included reasons for testing, how the results were relayed to the participants, how they reacted to the results, what they did with the information, and what they thought of the testing process as a whole. Twenty of the 42 participants had been referred because of a family history of VTE or thrombophilia; 10 had a prior history of VTE; and 5 were referred because of prior miscarriage. Seven participants were unaware they had been tested. Sixteen participants tested positive for FVL, although the results were for the most part not reported according to carriership status. Most participants did not consider thrombophilia testing to be different from the other tests ordered by their providers, even if they understood the increased risk of VTE.

The overall understanding of the FVL test and its implications fell along a spectrum: Most participants who understood the test well belonged predominantly to higher socio-economic strata, as compared to those with a poorer understanding of the test. Participants who received or obtained information about FVL prior to talking to their physicians (e.g., from relatives) were more likely to feel that the information given by their providers was sufficient or useful. Many did not receive written, detailed information about VTE risk factors or preventive behaviors from their providers when they were given their results. Participants also learned about the results of the tests in different ways, and this variability affected their behavior after receiving results. Those who were more informed about FVL sought information on their own, from relatives or the Internet, whereas the less-informed participants tended to assume that a lack of information from their providers meant that FVL was not an important condition. Most participants did not incorporate behaviors to reduce their risk for VTE into their daily routines as a result of the testing, although most participants who were aware of their positive status stated they had

undergone testing to inform their decision to take hormonal therapy or to advise relatives on the matter.

These studies by Saukko et al. once again suggest that information given to patients at the time of testing affects their understanding of the process and the implications of the results.[155,156] Furthermore, socio-economic factors such as income or literacy may potentially influence patients' understanding of thrombophilia testing by limiting their access to supplementary information about the various tests. It is worth noting that although all patients were referred for FVL testing, many were actually referred for a thrombophilia panel, and so the attitudes or behaviors reported may not necessarily have been in response to FVL testing.

Cost-effectiveness of FVL and Prothrombin G20210A Testing in the Case of Probands and Their Relatives (KQ 4d)

We identified six studies that assessed the cost-effectiveness of genetic testing and resultant changes in management[158-163] and one study that assessed only effectiveness.[164] Six of the studies employed decision-analytic modeling, with a single decision giving rise to a series of multiple possible outcomes, each of which was weighted according to the probability of its occurrence.[158,160-164] Of these six studies, only one did not incorporate a Markov state-transition model, a technique that permits the consideration of iterative events over time.[158] The seventh study used a prospective cohort to derive cost and outcomes data, with which the authors retrospectively modeled the costs and effectiveness of hypothetical changes in management.[160] Four of the studies considered testing for FVL alone,[160,161,163,164] while the remaining three evaluated testing for FVL along with prothrombin G20210A.[158,159,162] Five of the studies modeled probands with a personal history of VTE,[159,161-164] and the other two studies considered individuals with either a personal or family history of VTE.[158,160]

We first briefly describe the design, interventions, population, outcomes, results, conclusions, and quality deficits of each study (Table 12; Appendix G, Evidence Tables 25-27). In the tables, we present the results in 2007 U.S. dollars, although here in the text, we describe the results as reported in each paper.

The seven studies were of variable quality. They all had a clear statement of the objective and perspective, although the primary decision-maker for whom the study was conducted was specified only in the case of the United Kingdom National Health Service.[158,160] A societal perspective was stated twice, without the incorporation of any indirect costs.[159,161] The structure of the models was generally stated clearly and justified, together with the assumptions underlying the structure, the interventions examined, the time horizon, and the disease states included**.** When used, the health state quality weights were clearly stated and referenced, but no study critically discussed the health valuation methodology or options available. Only two of the five Markov models reported the cycle length.[162,163] Data were well identified, modeled, and incorporated, although only three studies undertook a systematic review with a meta-analysis to define at least one model parameter.[158,162,163] All studies assessed parameter uncertainty with sensitivity or scenario analysis, but none of the studies addressed structural uncertainty, and only one explicitly conducted subgroup analysis.[158] Finally, internal consistency (the methodology of the model development) was not discussed in any study, although all but two compared their results to previous models' results, providing a measure of external consistency.[160,163]

Table 12. Summary of studies evaluating the cost-effectiveness of testing for FVL and/or prothrombin G20210A mutations

Study	Design	Interventions Compared	Population	Outcomes	Results*	Quality Deficits
Sarasin, 1998[164]	Decision analysis with a Markov model	Extending oral anticoagulation up to 5 years *vs.* 3 months oral anticoagulation for carriers and non-carriers of FVL	Probands: Individuals having had a DVT and 3 months of anticoagulation	DVT/PE prevented, major bleeding induced	For those with FVL, for all durations of anticoagulation, the number of major iatrogenic bleeding episodes exceeded the number of PEs prevented, although the number of bleeding episodes was far less than the number of DVTs averted, by a ratio of 1:3 after 1 year, and decreased with longer duration. If the bleeding risk was <2.5% per year, continued anticoagulation was preferred.	*Structure* – the time horizon of this model may not have captured the full treatment effect, the rejection of quality weights for health states made comparison of the outcomes difficult, and the Markov cycle length was unspecified; *Data* – the authors did not address methodological or structural uncertainty; *Consistency* – internal and external consistency were not discussed
Marchetti, 2000[163]	CEA, decision analysis with a Markov model	Screening for FVL with 2 years of warfarin *vs.* no screening with 6 months of warfarin	Probands: 60-year-old males with a personal history of VTE	Costs, QALYs, DVT/PE averted, major bleeding induced, life years	Testing for FVL, followed by extended anticoagulation, was cost-effective ($15,451/QALY). ICER > $50,000/QALY for individuals with high bleeding risk (>0.34% per year), low VTE recurrence rate (<9% in first 2 years), low anticoagulation efficacy (<74%), or low anticoagulation compliance (<94%).	*Structure* – a single anticoagulant duration modeled without discussion of alternatives or justification of their exclusion; *Data* – half-cycle correction not discussed, structural uncertainty not addressed; *Consistency* – internal consistency not discussed.
Marchetti, 2001[162]	CEA, decision analysis with a Markov model	Screening for double heterozygosity with 2 years of warfarin *vs.* no screening and 6 months of warfarin	Probands: 60-year-old males with personal history of VTE	Costs, QALYs, DVT/PE averted, major bleeding induced, life years	Testing for both mutations, with subsequent extended anticoagulation for double heterozygous individuals, was cost-effective (ICER $15,959/QALY). ICER > $50,000/QALY for individuals with high bleeding risk (>1.6% in 6 months), low double-heterozygote prevalence (<1.4%), low PE mortality (<10%), or low anticoagulation efficacy (<65%).	*Structure* – a single anticoagulant duration modeled without discussion of alternatives or justification of their exclusion; *Data* – half-cycle correction not discussed, structural uncertainty not addressed; *Consistency* – internal consistency not discussed.

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Table 12. Summary of studies evaluating the cost-effectiveness of testing for FVL and/or prothrombin G20210A mutations (continued)

Study	Design	Interventions Compared	Population	Outcomes	Results	Quality Deficits
Clark, 2002[160]	CEA, prospective cohort	Universal FVL screening or selective FVL screening based on personal or family history of VTE (prophylactic enoxaparin for FVL carriers in both cases) vs. no screening	Pregnant women attending antenatal care	Costs, pregnancy-related vascular complications	$15,173/vascular complication prevented for selective screening with prophylactic anticoagulation relative to no screening; $26,744/vascular complication prevented for universal screening with prophylactic anticoagulation relative to no screening (not considered cost-effective). ICER for selective screening with 100% effective anticoagulant prophylaxis was $5758/vascular complication averted relative to no screening.	*Structure* – model not described; scope, outcomes, and time horizon not justified; *Data* – assumption of prophylactic efficacy not discussed or justified, no discussion of treatment alternatives or quality of life weighting, no assessment of uncertainty beyond the parameter of anticoagulant efficacy; *Consistency* – no assessment of internal or external consistency
Eckman, 2002[161]	CEA, decision analysis with a Markov model	Screening for FVL with warfarin for 3 years or lifelong vs. no screening and 6 months of warfarin	Probands: 35-year-old females with a personal history of VTE	Costs, QALYs	Testing and treating with 3 years of anticoagulation dominated (in base case); $20,937/QALY for testing and treating with lifelong anticoagulation relative to no screening with 6 months anticoagulation, with a constant VTE rate at 7.3%/year. Model very sensitive to rate of recurrent VTE.	*Structure* – societal perspective claimed, though only direct healthcare costs modeled; Markov cycle length not explicitly stated; *Data* – half-cycle correction was not mentioned, quality of life weights not justified, only costs were discounted, structural uncertainty not addressed; *Consistency* – internal consistency not addressed

Table 12. Summary of studies evaluating the cost-effectiveness of testing for FVL and/or prothrombin G20210A mutations (continued)

Study	Design	Interventions Compared	Population	Outcomes	Results	Quality Deficits
Auerbach, 2004[159]	CEA, decision analysis with a Markov model	Hypercoagulability testing with anticoagulation for 6 months to indefinite depending on results vs. no testing with anticoagulation for 6 months to indefinite	Probands: 40-year-old following a DVT and 6 months of anticoagulation	Costs, QALYs	$13,365/QALY for testing followed by 24 months anticoagulation for positive individuals relative to no testing followed by 24 months anticoagulation for all individuals (base case). Cost-effective to test for disorders conferring a RR of recurrent VTE > 1.25 with a prevalence > 5%, followed by 2 years of anticoagulation for 2 years (or indefinitely if the relative risk exceeds 6). This would include testing for FVL and for double heterozygosity.	*Structure* – societal perspective stated but only direct costs incorporated, Markov cycle length unspecified; *Data* – half-cycle correction not mentioned, utility weights not justified, structural uncertainty not addressed; *Consistency* – internal consistency not discussed
Wu, 2006[158]	CEA, decision analysis model	Universal thrombophilia screening *vs.* selective screening based on personal and family VTE history *vs.* no screening	(1) women prior to OC use, (2) women prior to HRT, (3) pregnant women at 6 weeks of gestation, (4) patients prior to major orthopedic surgery	Costs, adverse clinical events prevented	Selective screening relative to no screening: $136,604/complication prevented in cohort 1, $4,226/complication prevented in cohort 2, $140,344/complication prevented in cohort 3 (not cost-effective), $15,780/complication prevented in cohort 4.	*Structure* – the short time horizon of the model may not have captured the full treatment effects, the absence of quality weights makes comparing the health outcomes difficult, and the pregnancy cohort included an aggregate outcome that is difficult to interpret; *Data* – structural uncertainty not addressed; *Consistency* – internal consistency not discussed

* Monetary values converted into U.S. dollars (2007)

CEA = cost-effectiveness analysis; DVT = deep venous thrombosis; FVL = Factor V Leiden; ICER = incremental cost-effectiveness ratio; HRT = hormone replacement therapy; OC = oral contraceptive; PE = pulmonary embolism; QALYs = quality-adjusted life years; RR = relative risk; VTE = venous thromboembolism

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Sarasin and Bounameaux used a decision analysis with a Markov model and a 5-year time horizon to assess the effectiveness of extending anticoagulation from 3 months for FVL carriers and non-carriers following an initial lower-limb DVT to 1, 2, 3, 4, or 5 years in a hypothetical cohort.[164] Quality weighting was explicitly not employed, since the authors expressed doubt that sufficient evidence existed to make the adjustments "reliable." Instead, the results were provided in terms of the health outcomes: major bleeding events induced and recurrent DVT and pulmonary embolisms (PE) prevented. The best-case scenario, in which the point estimates most favorable for extended anticoagulation were modeled from the range of values identified in the literature (a high rate of PE, maximum anticoagulant efficacy, and low bleeding risk), revealed that the number of bleeding events induced outnumbered the number of pulmonary emboli prevented across all extended anticoagulant durations among carriers and non-carriers of FVL. However, the number of DVTs prevented among FVL carriers greatly exceeded the number of bleeding events induced across all extended anticoagulant durations, and the incremental ratio of DVT prevented to bleeding events induced was highest for 2 years of anticoagulation. Assuming that PE are of equal importance with bleeding events, the authors stated that the risk of bleeding must be below 2.5 percent/year in order for prolonged anticoagulation to be the more effective strategy. A major limitation of this analysis is that it did not take into consideration differences in how patients might view these different types of complications. The time horizon of this model may not have captured the full treatment effects, and the Markov cycle length was unspecified. The authors did not address methodological or structural uncertainty or internal or external consistency.

Marchetti et al used decision analysis with a Markov model and lifetime time horizon to assess the cost-effectiveness of testing for FVL with 2 years of warfarin anticoagulation for carriers following VTE in a hypothetical cohort of 60-year-old men.[163] FVL testing with 2 years of anticoagulation for carriers was a cost-effective strategy (ICER = $12,833/QALY) when compared to no testing and 6 months of anticoagulation. However, this intervention was not cost-effective (ICER > $50,000/QALY) for individuals with a high risk of fatal bleeding on warfarin (>0.34 percent/year), low VTE recurrence rate (<9 percent in first 2 years), low anticoagulation efficacy (<74 percent), or low anticoagulation compliance (<94 percent). Cost figures were derived from the Italian health system. Quality deficits are discussed with the next study.

Building on their previous study, Marchetti et al. used the same model to assess the cost-effectiveness of testing for prothrombin G20210A and FVL, followed by 2 years of warfarin anticoagulation, in doubly heterozygous individuals.[162] Testing for both mutations was cost-effective (ICER = $13,624/QALY) when compared to no testing and 6 months of anticoagulation, and if the testing costs were <$43, the testing strategy was cost-saving. Testing was not cost-effective (ICER > $50,000/QALY) for patient populations with a high bleeding risk (>1.6 percent in 6 months), low double-heterozygote prevalence (<1.4 percent), low pulmonary embolism mortality (<10 percent), or low anticoagulation efficacy (<65 percent). The quality deficits of this study apply to the previous study as well.[163] The model structure examined a single anticoagulant duration, and the choice of this strategy was not discussed or justified. A half-cycle correction was not discussed, and structural uncertainty was not addressed. Internal consistency was not discussed.

Eckman et al. used decision analysis with a Markov model and a lifetime time horizon to assess the cost-effectiveness of testing for FVL and extending warfarin anticoagulation for 3 years or for life for carriers following a first VTE in a hypothetical cohort of 35-year-old women.[161] The cost-effectiveness of extending the duration of anticoagulation was highly

dependent on the rate of VTE recurrence used in the model. Testing followed by 3 years of anticoagulation was the dominant strategy (more effective and less costly) in scenarios that assumed a high rate of recurrence (16.3 percent/year) for the first 3 years and no or low (2.3 percent/year) recurrence beyond. If the rate of recurrence remained constant (7.3 percent/year), lifelong anticoagulation was the most cost-effective strategy (ICER = $16,823/QALY) when compared to no testing with 6 months of anticoagulation. Lifelong anticoagulation would be less cost-effective in patient populations with a low FVL prevalence, low risk of recurrent VTE, or risk factors for bleeding on anticoagulant therapy. Structurally, the authors claimed a societal perspective, although only direct healthcare costs were modeled, and the cycle length of the Markov model was not explicitly stated. A half-cycle correction was not mentioned, the quality of life weights were not justified, and only the costs were discounted. Structural uncertainty and internal consistency were not addressed.

Auerbach et al. used decision analysis with a Markov model and lifetime time horizon to assess the cost-effectiveness of a hypercoagulability testing panel and warfarin anticoagulation for 6, 12, 18, 24, or 36 months, or for life, following an apparently idiopathic deep vein thrombosis in a hypothetical cohort of 40-year-old individuals.[159] In the base case analysis, extending warfarin anticoagulation for 24 months following a positive test result was the most cost-effective option (ICER $11,100/QALY) when compared to the least costly option of not testing and treating for 24 months. Each test was evaluated separately in the hypercoagulability panel, and the authors concluded that tests detecting disorders present in at least 5 percent of the population that confer a relative risk exceeding 1.25, including FVL and prothrombin G20210A, should be included. The authors claimed a societal perspective but incorporated only direct costs, and the cycle length of the Markov model was unspecified. A half-cycle correction was not mentioned, the utility weights were not justified, and structural uncertainty was not addressed. Internal consistency was not discussed, although the results were discussed in relation to similar studies for individual hypercoagulable disorders.

Clark et al. used the cost and outcomes data from a prospective cohort of 967 pregnant women in the United Kingdom to assess the cost-effectiveness of FVL testing and enoxaparin anticoagulant prophylaxis in preventing pregnancy-related vascular complications over the 8-month time horizon from 12 weeks gestation to 6 weeks postpartum.[160] All women were screened for FVL, but the results of the test were not disclosed to the women until the conclusion of the study. No women actually received anticoagulant prophylaxis, but the hypothetical impact of treating FVL carriers with an assumed efficacy of 50 percent was modeled *ex post facto*. While testing all women and treating the FVL carriers identified was the most effective approach, testing only those women with a personal or family history of VTE was a more cost-effective approach (ICER = £7,535/vascular complication prevented) when compared to no screening or prophylaxis. The structure of the model was not described, and the scope, outcomes, and time horizon were not justified. Data were largely derived from the cohort, but the assumption of prophylactic efficacy was stated without discussion or justification. There was no discussion of treatment alternatives, and quality of life weighting was not used or discussed. There was also no assessment of internal or external consistency or uncertainty beyond the parameter of anticoagulant efficacy.

Wu et al. used a decision analysis model with a 12-month time horizon to assess the cost-effectiveness of universal or selective screening for FVL and the resultant changes in management for carriers in four cohorts with high VTE risk: women prior to combined oral contraceptive prescription, women prior to hormone replacement therapy, pregnant women at 6

weeks of gestation, and patients prior to major orthopedic surgery.[158] FVL carriers in the first two cohorts did not receive hormonal therapy, and carriers in the second two cohorts received 3 months of thromboprophylaxis in an effort to prevent adverse events (VTE or pregnancy-related vascular complications). Selective screening was based on a personal or family history of VTE. While universal screening was more effective in all cohorts, selective screening was more cost-effective in all cohorts. Selective screening was most cost-effective when compared to no screening in the case of the hormonal therapy cohort (ICER = £2,447/VTE prevented), followed by the surgery cohort (£9,136/VTE prevented), and it was less cost-effective in the case of the oral contraceptive cohort (£79,085/VTE prevented) and the pregnancy cohort (£81,250/pregnancy-related vascular complication prevented). The short time horizon of the model may not have captured the full treatment effects, and the absence of quality weights made comparing the health outcomes difficult. Structural uncertainty was not addressed, and internal consistency was not discussed.

Summary of the evidence for Key Question 4

We summarize the evidence regarding the clinical utility of testing for FVL and prothrombin G20210A as follows:

- Low-grade evidence indirectly supported the hypothesis that patient management by physicians may change on the basis of the results of testing for FVL or prothrombin G20210A.

- There was no direct evidence that testing for these mutations, and the resultant management**,** can reduce VTE related-outcomes in individuals who have had VTE or in the probands' family members who have been tested.

- There was moderate-grade evidence that treatment can reduce recurrent events in patients with FVL or prothrombin G20210A; however, the magnitude of this relative reduction was comparable to that seen in individuals without mutations.

- There was moderate-grade evidence that neither harms nor benefits have been conclusively demonstrated in individuals with VTE or their family members when tested for FVL or prothrombin G20210A.

- There was only low-grade evidence, coming from models, to suggest that testing for FVL alone, prothrombin G20210A alone, or the two tests in combination is cost-effective when caring for selected patients with VTE (those with a high risk of recurrence and/or low risk of bleeding) or their family members.

Chapter 4. Discussion

Our literature review was designed to identify evidence to inform our Key Questions. We identified literature that was directly applicable to answering the questions about analytic and clinical validity (Key Questions 2 and 3), but these studies were only indirectly relevant to the overarching question (Key Question 1). The literature was also less directly applicable to addressing the question about the clinical utility of testing for these mutations (Key Question 4).

Key Question 1: Association of FVL Testing, Alone or in Combination With Prothrombin *G20210A* Testing, With Improved Clinical Outcomes in Adults With a Personal History of VTE or in Adult Family Members of Mutation-positive Individuals.

We found no evidence that directly informed our overarching question (Key Question 1): Does FVL testing, alone or in combination with prothrombin *G20210A* testing, lead to improved clinical outcomes (e.g., avoidance of a recurrent VTE) in adults with a personal history of VTE or to improved clinical outcomes (e.g., avoidance of an initial VTE) in adult family members of mutation-positive individuals? In our discussion of Key Question 4, below, we address the implications of the absence of direct evidence, the value of indirect evidence, and the implications for future research.

One recent study that did not meet our inclusion criteria nevertheless provides some information to answer this overarching question.[165] We could not include this study by Coppens et al. in our review because the patients had a variety of different thrombophilic conditions for which they were tested. Using a case-control design, the authors investigated whether thrombophilia testing reduced the rates of recurrent VTE among persons with a history of VTE. From a cohort of patients enrolled in the Dutch-based Multiple Environmental and Genetic Assessment (MEGA) trial, 197 patients with VTE recurrence and 324 without recurrence were studied. Physicians had ordered thrombophilia testing in 35 percent of cases and 30 percent of controls. The odds ratio for recurrence in the tested versus the non-tested patients was 1.2 (95 percent CI, 0.8-1.9) and changed little after adjusting for age, sex, and VTE risk factors. These results suggest that thrombophilia testing did not affect the rate of VTE recurrence.

Key Question 2: Analytic Validity of Tests to Identify FVL and Prothrombin G20210A Mutations

An assessment of the analytic validity of a genetic test refers not only to the test's accurate identification of genotypes but also its reliability and robustness. Many of the studies we reviewed were preclinical (and pre-commercial) evaluations of assays. The majority of laboratories in the United States still use PCR-RFLP or invader chemistry technology, and less frequently the assays described in this report. The studies we reviewed demonstrated the high analytic validity of both the commercially available and pre-commercial tests. Most of the

discordant results resolved with repetition of the respective test, suggesting that operator or administrative errors were responsible for the discordant results.

The majority of the studies reviewed used the well established and commonly used PCR-RFLP or AS-PCR as the reference (gold) standards. There is a theoretical concern that these non-sequence-based methods may not distinguish between the single nucleotide mutations of interest (e.g., FVL) and other nearby benign polymorphisms and could potentially yield false-positive results. For example, a rare silent A1692C Factor V polymorphism would be mistakenly genotyped as a FVL allele in an RFLP assay. We saw false-positives attributable to this mechanism only very infrequently in the studies we reviewed.

In the quality assurance studies, we found evidence that most laboratories are highly accurate, even perfectly accurate, when asked to classify a sample with a known mutation. The majority of errors came from a limited numbers of laboratories. As we described, among the responding laboratories in one study, 51 percent made at least 1 error; however, three of the 39 laboratories were responsible for 46 percent of all errors.[63] We suspect that this situation illustrates a systematic quality assurance defect in these isolated laboratories. We did not review the evidence about how to identify laboratories that may perform poorly. We suggest that our findings underscore the need for ongoing internal and external quality control programs.

There are abundant technologies that *can* be used to detect the FVL and prothrombin G20210A mutations. Given the almost equal analytic validity of these methods, considerations such as shorter turn-around time, cost-effectiveness, high throughput, and availability of user-friendly software become important factors in selecting one method over another. The choice of methodology will likely be driven by considerations other than analytic validity.

Key Question 3: Clinical Validity of Testing for FVL and Prothrombin G20210A Mutations

Testing Probands

We found moderate-grade evidence that individuals with at least one prior thrombotic event who are homozygous or heterozygous for FVL have a higher risk of recurrent VTE than do those without the mutation. For heterozygous individuals, the odds ratio was 1.56 (95 percent CI, 1.14-2.12); for homozygous individuals, it was 2.65 (95 percent CI, 1.2-6).

There was moderate-grade evidence that prothrombin G20210A is not predictive of recurrent thrombosis. The odds ratio was 1.45 (95 percent CI, 0.96-2.2), similar to that for heterozygous FVL, but the confidence interval overlapped 1.0. There were too few pieces of data to allow us to refute or support the contention that homozygosity for prothrombin G20210A, a rare condition, is associated with recurrent thrombosis.

There was insufficient evidence that double heterozygosity (FVL plus prothrombin G20210A) is predictive of recurrent thrombosis. The pooled odds ratio was 4.8, but the confidence interval was wide, with very few studied individuals having double heterozygosity.

When we separately evaluated patients with idiopathic VTE as the index event, we found that the odds ratio associated with heterozygous FVL was close to one (1.17; 95 percent CI, 0.63-2.18), suggesting that that there may be little predictive value in knowing the mutation status in patients with idiopathic events.

There were limitations in this body of evidence, although the quality of the studies overall was fairly high. There was often insufficient description of potential confounders of the relationship between the mutation and the recurrent event. However, in those studies employing multivariate models that included other potential predictors of recurrent thrombosis, statistical adjustment did not result in any attenuation of the mutation-specific effect size,[14, 111,116,120,127] suggesting that these other clinical variables were not driving the relationship between mutation status and recurrent thrombosis. Although not all of the studies were primarily designed to answer our question about risk among probands, the prospective design that we required for inclusion in this review is the optimal design for answering this question.

Testing Family Members

We found high-grade evidence that family members of probands who are homozygous for the FVL mutation have a substantially increased risk of VTE when compared to family members who do not have this mutation. Homozygous family members may have a thrombosis rate nearly 20-fold that of individuals without homoyzgosity, or an event rate of 2 percent per year. There was moderate evidence that the risk of an event for heterozygous individuals is increased roughly three-fold (odds ratio, 3.4; 95 percent CI, 2.4-4.9). The absolute increase in the rate of events, however, was low.

We saw that the annualized rate of venous thromboembolic events for family members without a mutation was approximately 0.1 percent per year. This value translates to an event rate in heterozygous family members of 0.3 percent per year, or an absolute increase of 0.2 percent per year (a change from an average of 1/1000 person-years to 3/1000 person-years).

There was insufficient evidence regarding family members who are heterozygous for prothrombin G20210A, with an odds ratio of 1.9 (95 percent CI, 0.35-10). Doubly heterozygous individuals, with one FVL and one prothrombin G20210A mutation, can be expected to have event rates that are higher than those for singly heterozygous individuals but lower than those of family members who are homozygous for FVL (odds ratio, 6.7; 95 percent CI, 2.9-15) This conclusion was supported by only low-grade evidence. There was little information about the rare condition of homozygosity for the prothrombin G20210A mutation.

There was insufficient evidence to allow us to draw any conclusions about the risks associated with heterozygosity in FVL among pregnant family members. The point estimates were above one in both studies, but with very wide CIs. Because pregnancy and the postpartum period are high-risk times for VTE, the absolute increase in risk with pregnancy is likely to be much greater than the absolute risk increase from FVL. We suspect that the risk attributable to FVL during pregnancy is very small. There was only low-grade evidence that homozygosity for this mutation *may* increase the risk of venous thrombosis in pregnancy beyond that which is usually seen. The evidence concerning women who are doubly heterozygous for FVL and prothrombin G20210A was insufficient, and we could not draw any conclusions about the risk. We reviewed one additional informative study that could not be included because the pregnant women were *either* those with a personal history of thrombosis *or* a family history of thrombosis *or* a family history of thrombophilia. This study suggested that double heterozygosity does modestly raise the risk to women during their pregnancies.[166]

Key Question 4
Clinical Utility of Testing for FVL and Prothrombin G20210A Mutations

Effect of Testing on Clinicians' Management

There was low-grade evidence that patient management by clinicians is altered on the basis of the results of FVL testing, although there was no evidence to indicate whether this action improves patient outcomes or not. No information was available on management practices resulting from testing for prothrombin G20210A.We identified a single study addressing this question, which assessed practitioners' responses when presented with clinical scenarios.[150] The evidence to address this question can be considered to be only indirect, as this study asked clinicians to respond to hypothetical cases; there was no observation of practice patterns. This study was performed in Canada, where clinicians' management decisions may be influenced by the availability of resources and the health policy environment specific to that country. Also, all of the scenarios described pregnant women, and therefore the results may not be applicable to other patient populations.

We identified one additional study published after the end of our search period.[167] In this study by Hindorff et al., 112 primary care physicians in Washington state (60 frequent and 52 infrequent prescribers of FVL testing) responded to a survey about their motivations for testing for FVL (response rate of 67 percent). Approximately 82 percent of the providers indicated they would order FVL in order to advise patients about VTE recurrence, while 67 percent would order the test to make decisions about VTE treatment or prevention. Fewer than 40 percent of the clinicians reported a high level of confidence in interpreting or communicating the results of FVL testing, or a high degree of confidence in determining when it is appropriate to order the test. As in the study by Rodger et al,[150] this study did not provide direct evidence that clinicians manage patients differently based on FVL test results. However, the results suggest that physicians ordering this test may use results to inform management decisions. This study also highlighted a significant level of uncertainty regarding when to order FVL testing as well as how to interpret the results.

Effect of Testing and Resultant Management on VTE-related Outcomes

We conclude that there is no direct evidence that testing for FVL and prothrombin G20210A, and the resultant management, reduce VTE related-outcomes. In our search for indirect evidence to support or refute this hypothesis, we found high-grade evidence that anticoagulation reduces recurrent events in patients with FVL or prothrombin G20210A. However, there was only low-grade evidence that the magnitude of this relative reduction in outcomes is comparable to that seen in individuals without these mutations. Thus, the mutation status of the patient apparently does not, of necessity, play a role in the decision to extend anticoagulation in a patient with a history of VTE. This conclusion is based on four studies, none of which was specifically designed to directly answer the question about testing or treatment changes based on testing. The studies did not describe bleeding associated with anticoagulation that was stratified according to mutation.

The four studies we identified were heterogeneous in their designs and treatments. This body of evidence is limited by the relative lack of data on patients with prothrombin G20210A. Although Ridker et al. included individuals with prothrombin G20210A, these authors pooled them with the individuals having FVL for the analysis, so it was not possible to assess the effect of each mutation on the relationship between treatment and outcome.[151] Also, some important safety outcomes were not analyzed according to the type of mutation of the participants. Of the three studies that addressed the risks associated with anticoagulation,[122,123,151] only one reported these results as a function of mutation status.[123]

Effect of Testing and Results on Other Outcomes

We found moderate-grade evidence that little change in knowledge and behavior results from testing for FVL or prothrombin G20210A. Four studies directly answered this question; each study was designed to explore how FVL or prothrombin G20210A testing affected non-thromboembolic outcomes. The main themes from these studies were: (1) an individual's understanding of the risk factors for VTE or the significance of the test results was not improved after testing, unless structured counseling as well as access to information before and after testing was provided; (2) daily life changes were uncommon, although some patients used the test results to make important medical decisions; (3) most individuals did not regard carrier status as a serious condition but tended to worry about the implications for their children and relatives. We conclude that there is moderate evidence that the process of testing for these mutations does *not* have serious adverse consequences but may possibly improve understanding of VTE risk factors.

The main limitation of these studies was that they were all performed outside the United States. The political and cultural factors in these countries, as well as the healthcare environment, may have affected how the patients experienced genetic testing, interpreted the results, or behaved in response to those results. Another limitation of this body of evidence is that all of the studies involved self-selected participants who were interested in the research question, a situation that could have skewed the study results. Only one study reported how outcomes differed based on the test *result*. It may be useful for clinicians to know how patients are affected by the results of the test, and not just by the process of testing.

Two other important limitations were the paucity of studies addressing outcomes for patients undergoing prothrombin G20210A testing and the fact that all of the results were exclusively based on patient perceptions and behaviors. No study (except for the one by Wahlander et al.[123]) addressed clinical outcomes arising from changes in management as a consequence of testing (such as bleeding, mortality, or hospitalization rates) or quantifiable nonclinical outcomes, such as cost to patients or utilization of healthcare services.

Cost-effectiveness of FVL and prothrombin G20210A testing in the care of probands and their relatives

The cost-effectiveness studies all used decision analytic models. These models can point to the need for further testing of the utility of an intervention if the assumptions in the models are compatible with actual practice. The data ranges explored in the sensitivity analyses demonstrate the variables to which the cost-effectiveness of the interventions are most sensitive.

The five studies that modeled the experience of probands following a VTE all suggested that testing for FVL alone or in combination with testing for prothrombin G20210A could be cost-effective in certain patients.[159,161-164] The strategy of testing for FVL alone[161,163] or in combination with prothrombin G20210A,[159,162] with extended anticoagulation for 2 or 3 years for identified carriers, was either the dominant or the most cost-effective option in patient populations having a high prevalence of the mutations (e.g., 5 percent or more), a high risk of recurrence (e.g., 10 percent or more within 2 years), and a low risk of bleeding. The models were not robust. They were extremely sensitive to the parameters chosen for the input, and we challenge some of the assumptions that were used for the base case analyses.

The models' results were most sensitive to the rate of venous thrombosis recurrence, the prevalence of FVL and prothrombin G20210A mutations, the risk of adverse bleeding events, and anticoagulant efficacy. We compared the model input to the summary results from our meta-analyses. In our analyses, the odds ratio for recurrence for individuals heterozygous for FVL relative to non-carriers was 1.56. This odds ratio is *above* the threshold suggested by Auerbach et al. at which a test for the mutation should be included in a hypercoagulability panel.[159] Also, our pooled odds ratio was slightly higher than the value used in the model by Marchetti et al.; using our data for input would have more strongly favored FVL testing and extended anticoagulation.[163]

However, there are examples in these models of the use of parameters that seem flawed. One model made the assumption that there is an average risk of recurrence of 16 percent per year for the first 3 years, followed by 0 percent in subsequent years.[161] These percentages were based on a single study with a rate of recurrence that we found to be an outlier.[120] Similarly, one model assumed that prophylaxis against recurrent VTE with enoxaparin would be 50 percent effective.[160] This assumption was based on a study of women with antiphospholipid syndrome and may not be applicable to women with FVL during pregnancy.

Two studies considered the use of universal or selective testing for FVL[160] with or without prothrombin G20210A[158] in cohorts at high risk for thrombosis recurrence. The absence of quality-of-life weighting makes interpretation challenging. Without a way to standardize outcomes, the models' results can only be meaningfully compared within a study, as in Wu et al,[158] or across similar studies, as in the pregnancy cohorts of Wu et al [158] and Clark et al.[160] Although these models shared many assumptions, the incremental cost-effectiveness ratios differed, presumably because of the different costs included in the models. However, *selective* screening based on personal or family history of VTE was preferred in both studies, although the authors of both studies acknowledged that it may be challenging to obtain an accurate family history.

While an incremental cost-effectiveness ratio must be interpreted carefully in the context of the study from which it is derived, the values obtained in these four studies, which ranged from $11,100 to $13,624/ QALY, were well within the range considered to be cost-effective in the economic evaluation literature. Lifelong anticoagulation was modeled in two studies[159,161] but was the preferred option only in scenarios of high recurrent VTE risk and low risk of anticoagulant-induced bleeding.

Testing for FVL or for FVL and prothrombin G20210A, followed by extended anticoagulation for 2 to 3 years in a carrier following a VTE *could* prove to be a cost-effective strategy, although these models have demonstrated that the level of cost-effectiveness depends heavily on the prevalence of the mutations, the risk of recurrent VTE, the risk of bleeding, and the effectiveness of anticoagulation. Lifelong anticoagulation after testing may prove to be cost-

effective for individuals with a very high risk of recurrent VTE and low risk of anticoagulant-induced bleeding events. The studies offer a robust model structure to assess the potential effectiveness and cost-effectiveness of various interventions.

The models are a useful starting point in the evaluation of cost-effectiveness, but since the literature did not strongly support the *effectiveness* of testing, the results of cost-effectiveness models are challenging to interpret and apply to patient care.

Summary of the Evidence

In Tables 13-15 (see also Appendix G, Evidence Table 28), we summarize the evidence to answer our Key Questions. In brief, we found no direct evidence to indicate whether testing for FVL and prothrombin G20210A improves outcomes for probands or family members. There was high-grade evidence to support the conclusion that existing laboratory assays accurately detect these mutations, and most laboratories do an adequate job of detecting these mutations in a clinical setting.

There was moderate-grade evidence that homozygosity or heterozygosity for FVL is predictive of recurrent thromboembolism among probands, and high-grade evidence that homozygosity is predictive of VTE events in family members of probands. There was moderate-grade evidence that heterozygosity for FVL is predictive of thromboembolic events in family members and that heterozygosity for prothrombin G20210A is not predictive of VTE in probands; there was insufficient evidence as to whether double heterozygosity is predictive. There was low-grade evidence that double heterozygosity is predictive of VTE in family members and insufficient information to allow us to draw any conclusions about the predictive value of homozygosity for prothrombin G20210A.

There was low-grade evidence that clinicians might change their practice based on testing results. There was high-grade evidence that anticoagulation can reduce VTE in individuals with these mutations, but only low-grade evidence that the relative risk reduction is comparable to that in individuals without mutations. There was moderate-grade evidence that there are neither harms nor benefits associated with these genetic tests. The modeling studies suggest that testing followed by treatment (for 2 to 3 years) for carriers of a mutation could be cost-effective, although the level of this evidence was only low-grade.

Table 13. Strength of the evidence regarding Key Questions 1 and 2: overarching question and analytic validity

KQ	Evidence statement	Strength of evidence
1	Overarching question: Testing for FVL testing alone, or in combination with prothrombin G20210A testing leads to improved clinical outcomes (e.g., avoidance of a recurrent VTE) in adults with a personal history of VTE, or to improved clinical outcomes (e.g., avoidance of an initial VTE) in adult family members of mutation-positive individuals.	No direct evidence supports this statement; the indirect evidence described below contributes to answering this question
2	Tests for detection of FVL have excellent analytic validity.	High-grade evidence
2	Tests for detection of prothrombin G20210A have excellent analytic validity.	High-grade evidence
2	Most, but not all, clinical laboratories can test for FVL and prothrombin G20210A very accurately.	High-grade evidence

Table 14. Strength of the evidence regarding Key Question 3: clinical validity

Factor V Leiden			Prothrombin G20210A		
Probands	**Pooled Odds Ratio [95% CI]**	**Evidence Grade**	**Probands**	**Pooled Odds Ratio [95% CI]**	**Evidence Grade**
Heterozygous	1.56 [1.14-2.12]	Moderate grade, predictive	Heterozygous	1.45 [0.96-2.2]	Moderate grade, not predictive
Homozygous	2.65 [1.2-6]	Moderate grade, predictive	Homozygous	---	---
Doubly heterozygous	4.8 [0.50-46]	Insufficient			
Family members	**Pooled Odds Ratio [95% CI]**	**Evidence Grade**	**Family members**	**Pooled Odds Ratio [95% CI]**	**Evidence Grade**
Heterozygous	3.4 [2.4-4.9]	Moderate grade, predictive	Heterozygous	1.9 [0.35-10]	Insufficient
Homozygous	18 [7.8-40]	High grade, predictive	Homozygous	---	
Doubly heterozygous	6.7 [2.9-15]	Low grade, predictive			
Pregnant family members	**Odds Ratios, Not Pooled [95% CI]**	**Evidence Grade**	**Pregnant Family Members**	**Odds Ratios, Not Pooled [95% CI]**	**Evidence Grade**
Heterozygous	5.4[0.65-46]; 3.4[0.35-33]	Insufficient	Heterozygous	3.0 [0.12-74]; 2.3 [0.21-25]	Insufficient
Homozygous	16 [0.9 - 278]; 41[5.5-419]	Low grade, predictive	Homozygous	---	
Doubly heterozygous	4.1 [0.37-44]; 15.3 [1-232]	Insufficient			

Table 15. Strength of the evidence regarding Key Question 4: clinical utility

KQ	Evidence statement	Strength of evidence
4	Patient management by physicians changes based on the results of testing for FVL or prothrombin G20210A.	Low-grade evidence
4	Knowledge of test results reduces VTE related-outcomes in individuals who have had VTE or in the probands' family members who have been tested.	No evidence supports this statement
4	Anticoagulation can reduce recurrent events in patients with FVL or prothrombin G20210A	High-grade evidence
4	The magnitude of this relative reduction with anticoagulation is comparable to that seen in individuals without mutations.	Low-grade evidence
4	Neither harms nor benefits have been demonstrated conclusively in individuals with VTE or their family members when tested for FVL or prothrombin G20210A.	Moderate-grade evidence
4	Testing for FVL alone, prothrombin G20210A alone, or the two tests in combination may be cost-effective when caring for selected patients with VTE (those with a high risk of recurrence and/or low risk of bleeding) or their family members.	Low-grade evidence (from models)

CI = confidence interval; FVL = Factor V Leiden; VTE = venous thromboembolism

Limitations of This Report

In addition to the reported deficits in the literature, there are limitations to this report. In our assessment of clinical validity, we used pooled odds ratios rather than time-dependent measures of recurrence (such as hazard ratios or incident rate ratios). This approach necessarily excluded some studies from the pooled estimates. We recognize that odds ratios may be biased if the follow-up duration varied systematically between individuals with and without the mutations. However, there is little reason to suspect that follow-up duration varied according to mutation status in these prospective studies. In the studies that reported time-dependent analyses, the results of these analyses were generally similar in direction and magnitude to the unadjusted odds ratios that we calculated from the raw event data.[113] Odds ratios are often misinterpreted as being highly clinically significant when the absolute difference in the rates of events is very low. We did not calculate pooled rates of events, since we expected the rates of events in the probands to be very dependent on the timing of the study relative to the index event and change over time.[26,110,111,125,138]

The odds ratios should approximate the relative rates of events in most studies, since these were relatively rare outcomes. We pooled the results using the DerSimonian and Laird random effects methods, a conservative method that often results in wide confidence intervals. In our sensitivity analyses, we also repeated the pooling using several fixed effect methods. Given the near-absence of heterogeneity among the studies in our comparisons, the results were very similar. We opted to report the results from our pooling incorporating random effects because we think this more accurately represents the truth (the odds ratios for the individual studies coming from a distribution of the odds ratios).

We opted not to pool time-dependent outcomes, including the rates of VTE. The reporting of time-dependent outcomes has inherent limitations in a study of thrombosis recurrence, since recurrence rates are highest in the months following anticoagulation cessation. This situation

renders absolute event rates (such as the number of events per 100-patient years) challenging to interpret, because a longer duration of follow-up after termination of anticoagulation will tend to bias the results in the direction of lower annualized incidence rates. For this reason, we did not primarily compare incidence rates across studies when the follow-up or anticoagulation duration varied or was unstated.

Another potential source of bias was that anticoagulation practices are *not* independent of mutation status (e.g., the longer-duration anticoagulation or more aggressive preventive strategies in those with mutations). Most studies mitigated this potential difficulty by excluding patients who were chronically anticoagulated[14,110,113,114,118,119,121,123-125] or by using a pre-defined anticoagulation approach.[116,119,123-125]

In those studies that reported the duration of anticoagulation after the index event in mutation-positive and -negative subgroups, there was no obvious discordance in the anticoagulation duration between the two groups.[111,112,120,121,127] If any bias was introduced by changes in clinical management based on knowledge of mutation status, it would tend to reduce the association between the mutation(s) and recurrent events, presuming that mutation positivity led to more intensive anticoagulation.

There was substantial heterogeneity in the composition of the control groups across studies, a matter of concern in that the rates of events in the control groups could have differed substantially. However, all studies were internally consistent, in that those that included other prothrombotic defects in the control group included those same defects in the group with mutations. When data were presented on the prevalence of other defects in groups with and without mutations, there was no evidence that the prevalence of other thrombophilic defects differed across groups.[108,112,113] We cannot exclude, however, *an interaction* between other thrombophilic defects and our mutations of interest and thrombosis recurrence. In these cohort studies, ascertainment bias is possible. In the studies of probands, the individuals were not blinded to their mutation status. It is possible that patients with mutations were more likely to seek medical attention for symptoms consistent with deep vein thrombosis or pulmonary embolism and might have been over-diagnosed with recurrence (due to false-positive testing), or that those without mutations were under-diagnosed (because they did not seek medical attention for a thrombotic event that ultimately resolved without therapy). Ascertainment bias would tend to augment the association between the mutations and recurrent thrombosis. None of the studies we included had scheduled periodic radiographic testing to limit the potential for ascertainment bias.

The majority of the observational studies concerning family members were retrospective, with some notable exceptions.[129-133] Retrospective studies are prone to important biases, including recall bias. Although this potential source of bias can be mitigated by interviewing participants before they have knowledge of their mutation status, this process was variably described in these studies.

The limitations that are specific to Key Question 4 – the clinical utility question – are described above in the discussion of that question.

Implications for Future Research

Studies to directly address our overarching question (Key Question 1) would ideally be designed as trials in which participants with venous thrombosis, and/or their family members, would be randomized to a test arm or a no-test arm. Individuals would be managed by their

physicians on the basis of the test results (with evidence-based recommendations). Sufficient follow-up time would be included in the study design so that VTE events could be witnessed and compared between the tested and untested groups. An alternative approach would be a well-designed prospective cohort study. The population of interest would be defined as individuals with recent VTE. The subgroup of these individuals who had testing for the mutation would be considered the "exposed" group. A comparison group of closely comparable individuals who were not "exposed" to testing would also be defined, and the outcomes in the groups would be compared, with careful attention to complete follow-up in both groups.

Analytic Validity

Although the mutation detection methods were found to have high analytic validity, a small minority of laboratories accounted for a disproportionate percentage of the errors in the performance of these tests. This result suggests an ongoing need for participation of molecular diagnostic laboratories in external quality assurance programs to assure consistent provision of high-quality genetic testing services. In the United States, laboratories doing molecular testing on human samples are required to follow the guidelines set by the Clinical Laboratory Improvement Amendments (CLIA) 1988 that include requirements for proficiency testing. In addition, a large majority of the laboratories performing these tests have additional accreditation from bodies such as from the College of American Pathologists, which also requires semi-annual proficiency testing to ensure accurate and precise testing.

Clinical Validity

Future studies should report event rates over time (and relative rates of recurrence between specified groups), rather than just the number of events. Studies should consistently differentiate between heterozygous and homozygous individuals, since there is a different rate of recurrence in these two groups. Future studies should continue to use objectively measured thrombosis (radiographically proven) as a criterion for the index and recurrent thromboses but should provide more detail about both the index events and the recurrence, including whether the events had other precipitants (e.g., peri-procedural, idiopathic, associated with hospitalization, or cancer-associated). Such data were presented in an inconsistent fashion in the studies we reviewed, and when reported, they were generally given for the entire study cohort, rather than separately for the groups of interest. By examining specific subsets of patients, it may be possible to clarify whether there are any interactions between mutation status and clinical variables in terms of predicting recurrence. With regard to the prothrombin G20210A mutation (alone or in conjunction with FVL), additional studies are needed to more precisely quantify the effect size.

There remains uncertainty about the estimates of risk for family members, given the very wide confidence intervals surrounding the odds ratios and the rarity with which the studies reported actual rates of events (rather than counts). Also, the studies that we included were exclusively studies of European populations. It is well known that the mutation frequency varies markedly across populations (and is particularly low in African-derived populations), but it is still unclear whether the risk attributable to the mutation differs in other populations having different genetic or environmental contributors to VTE risk. Future research would be

appropriate in Caucasian populations outside of Europe or in other populations with appreciable frequencies of mutations. Also, future research could better explore the age-mutation interaction.

Clinical Utility

Future studies should directly address whether clinicians change their recommendations in response to the results of FVL and prothrombin G20210A testing. Rather than surveys based on hypothetical situations, we suggest that chart reviews or analyses of utilization data (such as tracking prescriptions or the number of referrals) based on actual patients referred for testing would more directly and cogently answer this question.

To assist clinicians in the management of patients with VTE, future studies should be powered sufficiently to evaluate the *risks* associated with prolonged anticoagulation, as they relate to patients with specific thrombophilic mutations. Future studies addressing this question might move away from primarily focusing on the effect of treatment on absolute recurrence rate toward whether *management decisions* based on testing results affect the rates of recurrence in carriers of each of these mutations. Even though the evidence suggested that neither FVL nor prothrombin G20210A attenuates the prevention of recurrence during ongoing anticoagulation in probands, future studies in both probands and family members might focus on whether management decisions (duration of therapy, use of thromboprophylaxis) affect rates of VTE, particularly during times of heightened thromboembolic risk.

Future studies should ensure an adequate representation of patients with FVL and prothrombin G20210A. Studies based in the United States may give a clear understanding of how patients here might respond to the testing process and results. Larger sample sizes should also be used to increase the ability to detect rarer events, such as stigmatization and discrimination by insurers. Efforts should be made to recruit representative patient populations, and relevant comparison groups should be included (e.g., carriers and non-carriers) to increase the practical applicability of the study findings. Quantitative studies may be preferable, involving the use of standardized, validated questionnaires to evaluate patients' experiences.

The cost-effectiveness analyses should be updated when there are additional data to support the assumptions of the models and the factors on which the results most depend, including the magnitude and duration of VTE recurrence risk, anticoagulant efficacy in preventing recurrent VTE, and anticoagulant-induced bleeding risk. To more definitively determine the cost-effectiveness of testing for these mutations, clinical trials could include an assessment of the costs associated with a testing strategy, as compared to care without testing.

Our literature review included articles through December 2008. We do not anticipate any important secular changes in the event rate that would markedly change the event rates in the upcoming years. We also do not expect major changes in the coming years in terms of the methods used to detect mutations. The most anticipated change would be an increase in options to reduce risk as new drugs become available. Future research will need to include an evaluation of the risks and benefits associated with use of new anticoagulant drugs in probands and family members at high risk of events.

References

1. Silverstein MD, Heit JA, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ 3rd. Trends in the incidence of deep vein thrombosis and pulmonary embolism: a 25-year population-based study. Arch Intern Med. 1998; 158(6):585-93.
2. White RH. The epidemiology of venous thromboembolism. Circulation 2003; 107(23 Suppl 1):I4-8.
3. Seligsohn U, Lubetsky A. Genetic susceptibility to venous thrombosis. N Engl J Med 2001; 344(16):1222-31.
4. Eldor A. The use of low-molecular-weight heparin for the management of venous thromboembolism in pregnancy. Eur J Obstet Gynecol Reprod Biol 2002; 104(1):3-13.
5. Warkentin TE. Heparin-induced thrombocytopenia: a clinicopathologic syndrome. Thromb Haemost. 1999; 82(2):439-47.
6. Welch GN, Loscalzo J. Homocysteine and atherothrombosis. N Engl J Med. 1998; 338(15):1042-50.
7. Khajuria A, Houston DS. Induction of monocyte tissue factor expression by homocysteine: a possible mechanism for thrombosis. Blood 2000; 96(3):966-72.
8. Undas A, Williams EB, Butenas S, Orfeo T, Mann KG. Homocysteine inhibits inactivation of factor Va by activated protein C. J Biol Chem. 2001; 276(6):4389-97.
9. Stein PD, Hull RD, Kayali F, Ghali WA, Alshab AK, Olson RE. Venous thromboembolism according to age: the impact of an aging population. Arch Intern Med 2004; 164(20):2260-5.
10. Crowther MA, Roberts J, Roberts R *et al*. Fibrinolytic variables in patients with recurrent venous thrombosis: a prospective cohort study. Thromb Haemost. 2001; 85(3):390-4.
11. Koster T, Rosendaal FR, Briet E, Vandenbroucke JP. John Hageman's factor and deep-vein thrombosis: Leiden thrombophilia Study. Br J Haematol. 1994; 87(2):422-4.
12. Ridker PM, Vaughan DE, Stampfer MJ *et al*. Baseline fibrinolytic state and the risk of future venous thrombosis. A prospective study of endogenous tissue-type plasminogen activator and plasminogen activator inhibitor. Circulation 1992; 85(5):1822-7.
13. Roberts HR, Stinchcombe TE, Gabriel DA. The dysfibrinogenaemias. Br J Haematol. 2001; 114(2):249-57.
14. Kyrle PA, Minar E, Bialonczyk C, Hirschl M, Weltermann A, Eichinger S. The risk of recurrent venous thromboembolism in men and women. N Engl J Med 2004; 350(25):2558-63.
15. Hansson PO, Sorbo J, Eriksson H. Recurrent venous thromboembolism after deep vein thrombosis: incidence and risk factors. Arch Intern Med 2000; 160(6):769-74.
16. Heit JA, Mohr DN, Silverstein MD, Petterson TM, O'Fallon WM, Melton LJ 3rd. Predictors of recurrence after deep vein thrombosis and pulmonary embolism: a population-based cohort study. Arch Intern Med. 2000; 160(6):761-8.
17. Kearon C, Ginsberg JS, Kovacs MJ *et al*. Comparison of low-intensity warfarin therapy with conventional-intensity warfarin therapy for long-term prevention of recurrent venous thromboembolism . N Engl J Med 2003; 349(7):631-9.
18. Lee AY, Levine MN, Baker RI *et al*. Low-molecular-weight heparin versus a coumarin for the prevention of recurrent venous thromboembolism in patients with cancer. N Engl J Med 2003; 349(2):146-53.
19. Palareti G, Legnani C, Cosmi B *et al*. Predictive value of D-dimer test for recurrent venous thromboembolism after anticoagulation withdrawal in subjects with a previous idiopathic event and in carriers of congenital thrombophilia. Circulation 2003; 108(3):313-8.
20. Prandoni P, Lensing AW, Prins MH *et al*. Residual venous thrombosis as a predictive factor of recurrent venous thromboembolism. Ann Intern Med 2002; 137(12):955-60.
21. Kujovich JL. Factor V Leiden Thrombophilia. GeneReview on GeneTests, Last Updated 5/20/04 .
22. Spector EB, Grody WW, Matteson CJ *et al*. Technical standards and guidelines: venous thromboembolism (Factor V Leiden and prothrombin 20210G >A testing): a disease-specific supplement to the standards and guidelines for clinical genetics laboratories. Genet Med 2005; 7(6):444-53.
23. Ridker PM, Miletich JP, Hennekens CH, Buring JE. Ethnic distribution of factor V Leiden in 4047 men and women. Implications for venous thromboembolism screening. JAMA 1997; 277(16):1305-7.
24. Chang MH, Lindegren ML, Butler MA *et al*. Prevalence in the United States of selected candidate gene variants: Third National Health and Nutrition Examination Survey, 1991-1994. Am J Epidemiol 2009; 169(1):54-66.

25. Emmerich J, Rosendaal FR, Cattaneo M *et al*. Combined effect of factor V Leiden and prothrombin 20210A on the risk of venous thromboembolism--pooled analysis of 8 case-control studies including 2310 cases and 3204 controls. Study Group for Pooled-Analysis in Venous Thromboembolism. Thromb Haemost 2001; 86(3):809-16.

26. Martinelli I, Bucciarelli P, Margaglione M, De Stefano V, Castaman G, Mannucci PM. The risk of venous thromboembolism in family members with mutations in the genes of factor V or prothrombin or both. Br J Haematol 2000; 111(4):1223-9.

27. http:, www.fda.gov/cdrh/pdf6/K060564.pdf.

28. http:, www.fda.gov/cdrh/pdf3/k033612.pdf.

29. Sanderson S, Zimmern R, Kroese M, Higgins J, Patch C, Emery J. How can the evaluation of genetic tests be enhanced? Lessons learned from the ACCE framework and evaluating genetic tests in the United Kingdom. Genet Med 2005; 7(7):495-500.

30. Berlin JA. Does blinding of readers affect the results of meta-analyses? University of Pennsylvania Meta-analysis Blinding Study Group. Lancet 1997; 350(9072):185-6.

31. Bossuyt PM, Reitsma JB, Bruns DE *et al*. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Fam Pract 2004; 21(1):4-10.

32. Segal J, Strouse J, Beach M *et al*. Hydroxyurea for the Treatment of Sickle Cell Disease. Evidence Report/Technology Assessment No. 165. (Prepared by Johns Hopkins University Evidence-Based Practice Center Under Contract No. 290-02-0018). AHRQ Publication No. 08-E007. Rockville, MD. Agency for Healthcare Research and Quality 2008.

33. Marchionni L , Wilson R, Marinopoulos S *et al*. Impact of Gene Expression Profiling Tests on Breast Cancer Outcomes. Evidence Report/Technology Assessment No. 160. (Prepared by The Johns Hopkins University Evidence-Based Practice Center Under Contract No. 290-02-0018). AHRQ Publication No. 08-E002. Rockville, MD. Agency for Healthcare Research and Quality 2008.

34. The Joanna Briggs Institute. Appraisal, extraction and pooling of evidence arising out of qualitative research. Accessed December 01, 2008 from www.joannabriggs.edu.au/cqrmg/documents/QARI_CQRMG.pps .

35. Philips Z, Ginnelly L, Sculpher M *et al*. Review of guidelines for good practice in decision-analytic modelling in health technology assessment. Health Technol Assess 2004; 8(36):iii-iv, ix-xi, 1-158.

36. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986; 7(3):177-88.

37. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003; 327(7414):557-60.

38. Duval S, Tweedie R. Trim and fill: A simple funnel-plot-based method of testing and adjusting for publication bias in meta-analysis. Biometrics 2000; 56(2):455-63.

39. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ 1997; 315(7109):629-34.

40. Atkins D, Best D, Briss PA *et al*. Grading quality of evidence and strength of recommendations. BMJ 2004; 328(7454):1490.

41. Teutsch SM, Bradley LA, Palomaki GE *et al*. The Evaluation of Genomic Applications in Practice and Prevention (EGAPP) initiative: methods of the EGAPP Working Group. Genet Med 2008.

42. Coppens M, van de Poel MH, Bank I *et al*. A prospective cohort study on the absolute incidence of venous thromboembolism and arterial cardiovascular disease in asymptomatic carriers of the prothrombin 20210A mutation. Blood 2006; 108(8):2604-7.

43. Hessner MJ, Budish MA, Friedman KD. Genotyping of factor V G1691A (Leiden) without the use of PCR by invasive cleavage of oligonucleotide probes. Clin Chem 2000; 46(8 Pt 1):1051-6.

44. Ledford M, Friedman KD, Hessner MJ, Moehlenkamp C, Williams TM, Larson RS. A multi-site study for detection of the factor V (Leiden) mutation from genomic DNA using a homogeneous invader microtiter plate fluorescence resonance energy transfer (FRET) assay. J Mol Diagn 2000; 2 (2):97-104.

45. Nauck M, Marz W, Wieland H. Evaluation of the Roche diagnostics LightCycler-Factor V Leiden Mutation Detection Kit and the LightCycler-Prothrombin Mutation Detection Kit. Clin Biochem 2000; 33(3):213-6.

46. Pecheniuk NM, Marsh NA, Walsh TP. Multiple analysis of three common genetic alterations associated with thrombophilia. Blood Coagul Fibrinolysis 2000; 11(2):183-9.

47. Benson JM, Ellingsen D, El-Jamil M *et al*. Factor V Leiden and factor V R2 allele: high-throughput analysis and association with venous thromboembolism. Thromb Haemost 2001; 86(5):1188-92.

48. Parks SB, Popovich BW, Press RD. Real-time polymerase chain reaction with fluorescent hybridization probes for the detection of prevalent mutations causing common thrombophilic and iron overload phenotypes. Am J Clin Pathol 2001; 115(3):439-47.

49. Potter CG, Liu YT, Rees DC. Factor V Leiden mutation screened by PCR and detected with lanthanide-labeled probes. Genet Test 2001; 5(4):291-7.

50. Hung K, Sun X, Ding H, Kalafatis M, Simioni P, Guo B. A matrix-assisted laser desorption/ionization time-of-flight based method for screening the 1691G --> A mutation in the factor V gene. Blood Coagul Fibrinolysis 2002; 13(2):117-22.

51. Angeline T, Bentley HA, Hawk AB *et al*. Prevalence of the Factor V G1691A and the Factor II/prothrombin G20210A gene polymorphisms among Tamilians. Exp Mol Pathol 2005; 79(1):9-13.

52. Meyer M, Kutscher G, Vogel G. The prothrombin nt20210 A allele as a risk factor for venous thromboembolism: detection of heterozygous and homozygous carriers by alternative methods. Thromb Res 2000; 97(5):359-63.

53. Gellings A, Holzem G, Wielckens K, Klingler KR. Prothrombin mutation: Employing the electrochemiluminescence technology of the elecsys(registered trademark) system for the detection of the point mutation at position 20210 in the 3' untranslated region of the prothrombin gene: LaboratoriumsMedizin 2001; 25(1-2):26-30.

54. Gilchrist A, Solomon N, Erickson D *et al*. Automated detection of the G20210A prothrombin mutation using the LCx microparticle enzyme immunoassay. Clin Chim Acta 2001; 314(1-2):249-54.

55. Humeny A, Bonk T, Berkholz A, Wildt L, Becker CM. Genotyping of thrombotic risk factors by MALDI-TOF mass spectrometry. Clin Biochem 2001; 34(7):531-6.

56. Endler G, Kyrle PA, Eichinger S, Exner M, Mannhalter C. Multiplexed mutagenically separated PCR: simultaneous single-tube detection of the factor V R506Q (G1691A), the prothrombin G20210A, and the methylenetetrahydrofolate reductase A223V (C677T) variants. Clin Chem 2001; 47(2):333-5.

57. Koksal V, Baris I, Etlik O. Primer-engineered multiplex PCR-RFLP for detection of MTHFR C677T, prothrombin G20210A and factor V Leiden mutations. Exp Mol Pathol 2007; 83(1):1-3.

58. Kowalski A, Radu D, Gold B. Colorimetric microwell plate detection of the factor V Leiden mutation. Clin Chem 2000; 46(8 Pt 1):1195-8.

59. Rhodes RB, Lewis K, Shultz J *et al*. Analysis of the factor V Leiden mutation using the READIT Assay. Mol Diagn 2001; 6(1):55-61.

60. Moser MJ, Marshall DJ, Grenier JK *et al*. Exploiting the enzymatic recognition of an unnatural base pair to develop a universal genetic analysis system. Clin Chem 2003; 49(3):407-14.

61. Murphy K, Hafez M, Philips J, Yarnell K, Gutshall K, Berg K. Evaluation of temperature gradient capillary electrophoresis for detection of the Factor V Leiden mutation: coincident identification of a novel polymorphism in Factor V. Mol Diagn 2003; 7(1):35-40.

62. Louis M, Dekairelle AF, Gala JL. Rapid combined genotyping of factor V, prothrombin and methylenetetrahydrofolate reductase single nucleotide polymorphisms using minor groove binding DNA oligonucleotides (MGB probes) and real-time polymerase chain reaction. Clin Chem Lab Med 2004; 42(12):1364-9.

63. Hertzberg M, Neville S, Favaloro E, McDonald D. External quality assurance of DNA testing for thrombophilia mutations. Am J Clin Pathol 2005; 123(2):189-93.

64. DelRio-LaFreniere SA, McGlennen RC. Simultaneous allele-specific amplification: a strategy using modified primer-template mismatches for SNP detection--application to prothrombin 20210A (factor II) and factor V Leiden (1691A) gene mutations. Mol Diagn 2001; 6(3):201-9.

65. Ozkan D, Erdem A, Kara P *et al*. Allele-specific genotype detection of factor V Leiden mutation from polymerase chain reaction amplicons based on label-free electrochemical genosensor. Anal Chem 2002; 74(23):5931-6.

66. Lopez M, Giraldo P, Alvarez P *et al*. Multiplex assay for genetic testing of thrombophilia: a method for routine clinical care. J Clin Lab Anal 2007; 21(6):349-55.

67. Patnaik M, Dlott JS, Fontaine RN *et al*. Detection of genomic polymorphisms associated with venous thrombosis using the invader biplex assay. J Mol Diagn 2004; 6(2):137-44.

68. Schroell-Metzger B, Dicato M, Bosseler M, Berchem G. Comparison of standard PCR and the LightCycler technique to determine the thrombophilic mutations: an efficiency and cost study. Clin Chem Lab Med 2003; 41(4):482-5.

69. Mammo L, Sheereen A, Saour T, Saour J. Comparative study between the Light Cycler and the PCR-restriction fragment length polymorphism in detecting factor V Leiden and factor II 20210G>A mutations. Clin Biochem 2006; 39(7):767-9.

70. Cooper PC, Cooper SM, Smith JM, Kitchen S, Makris M. Evaluation of the Roche LightCycler: a simple and rapid method for direct detection of factor V Leiden and prothrombin G20210A genotypes from blood samples without the need for DNA extraction. Blood Coagul Fibrinolysis 2003; 14(5):499-503.

71. Behrens M, Lange R. A highly reproducible and economically competitive SNP analysis of several well characterized human mutations. Clin Lab 2004; 50(5-6):305-16.

72. Ozsoz M, Erdem A, Kerman K *et al*. Electrochemical genosensor based on colloidal gold nanoparticles for the detection of Factor V Leiden mutation using disposable pencil graphite electrodes. Anal Chem 2003; 75(9):2181-7.

73. Huang TJ, Liu M, Knight LD, Grody WW, Miller JF, Ho CM. An electrochemical detection scheme for identification of single nucleotide polymorphisms using hairpin-forming probes. Nucleic Acids Res 2002; 30(12):e55.

74. Simundic AM, Topic E, Stefanovic M. Detection of factor V Leiden by PCR-SSCP using GMA precast Elchrom scientific gels. Clin Appl Thromb Hemost 2003; 9(3):227-31.

75. Sanders Sevall J. Factor V Leiden genotyping using real-time fluorescent polymerase chain reaction. Mol Cell Probes 2000; 14(4):249-53.

76. Liew M, Pryor R, Palais R *et al*. Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons. Clin Chem 2004; 50(7):1156-64.

77. Verri A, Focher F, Tettamanti G, Grazioli V. Two-step genetic screening of thrombophilia by pyrosequencing. Clin Chem 2005; 51(7):1282-4.

78. Reyes AA, Ugozzoli LA, Lowery JD *et al*. Linked linear amplification: a new method for the amplification of DNA . Clin Chem 2001; 47(1):31-40.

79. Castley A, Higgins M, Ivey J, Mamotte C, Sayer DC, Christiansen FT. Clinical applications of whole-blood PCR with real-time instrumentation. Clin Chem 2005; 51(11):2025-30.

80. El Housni H, Heimann P, Parma J, Vassart G. Single-nucleotide polymorphism genotyping by melting analysis of dual-labeled probes: examples using factor V Leiden and prothrombin 20210A mutations. Clin Chem 2003; 49(10):1669-72.

81. Vaughn CP, Elenitoba-Johnson KS. Hybridization-induced dequenching of fluorescein-labeled oligonucleotides: a novel strategy for PCR detection and genotyping. Am J Pathol 2003; 163(1):29-35.

82. Crockett AO, Wittwer CT. Fluorescein-labeled oligonucleotides for real-time pcr: using the inherent quenching of deoxyguanosine nucleotides. Anal Biochem 2001; 290(1):89-97.

83. Alsmadi OA, Bornarth CJ, Song W *et al*. High accuracy genotyping directly from genomic DNA using a rolling circle amplification based assay. BMC Genomics 2003; 4 (1):21.

84. Tsongalis GJ, Rainey BJ, Hodges KA. READIT: a novel technology used in the interrogation of nucleic acid sequences for single-nucleotide polymorphisms. Exp Mol Pathol 2001; 71(3):222-5.

85. Carmi N, Cohen D, Zvang E, Naparstek E, Deutsch V. Pronto ThromboRisk--a novel primer-extension ELISA based assay for the detection of mutations associated with increased risk for thrombophilia. J Clin Lab Anal 2004; 18(5):259-64.

86. Leyte A, Smits PH, van Straalen JP, van Doorn LJ, Quint WG. Automated, simultaneous detection of the factor V Leiden and prothrombin (G20210A) variants using multiplex PCR and a line probe assay. Thromb Haemost 2000; 83(2):354-5.

87. Schrijver I, Lay MJ, Zehnder JL. Diagnostic single nucleotide polymorphism analysis of factor V Leiden and prothrombin 20210G > A. A comparison of the Nanogen Eelectronic Microarray with restriction enzyme digestion and the Roche LightCycler. Am J Clin Pathol 2003; 119(4):490-6.

88. Erali M, Schmidt B, Lyon E, Wittwer C. Evaluation of electronic microarrays for genotyping factor V, factor II, and MTHFR. Clin Chem 2003; 49(5):732-9.

89. Evans JG, Lee-Tataseo C. Determination of the factor V Leiden single-nucleotide polymorphism in a commercial clinical laboratory by use of NanoChip microelectronic array technology. Clin Chem 2002; 48(9):1406-11.

90. French C, Li C, Strom C *et al*. Detection of the factor V Leiden mutation by a modified photo-cross-linking oligonucleotide hybridization assay. Clin Chem 2004; 50(2):296-305.

91. French DJ, McDowell DG, Debenham P, Gale N, Brown T. HyBeacon probes for rapid DNA sequence detection and allele discrimination. Methods Mol. Biol. 2008; 429(-):171-85.

92. van den Bergh FA, van Oeveren-Dybicz AM, Bon MA. Rapid single-tube genotyping of the factor V Leiden and prothrombin mutations by real-time PCR using dual-color detection. Clin Chem 2000; 46(8 Pt 1):1191-5.

93. Hobson-Peters J, Toye P. A whole-blood homogeneous assay for the multiplex detection of the factor V G1691A and the prothrombin G20210A mutations. Mol Cell Probes 2005; 19(4):290-7.

94. Ameziane N, Lamotte M, Lamoril J *et al*. Combined factor V leiden (G1691A) and prothrombin (G20210A) genotyping by multiplex real-time polymerase chain reaction using fluorescent resonance energy transfer hybridization probes on the Rotor-Gene 2000. Blood Coagul Fibrinolysis 2003; 14(4):421-4.

95. Baris I, Koksal V, Etlik O. Multiplex PCR-RFLP assay for detection of factor V Leiden and prothrombin G20210A. Genet Test 2004; 8(4):381-3.

96. Huber S, McMaster KJ, Voelkerding KV. Analytical evaluation of primer engineered multiplex polymerase chain reaction-restriction fragment length polymorphism for detection of factor V Leiden and prothrombin G20210A. J Mol Diagn 2000; 2(3):153-7.

97. Lucotte G, Champenois T. Duplex PCR-RFLP for simultaneous detection of factor V Leiden and prothrombin G20210A. Mol Cell Probes 2003; 17(5):267-9.

98. Corral J, Hernandez-Espinosa D, Soria JM *et al*. Antithrombin Cambridge II (A384S): an underestimated genetic risk factor for venous thrombosis. Blood 2007; 109(10):4258-63.

99. Neitzel B, Matern C, Holinski-Feder E. Easy, accurate and reliable screening for SNPs by ion pair/reverse phase HPLC: simultaneous detection of factor V G1691A, prothrombin G20210A and methylenetetrahydrofolate reductase C677T variants. Clin Lab 2003; 49(7-8):313-8.

100. Ugozzoli LA, Hamby K. Four-color multiplex 5' nuclease assay for the simultaneous detection of the factor V Leiden and the prothrombin G20210A mutations. Mol Cell Probes 2004; 18(3):161-6.

101. Jennings I, Kitchen S, Woods TA, Preston FE. Multilaboratory testing in thrombophilia through the United Kingdom National External Quality Assessment Scheme (Blood Coagulation) Quality Assurance Program. Semin Thromb Hemost 2005; 31(1):66-72.

102. Tripodi A, Chantarangkul V, Menegatti M, Tagliabue L, Peyvandi F. Performance of clinical laboratories for DNA analyses to detect thrombophilia mutations. Clin Chem 2005; 51(7):1310-1.

103. Lutz CT, Foster PA, Noll WW *et al*. Multicenter evaluation of PCR methods for the detection of factor V Leiden (R506Q) genotypes. Clin Chem 1998; 44(6 Pt 1):1356-8.

104. Bravo-Osorio M, Bydlowski SP. Detection of methylenetetrahydrofolate reductase (MTHFR) C677T and prothrombin G20210A mutations: second restriction site for digestion control of PCR products. Clin Chim Acta 2000; 301(1-2):219-23.

105. Hessner MJ, Friedman KD, Voelkerding KV *et al*. Multisite study for genotyping of the factor II (prothrombin) G20210A mutation by the invader assay. Clin Chem 2001; 47(11):2048-50.

106. Happich D, Madlener K, Schwaab R, Hanfland P, Potzsch B. Application of the TaqMan-PCR for genotyping of the prothrombin G20210A mutation and of the thermolabile methylenetetrahydrofolate reductase mutation. Thromb Haemost 2000; 84(1):144-5.

107. Preston FE, Kitchen S, Jennings I, Woods TA. A UK National External Quality Assessment scheme (UK Neqas) for molecular genetic testing for the diagnosis of familial thrombophilia. Thromb Haemost 1999; 82(5):1556-7.

108. Is recurrent venous thromboembolism more frequent in homozygous patients for the factor V Leiden mutation than in heterozygous patients? Blood Coagul Fibrinolysis 2003; 14(6):523-9.

109. Baarslag HJ, Koopman MM, Hutten BA *et al*. Long-term follow-up of patients with suspected deep vein thrombosis of the upper extremity: survival, risk factors and post-thrombotic syndrome. Eur J Intern Med 2004; 15(8):503-7.

110. Baglin T, Luddington R, Brown K, Baglin C. Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. Lancet 2003; 362(9383):523-6 .

111. Christiansen SC, Cannegieter SC, Koster T, Vandenbroucke JP, Rosendaal FR. Thrombophilia, clinical factors, and recurrent venous thrombotic events. JAMA 2005; 293(19):2352-61.

112. Eichinger S, Weltermann A, Mannhalter C *et al*. The risk of recurrent venous thromboembolism in heterozygous carriers of factor V Leiden and a first spontaneous venous thromboembolism. Arch Intern Med 2002; 162(20):2357-60.

113. Gonzalez-Porras JR, Garcia-Sanz R, Alberca I *et al*. Risk of recurrent venous thrombosis in patients with G20210A mutation in the prothrombin gene or factor V Leiden mutation. Blood Coagul Fibrinolysis 2006; 17(1):23-8.

114. Hoibraaten E, Mowinckel MC, de Ronde H, Bertina RM, Sandset PM. Hormone replacement therapy and acquired resistance to activated protein C: results of a randomized, double-blind, placebo-controlled trial. Br J Haematol 2001; 115(2):415-20.

115. Hron G, Eichinger S, Weltermann A *et al*. Family history for venous thromboembolism and the risk for recurrence. Am J Med 2006; 119(1):50-3.

116. Lindmarker P, Schulman S, Sten-Linder M, Wiman B, Egberg N, Johnsson H. The risk of recurrent venous thromboembolism in carriers and non-carriers of the G1691A allele in the coagulation factor V gene and the G20210A allele in the prothrombin gene. DURAC Trial Study Group. Duration of Anticoagulation. Thromb Haemost 1999; 81(5):684-9.

117. Mansilha A, Araujo F, Severo M, Sampaio SM, Toledo T, Albuquerque R. Genetic polymorphisms and risk of recurrent deep venous thrombosis in young people: prospective cohort study. Eur J Vasc Endovasc Surg 2005; 30(5):545-9.

118. Prandoni P, Noventa F, Ghirarduzzi A *et al*. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. Haematologica 2007; 92(2):199-205.

119. Santamaria MG, Agnelli G, Taliani MR *et al*. Thrombophilic abnormalities and recurrence of venous thromboembolism in patients treated with standardized anticoagulant treatment. Thromb Res 2005; 116(4):301-6.

120. Simioni P, Prandoni P, Lensing AW *et al*. Risk for subsequent venous thromboembolic complications in carriers of the prothrombin or the factor V gene mutation with a first episode of deep-vein thrombosis. Blood 2000; 96(10):3329-33.

121. Strandberg K, Svensson PJ, Ohlin AK. Venous thromboembolism in carriers of the Factor V Leiden mutation and in patients without known thrombophilic risk factor; prediction of recurrence and APC-PCI complex concentration and/or soluble thrombomodulin antigen and activity. Thromb Res 2007; 121(2):145-51.

122. Vossen CY, Walker ID, Svensson P *et al*. Recurrence rate after a first venous thrombosis in patients with familial thrombophilia. Arterioscler Thromb Vasc Biol 2005; 25(9):1992-7.

123. Wahlander K, Eriksson H, Lundstrom T *et al*. Risk of recurrent venous thromboembolism or bleeding in relation to thrombophilic risk factors in patients receiving ximelagatran or placebo for long-term secondary prevention of venous thromboembolism. Br J Haematol 2006; 133(1):68-77.

124. Kearon C, Gent M, Hirsh J *et al*. A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. N Engl J Med 1999; 340(12):901-7.

125. Kearon C, Julian JA, Kovacs MJ *et al*. Influence of thrombophilia on risk of recurrent venous thromboembolism while on warfarin: Results from a randomized trial. Blood 2008.

126. Miles JS, Miletich JP, Goldhaber SZ, Hennekens CH, Ridker PM. G20210A mutation in the prothrombin gene and the risk of recurrent venous thromboembolism . J Am Coll Cardiol 2001; 37(1):215-8.

127. Ridker PM, Miletich JP, Stampfer MJ, Goldhaber SZ, Lindpaintner K, Hennekens CH. Factor V Leiden and risks of recurrent idiopathic venous thromboembolism. Circulation 1995; 92(10):2800-2.

128. Eichinger S, Minar E, Hirschl M *et al*. The risk of early recurrent venous thromboembolism after oral anticoagulant therapy in patients with the G20210A transition in the prothrombin gene. Thromb Haemost 1999; 81(1):14-7.

129. Middeldorp S, Meinardi JR, Koopman MM *et al*. A prospective study of asymptomatic carriers of the factor V Leiden mutation to determine the incidence of venous thromboembolism. Ann Intern Med 2001; 135(5):322-7.

130. Simioni P, Tormene D, Prandoni P *et al*. Incidence of venous thromboembolism in asymptomatic family members who are carriers of factor V Leiden: a prospective cohort study. Blood 2002; 99(6):1938-42.

131. Vossen CY, Conard J, Fontcuberta J *et al*. Risk of a first venous thrombotic event in carriers of a familial thrombophilic defect. The European Prospective Cohort on Thrombophilia (EPCOT). J Thromb Haemost 2005; 3(3):459-64.

132. Couturaud F, Kearon C, Leroyer C *et al*. Incidence of venous thromboembolism in first-degree relatives of patients with venous thromboembolism who have factor V Leiden. Thromb Haemost 2006; 96(6):744-9.

133. Simioni P, Prandoni P, Girolami A. Low rate of venous thromboembolism in asymptomatic relatives of probands with factor V Leiden mutation. Ann Intern Med 1999; 130(6):538.

134. Simioni P, Tormene D, Luni S, Caldato M, Girolami A. Clinical and laboratory expression of associated thrombophilic conditions (homozygous/heterozygous factor V Leiden mutation and heterozygous prothrombin variant 20210A) in an Italian family. Blood Coagul Fibrinolysis 2000; 11(4):379-84.

135. Samama MM, Simon D, Horellou MH, Trossaert M, Elalamy I, Conard J. Diagnosis and clinical characteristics of inherited activated protein C resistance. Haemostasis 1996; 26 Suppl 4:315-30.

136. Lensen R, Bertina RM, Vandenbroucke JP, Rosendaal FR. High factor VIII levels contribute to the thrombotic risk in families with factor V Leiden. Br J Haematol 2001; 114(2):380-6.

137. Rintelen C, Pabinger I, Bettelheim P *et al*. Impact of the factor II: G20210A variant on the risk of venous thromboembolism in relatives from families with the factor V: R506Q mutation. Eur J Haematol 2001; 67(3):165-9.

138. Lensen R, Rosendaal F, Vandenbroucke J, Bertina R. Factor V Leiden: the venous thrombotic risk in thrombophilic families. Br J Haematol 2000; 110(4):939-45.

139. Le Cam-Duchez V, Gandrille S, Tregouet D *et al*. Influence of three potential genetic risk factors for thrombosis in 43 families carrying the factor V Arg 506 to Gln mutation. Br J Haematol 1999; 106(4):889-97.

140. Middeldorp S, Henkens CM, Koopman MM *et al*. The incidence of venous thromboembolism in family members of patients with factor V Leiden mutation and venous thrombosis. Ann Intern Med 1998; 128(1):15-20.

141. Meinardi JR, Middeldorp S, de Kam PJ *et al*. The incidence of recurrent venous thromboembolism in carriers of factor V Leiden is related to concomitant thrombophilic disorders. Br J Haematol 2002; 116(3):625-31.

142. Vossen CY, Conard J, Fontcuberta J *et al*. Familial thrombophilia and lifetime risk of venous thrombosis. J Thromb Haemost 2004; 2(9):1526-32.

143. Simioni P, Sanson BJ, Prandoni P *et al*. Incidence of venous thromboembolism in families with inherited thrombophilia. Thromb Haemost 1999; 81(2):198-202.

144. Castaman G, Tosetto A, Cappellari A, Ruggeri M, Rodeghiero F. The A20210 allele in the prothrombin gene enhances the risk of venous thrombosis in carriers of inherited protein S deficiency. Blood Coagul Fibrinolysis 2000; 11(4):321-6.

145. Marik PE, Plante LA. Venous thromboembolic disease and pregnancy. N Engl J Med 2008; 359(19):2025-33.

146. Martinelli I, Legnani C, Bucciarelli P, Grandone E, De Stefano V, Mannucci PM. Risk of pregnancy-related venous thrombosis in carriers of severe inherited thrombophilia. Thromb Haemost 2001; 86(3):800-3.

147. Tormene D, Simioni P, Prandoni P *et al*. Factor V Leiden mutation and the risk of venous thromboembolism in pregnant women. Haematologica 2001; 86(12):1305-9.

148. Tormene D, De Stefano V, Grandone E *et al*. The G20210A prothrombin variant and the risk of venous thromboembolism or fetal loss in pregnant women: a family study. J Thromb Haemost 2007; 5(11):2193-6.

149. Martinelli I, Battaglioli T, De Stefano V *et al*. The risk of first venous thromboembolism during pregnancy and puerperium in double heterozygotes for factor V Leiden and prothrombin G20210A. J Thromb Haemost 2008; 6(3):494-8.

150. Rodger MA, Carrier M, Keely E *et al*. The management of thrombophilia during pregnancy: a Canadian survey. J Obstet Gynaecol Can 2002; 24(12):946-52.

151. Ridker PM, Goldhaber SZ, Danielson E *et al*. Long-term, low-intensity warfarin therapy for the prevention of recurrent venous thromboembolism. N Engl J Med 2003; 348(15):1425-34.

152. Schulman S, Wahlander K, Lundstrom T, Clason SB, Eriksson H. Secondary prevention of venous thromboembolism with the oral direct thrombin inhibitor ximelagatran. N Engl J Med 2003; 349(18):1713-21 .

153. Heshka J, Palleschi C, Wilson B *et al*. Cognitive and Behavioural Effects of Genetic Testing for Thrombophilia . J Genet Couns 2008.

154. Kaptein AA, van Korlaar IM, Cameron LD, Vossen CY, van der Meer FJ, Rosendaal FR. Using the common-sense model to predict risk perception and disease-related worry in individuals at increased risk for venous thrombosis. Health Psychol 2007; 26(6):807-12.

155. Saukko PM, Ellard S, Richards SH, Shepherd MH, Campbell JL. Patients' understanding of genetic susceptibility testing in mainstream medicine: qualitative study on thrombophilia. BMC Health Serv Res 2007; 7:82.

156. Saukko PM, Richards SH, Shepherd MH, Campbell JL. Are genetic tests exceptional? Lessons from a qualitative study on thrombophilia. Soc Sci Med 2006; 63(7):1947-59.

157. Bank I, Scavenius MP, Buller HR, Middeldorp S. Social aspects of genetic testing for factor V Leiden mutation in healthy individuals and their importance for daily practice. Thromb Res 2004; 113(1):7-12.

158. Wu O, Robertson L, Twaddle S *et al*. Screening for thrombophilia in high-risk situations: systematic review and cost-effectiveness analysis. The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) study. Health Technol Assess 2006; 10(11):1-110.

159. Auerbach AD, Sanders GD, Hambleton J. Cost-effectiveness of testing for hypercoagulability and effects on treatment strategies in patients with deep vein thrombosis. Am J Med 2004; 116(12):816-28.

160. Clark P, Twaddle S, Walker ID, Scott L, Greer IA. Cost-effectiveness of screening for the factor V Leiden mutation in pregnant women. Lancet 2002; 359(9321):1919-20.

161. Eckman MH, Singh SK, Erban JK, Kao G. Testing for factor V Leiden in patients with pulmonary or venous thromboembolism: a cost-effectiveness analysis. Med Decis Making 2002; 22(2):108-24.

162. Marchetti M, Quaglini S, Barosi G. Cost-effectiveness of screening and extended anticoagulation for carriers of both factor V Leiden and prothrombin G20210A. QJM 2001; 94(7):365-72.

163. Marchetti M, Pistorio A, Barosi G. Extended anticoagulation for prevention of recurrent venous thromboembolism in carriers of factor V Leiden--cost-effectiveness analysis. Thromb Haemost 2000; 84(5):752-7.

164. Sarasin FP, Bounameaux H. Decision analysis model of prolonged oral anticoagulant treatment in factor V Leiden carriers with first episode of deep vein thrombosis. BMJ 1998; 316(7125):95-9.

165. Coppens M, Reijnders JH, Middeldorp S, Doggen CJ, Rosendaal FR. Testing for inherited thrombophilia does not reduce the recurrence of venous thrombosis. J Thromb Haemost 2008; 6(9):1474-7.

166. Samama MM, Rached RA, Horellou MH *et al*.Pregnancy-associated venous thromboembolism (VTE) in combined heterozygous factor V Leiden (FVL) and prothrombin (FII) 20210 A mutation and in heterozygous FII single gene mutation alone. Br J Haematol 2003; 123(2):327-34.

167. Hindorff LA, Burke W, Laberge AM *et al*. Motivating factors for physician ordering of factor V Leiden genetic tests. Arch Intern Med 2009; 169(1):68-74.

Acronyms

Acronym	Definition
ACCE	Analytic validity, Clinical validity, Clinical utility and Ethical, legal and social implications
AHRQ	Agency for Healthcare Research and Quality
APC	Activated protein C
AS-PCR	Allele-specific polymerase chain reaction
CDC	Center for Disease Control and Prevention
CI	Confidence interval
CISMEL	Italian Committee for Standardization of Laboratory Tests
DNA	Deoxyribonucleic acid
DVT	Deep venous thrombosis
EGAPP	Evaluation of Genomic Applications in Practice and Prevention
ELATE	Extended Low-Intensity Anticoagulation for Unprovoked Thromboembolism
ELISA	Enzyme-linked immunosorbent assay
EPC	Evidence-based Practice Center
EPCOT	European Prospective Cohort on Thrombophilia
FDA	Food and Drug Administration
FRET	Fluorescent resonance energy transfer
FVL	Factor V Leiden
GRADE	The Grading of Recommendations Assessment, Development and Evaluation
HR	Hazard ratio
ICER	Incremental cost-effectiveness ratio
INR	International normalized ratio
MeSH	Medical Subject Heading
M-H	Mantel-Haenszel
PCR	Polymerase chain reaction
PCR-RFLP	Polymerase chain reaction and restriction fragment length polymorphism
PE	Pulmonary embolism
PT 20210A	Prothrombin G20210A
QA	Quality assurance
QALY	Quality-adjusted life year
READIT	Reversed Enzyme Activity DNA Interrogation Test
STARD	Standards of Reporting of Diagnostic Accuracy
TEP	Technical Expert Panel
TGCE	Temperature gradient capillary electrophoresis
THRIVE	Thrombin Inhibitor in Venous Thromboembolism
UK NEQAS	United Kingdom National External Quality Assessment Scheme
VTE	Venous thromboembolism

Appendix A: Technical Experts and Peer Reviewers

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Appendix B: Hand Searched Journals

Clinical Chemistry
British Journal of Haematology
Thrombosis and Heamostasis
Blood Coagulation and Fibrinolysis
Journal of Thrombosis and Haemostasis
Thrombosis Research
Molecular and Cellular Probes
Haematologica
NEJM
Blood
Annals of Internal Medicine

Appendix C: Detailed Search Strategies

Database	Terms	Date	Returns
PubMed	(((Inherit*[tiab] OR herit*[tiab] OR genetic*[tiab] OR genotype[tiab] OR familial[tiab]) AND (thrombophil*[tiab] OR coagula*[tiab] OR (venous[tiab] AND (thromboemboli*[tiab] OR thrombophili*[tiab])) OR "deep vein thrombosis"[tiab] OR "deep-vein thrombosis"[tiab] OR "deep venous thrombosis"[tiab] OR VTE[tiab] OR "pulmonary embolism"[tiab])) OR ("factor v/genetics"[mh] OR ("factor v"[tiab] AND (leiden[tiab] OR g1691a[tiab] OR Arg506[tiab] OR R506Q[tiab] OR (ARG[tiab] AND 506[tiab]))) OR "prothrombin/genetics"[mh] OR (prothrombin[tiab] AND (G20210A[tiab] OR 20210[tiab] OR 20210GA[tiab]))) AND 1993:2008[dp] NOT (animals[mh] NOT humans[mh]) NOT (editorial[pt] OR comment[pt] OR letter[pt])	5/9/08	5704
EMBASE	(((Inherit*:ti,ab OR herit*:ti,ab OR genetic*:ti,ab OR genotype:ti,ab OR familial:ti,ab) AND (thrombophil*:ti,ab OR coagula*:ti,ab OR (venous:ti,ab AND (thromboemboli*:ti,ab OR thrombophili*:ti,ab)) OR "deep vein thrombosis":ti,ab OR "deep-vein thrombosis":ti,ab OR "deep venous thrombosis":ti,ab OR VTE:ti,ab OR "pulmonary embolism":ti,ab)) OR ('blood clotting factor v leiden'/exp OR ("factor v":ti,ab AND (leiden:ti,ab OR g1691a:ti,ab OR Arg506:ti,ab OR R506Q:ti,ab OR (ARG:ti,ab AND 506:ti,ab))) OR ((prothrombin/exp OR prothrombin:ti,ab) AND (G20210A:ti,ab OR 20210:ti,ab OR 20210GA:ti,ab)))) AND [1993-2008]/py NOT ([conference paper]/lim OR [editorial]/lim OR [erratum]/lim OR [letter]/lim OR [note]/lim) NOT ([animals]/lim NOT [humans]/lim)	5/12/08	6297
Cochrane Library	(((Inherit*:ti,ab OR herit*:ti,ab OR genetic*:ti,ab OR genotype:ti,ab OR familial:ti,ab) AND (thrombophil*:ti,ab OR coagula*:ti,ab OR (venous:ti,ab AND (thromboemboli*:ti,ab OR thrombophili*:ti,ab)) OR "deep vein thrombosis":ti,ab OR "deep-vein thrombosis":ti,ab OR "deep venous thrombosis":ti,ab OR VTE:ti,ab OR "pulmonary embolism":ti,ab)) OR ("factor v":genetics[mh] OR ("factor v":ti,ab AND (leiden:ti,ab OR g1691a:ti,ab OR Arg506:ti,ab OR R506Q:ti,ab OR (ARG:ti,ab AND 506:ti,ab))) OR "prothrombin:genetics"[mh] OR (prothrombin[tiab] AND (G20210A[tiab] OR 20210[tiab] OR 20210GA[tiab]))) , from 1993 to 2008	5/12/08	129 Cochrane reviews 7 DARE 3 CENTRAL 108 Economic Evaluations 5
PsycInfo	(((AB Inherit* OR AB herit* OR AB genetic* OR AB genotype OR AB familial) AND (AB thrombophil* OR AB coagula* OR (AB venous AND (AB thromboemboli* OR AB thrombophili*)) OR AB "deep vein thrombosis" OR AB "deep-vein thrombosis" OR AB "deep venous thrombosis" OR AB VTE)) OR ((TI "factor V" OR AB "factor v") AND (AB leiden OR AB g1691a OR AB Arg506 OR AB R506Q OR (AB ARG AND AB 506))) OR ((TI prothrombin OR AB prothrombin) AND (AB G20210A OR AB 20210 OR AB 20210GA))) AND DT 199301-200812 NOT (PZ animal NOT PZ human) Not ((ZZ "BIBLIOGRAPHY") or (ZZ "CHAPTER") or (ZZ "COLUMN/OPINION") or (ZZ "COMMENT/REPLY") or (ZZ "EDITORIAL") or (ZZ "ENCYCLOPEDIA ENTRY") or (ZZ "LETTER") or (ZZ "OBITUARY") or (ZZ "PUBLICATION INFORMATION"))	5/12/08	25
CINAHL	(((TX Inherit* OR TX herit* OR TX genetic* OR TX genotype OR TX familial) AND (TX thrombophil* OR TX coagula* OR (TX venous AND (TX thromboemboli* OR TX thrombophili*)) OR TX "deep vein thrombosis" OR TX "deep-vein thrombosis" OR TX "deep venous thrombosis" OR TX VTE)) OR ((TI "factor V" OR TX "factor v") AND (TX leiden OR TX g1691a OR TX Arg506 OR TX R506Q OR (TX ARG AND TX 506))) OR ((TI prothrombin OR TX prothrombin) AND (TX G20210A OR TX 20210 OR TX 20210GA))) AND (PY 1993-2008) AND (ZT "CLINICAL TRIAL" or ZT "JOURNAL ARTICLE" or ZT "RESEARCH" or ZT "REVIEW" or ZT "SYSTEMATIC REVIEW")	5/12/08	660

Appendix D: List of Excluded Articles

Medical devices; hematology and pathology devices; classification of the Factor V Leiden DNA mutation detection systems devices. Final rule. Fed Regist 2004;69(51):12271-3
Other reason

Investigation and management of heritable thrombophilia. Br J Haematol 2001;114(3):512-28
No original data

Comparison of thrombotic risk between 85 homozygotes and 481 heterozygotes carriers of the factor V Leiden mutation: retrospective analysis from the Procare Study. Blood Coagul Fibrinolysis 2000;11(6):511-8
Doesn't address study question

Adkins K K, Strom D A, Jacobson T E et al. Utilizing genomic DNA purified from clotted blood samples for single nucleotide polymorphism genotyping. Arch Pathol Lab Med 2002;126(3):266-70
Evaluates only DNA extraction methods

Agnelli G. Long-term low-dose warfarin use is effective in the prevention of recurrent venous thromboembolism: No. J. Thromb. Haemost. 2004;2(7):1038-1040
No original data

Aliev M A, Mierbekov E M, Sviatova G S et al. [Venous thromboembolic events in surgery: a role of genetic disorders]. Anesteziol Reanimatol 2008;(1):71-3
Non-English article

Altes A, Souto J C, Mateo J et al. Activated protein C resistance assay when applied in the general population. Am J Obstet Gynecol 97;176(2):358-9
Published before 2000

Amberger N, Masuhr F, Valdueza J M et al. A negative personal and family history for venous thrombotic events is not sufficient to exclude thrombophilia in patients with cerebral venous thrombosis. Eur J Neurol 2004;11(8):555-8
Does not report results separately for FV Leiden and/or PT 20210A.

Andersson H C, Krousel-Wood M A, Jackson K E et al. Medical genetic test reporting for cystic fibrosis (deltaF508) and factor V Leiden in North American laboratories. Genet Med 2002;4(5):324-7
Doesn't address study question

Angelini A, Di Febbo C, Baccante G et al. Identification of three genetic risk factors for venous thrombosis using a multiplex allele-specific PCR assay: comparison of conventional and new alternative methods for the preparation of DNA from clinical samples. J Thromb Thrombolysis 2003;16(3):189-93
Evaluates only DNA extraction methods

Angelini A, Di Febbo C, Rullo A et al. New method for the extraction of DNA from white blood cells for the detection of common genetic variants associated with thrombophilia. Pathophysiol Haemost Thromb 2002;32(4):180-3
Evaluates only DNA extraction methods

Arruda V R, von Zuben P M, Annichino-Bizzachi J M et al. Rapid detection of factor V Leiden (FVQ506) by non-radioactive single strand conformation polymorphism (SSCP). Sangre (Barc) 96;41(5):379-81
Published before 2000

Aslanidis C, Nauck M, Schmitz G. High-speed prothrombin G-->A 20210 and methylenetetrahydrofolate reductase C-->T 677 mutation detection using real-time fluorescence PCR and melting curves. Biotechniques 99;27(2):234-6, 238
Published before 2000

Aznar J, Vaya A, Estelles A et al. Risk of venous thrombosis in carriers of the prothrombin G20210A variant and factor V Leiden and their interaction with oral contraceptives. Haematologica 2000;85(12):1271-6
Case-control or retrospective cohort of probands

Baglin C, Brown K, Luddington R et al. Risk of recurrent venous thromboembolism in patients with the factor V Leiden (FVR506Q) mutation: effect of warfarin and prediction by precipitating factors. East Anglian Thrombophilia Study Group. Br J Haematol 98;100(4):764-8
Other reason

Bank I, Libourel E J, Middeldorp S et al. Prothrombin 20210A mutation: a mild risk factor for venous thromboembolism but not for arterial thrombotic disease and pregnancy-related complications in a family study. Arch Intern Med 2004;164(17):1932-7
Other reason

Bathelier C, Champenois T, Lucotte G. ARMS test for diagnosis of factor V Leiden mutation and allele frequencies in France. Mol Cell Probes 98;12(2):121-3
Published before 2000

Beauchamp N J, Daly M E, Cooper P C et al. Rapid two-stage PCR for detecting factor V G1691A mutation. Lancet 94;344(8923):694-5
Published before 2000

Behn M, Schuermann M. Simple and reliable factor V genotyping by PNA-mediated PCR clamping. Thromb Haemost 98;79(4):773-7
Published before 2000

Bennett C D, Campbell M N, Cook C J et al. The LightTyper: high-throughput genotyping using fluorescent melting curve analysis. Biotechniques 2003;34(6):1288-92, 1294-5
Other reason

Benson J M, Ellingsen D, Renshaw M A et al. Multiplex

analysis of mutations in four genes using fluorescence scanning technology. Thromb Res 99;96(1):57-64
Published before 2000

Benson J M, Phillips D J, Holloway B P et al. Oligonucleotide ligation assay for detection of the factor V mutation (Arg506-->Gln) causing protein C resistance. Thromb Res 96;83(1):87-96
Published before 2000

Bertina R M. Molecular risk factors for thrombosis. Thromb Haemost 99;82(2):601-9
No original data

Biswas A, Bajaj J, Ranjan R et al. Factor V Leiden: Is it the chief contributor to activated protein C resistance in Asian-Indian patients with deep vein thrombosis?. Clin Chim Acta 2008;392(1-2):21-4
Doesn't address study question

Blasczyk R, Ritter M, Thiede C et al. Simple and rapid detection of factor V Leiden by allele-specific PCR amplification. Thromb Haemost 96;75(5):757-9
Published before 2000

Blasczyk R, Wehling J, Ritter M et al. Allele-specific PCR amplification of factor V Leiden to identify patients at risk for thromboembolism. Beitr Infusionsther Transfusionsmed 97;34236-41
Published before 2000

Boda Z, Laszlo P, Rejto L et al. Low molecular weight heparin as thromboprophylaxis in familial thrombophilia during the whole period of pregnancy. Thromb Haemost 96;76(1):128
Doesn't address study question

Bokarewa M I, Bremme K, Blomback M. Arg506-Gln mutation in factor V and risk of thrombosis during pregnancy. Br J Haematol 96;92(2):473-8
Other reason

Bokarewa M I, Falk G, Sten-Linder M et al. Thrombotic risk factors and oral contraception. J Lab Clin Med 95;126(3):294-8
Does not report results separately for FV Leiden and/or PT 20210A

Bounameaux H. Factor V Leiden paradox: risk of deep-vein thrombosis but not of pulmonary embolism. Lancet 2000;356(9225):182-3
Doesn't address study question

Bowen D J, Bowley S, John M et al. Factor V Leiden (G1691A), the prothrombin 3'-untranslated region variant (G20210A) and thermolabile methylenetetrahydrofolate reductase (C677T): a single genetic test genotypes all three loci--determination of frequencies in the S. Wales population of the UK. Thromb Haemost 98;79(5):949-54
Published before 2000

Bowen D J, Standen G R, Granville S et al. Genetic diagnosis of factor V Leiden using heteroduplex technology. Thromb Haemost 97;77(1):119-22
Published before 2000

Brenner B, Zivelin A, Lanir N et al. Venous thromboembolism associated with double heterozygosity for R506Q mutation of factor V and for T298M mutation of protein C in a large family of a previously described homozygous protein C-deficient newborn with massive thrombosis. Blood 96;88(3):877-80
Other reason

Brouwer J L, Bijl M, Veeger N J et al. The contribution of inherited and acquired thrombophilic defects, alone or combined with antiphospholipid antibodies, to venous and arterial thromboembolism in patients with systemic lupus erythematosus. Blood 2004;104(1):143-8
Does not report results separately for FV Leiden and/or PT 20210A

Buller H R, Davidson B L, Decousus H et al. Fondaparinux or enoxaparin for the initial treatment of symptomatic deep venous thrombosis: a randomized trial. Ann Intern Med 2004;140(11):867-73
Doesn't address study question

Buller H R, Davidson B L, Decousus H et al. Subcutaneous fondaparinux versus intravenous unfractionated heparin in the initial treatment of pulmonary embolism. N Engl J Med 2003;349(18):1695-702
Doesn't address study question

Caprini J A, Goldshteyn S, Glase C J et al. Thrombophilia testing in patients with venous thrombosis. Eur J Vasc Endovasc Surg 2005;30(5):550-5
Evaluates less than 10 probands or 10 family members

Castaman G, Tosetto A, Ruggeri M et al. Pseudohomozygosity for activated protein C resistance is a risk factor for venous thrombosis. Br J Haematol 99;106(1):232-6
Other reason

Chakravarty A, Hansen T S, Horder M et al. A fast and robust dual-label nonradioactive oligonucleotide ligation assay for detection of factor V Leiden. Thromb Haemost 97;78(4):1234-6
Published before 2000

Charakidas A, Brouzas D, Andrioti E et al. Papillophlebitis associated with coexisting factor V Leiden and prothrombin G20210A mutations. Retina 2002;22(2):239-40
Doesn't address study question

Cibula D, Kvasnicka J, Salaj P et al. [Informed consent for factor V Leiden mutation screening before recommending use of combined hormonal contraceptives or hormone replacement therapy]. Ceska Gynekol 2003;68(3):212
Non-English article

Appendix D: List of Excluded Articles

Clayton E W. Ethical, legal, and social implications of genomic medicine. N Engl J Med 2003;349(6):562-9
Doesn't address study question

Cleary-Goldman J, Bettes B, Robinson J N et al. Thrombophilia and the obstetric patient. Obstet Gynecol 2007;110(3):669-74
Does not study probands or family members

Conard J, Plu-Bureau G, Bahi N et al. Progestogen-only contraception in women at high risk of venous thromboembolism. Contraception 2004;70(6):437-41
Evaluates less than 10 probands or 10 family members

Coppens M, van de, Poel M H et al. A prospective cohort study on the absolute incidence of venous thromboembolism and arterial cardiovascular disease in asymptomatic carriers of the prothrombin 20210A mutation. Blood 2006;108(8):2604-7
Other reason

Corral J, Iniesta J A, Gonzalez-Conejero R et al. Detection of factor V Leiden from a drop of blood by PCR-SSCP. Thromb Haemost 96;76(5):735-7
Published before 2000

Corral J, Zuazu-Jausoro I, Rivera J et al. Clinical and analytical relevance of the combination of prothrombin 20210A/A and factor V Leiden: results from a large family. Br J Haematol 99;105(2):560-3
No objective confirmation of VTE event

Correa M F, Rezende S M. A very rapid multiplex polymerase chain reaction for simultaneous detection of factor V Leiden and G20210A prothrombin mutation. Clin Lab Haematol 2004;26(3):239-40
Evaluates less than 10 samples

Coude M, Pineau-Vincent F. Prothombin gene: Comparative study between PCR-RFLP/Allele specific PCR: ETUDE DU GENE DE LA PROTHROMBINE: ETUDE COMPARATIVE DE DEUX TECHNIQUES PCR-RFLP/PCR ALLELES SPECIFIQUES. Immuno-Anal. Biol. Spec. 2004;19(3):177-179
Non-English article

Couturaud F, Leroyer C, Mottier D. Risk factors and clinical presentation of venous thromboembolism according to the age of relatives of patients with factor V Leiden. Thromb Haemost 2008;99(4):793-794
Case-control or retrospective cohort of family members

Dahlback B. Procoagulant and anticoagulant properties of coagulation factor V: factor V Leiden (APC resistance) causes hypercoagulability by dual mechanisms. J Lab Clin Med 99;133(5):415-22
No original data

Danneberg J, Abbes A P, Bruggeman B J et al. Reliable genotyping of the G-20210-A mutation of coagulation factor II (prothrombin). Clin Chem 98;44(2):349-51
Published before 2000

David D, Ribeiro S, Ferrao L et al. Molecular basis of inherited antithrombin deficiency in Portuguese families: identification of genetic alterations and screening for additional thrombotic risk factors. Am J Hematol 2004;76(2):163-71
Case-control or retrospective cohort of probands

De Bel A V, Van der, Cruyssen G A et al. A 2-year retrospective analysis of laboratory testing for activated protein C resistance with a factor V-corrected activated partial thromboplastin time-based method. Blood Coagul Fibrinolysis 2006;17(2):155-60
Evaluates only APC resistance

De Stefano V. Inherited thrombophilia and life-time risk of venous thromboembolism: Is the burden reducible?. J. Thromb. Haemost. 2004;2(9):1522-1525
No original data

De Stefano V, Martinelli I, Mannucci P M et al. The risk of recurrent deep venous thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. N Engl J Med 99;341(11):801-6
Other reason

De Stefano V, Martinelli I, Mannucci P M et al. The risk of recurrent venous thromboembolism among heterozygous carriers of the G20210A prothrombin gene mutation. Br J Haematol 2001;113(3):630-5
Other reason

De Stefano V, Martinelli I, Rossi E et al. The risk of recurrent venous thromboembolism in pregnancy and puerperium without antithrombotic prophylaxis. Br J Haematol 2006;135(3):386-91
Other reason

Dietrich J E, Hertweck S P, Perlman S E. Efficacy of family history in determining thrombophilia risk. J Pediatr Adolesc Gynecol 2007;20(4):221-4
No objective confirmation of VTE event

Djordjevic V, Rakicevic L, Gagic M et al. Simultaneous detection of factor V Leiden and factor II G20210A variants by a multiplex PCR on whole blood. Balk. J. Med. Genet. 2001;4(1-2):15-17
Does not report concordance, discordance, or reproducibility

Dordevic V, Rakicevic L, Miljic P et al. Impact of acquired and genetic factors on thrombophilic phenotype in FV Leiden mutation carriers. Jugosl. Med. Biohem. 2005;24(2):141-146
Case-control or retrospective cohort of probands

Douketis J D, Foster G A, Crowther M A et al. Clinical risk factors and timing of recurrent venous thromboembolism during the initial 3 months of anticoagulant therapy. Arch Intern Med 2000;160(22):3431-6

Appendix D: List of Excluded Articles

Doesn't address study question

Dubreuil Lastrucci R M, Dawson D A, Bowden J H et al. Development of a simple multiplex polymerase chain reaction for the simultaneous detection of factor V Leiden and prothrombin 20210A mutations. Mol Diagn 99;4(3):247-50
Published before 2000

Dulicek P, Maly J, Safarova M. Risk of thrombosis in patients homozygous and heterozygous for factor V Leiden in the East Bohemian region. Clin Appl Thromb Hemost 2000;6(2):87-9
Doesn't address study question

Duperat V G, Fauchon M, Nurden A T et al. Simple and rapid combined genetic diagnosis of mutation (1691 G-->A) of the factor V gene and (20210 G-->A) of the prothrombin gene. Blood Coagul Fibrinolysis 98;9(6):549-51
Published before 2000

Dzhafarov T A, Rustamov R S h. [Simultaneous determination of mutations 1691 G-A of the blood coagulation system factor V gene and 20210 G-A gene, coding for prothrombin, by means of complex PCR from whole blood using one restriction enzyme (HindIII)]. Mol Biol (Mosk) 2000;34(3):486-7
Non-English article

Eichinger S, Pabinger I, Stumpflen A et al. The risk of recurrent venous thromboembolism in patients with and without factor V Leiden. Thromb Haemost 97;77(4):624-8
Other reason

Eichinger S, Weltermann A, Minar E et al. Symptomatic pulmonary embolism and the risk of recurrent venous thromboembolism. Arch Intern Med 2004;164(1):92-6
Other reason

Eikelboom J W, Ivey L, Ivey J et al. Familial thrombophilia and the prothrombin 20210A mutation: association with increased thrombin generation and unusual thrombosis. Blood Coagul Fibrinolysis 99;10(1):1-5
Evaluates less than 10 probands or 10 family members

Emmerich J, Alhenc-Gelas M, Aillaud M F et al. Clinical features in 36 patients homozygous for the ARG 506-->GLN factor V mutation. Thromb Haemost 97;77(4):620-3
Other reason

Eriksson H, Lundstrom T, Wahlander K et al. Prognostic factors for recurrence of venous thromboembolism (VTE) or bleeding during long-term secondary prevention of VTE with ximelagatran. Thromb Haemost 2005;94(3):522-7
Doesn't address study question

Faioni E M, Franchi F, Bucciarelli P et al. Coinheritance of the HR2 haplotype in the factor V gene confers an increased risk of venous thromboembolism to carriers of factor V R506Q (factor V Leiden). Blood 99;94(9):3062-6
Other reason

Favaloro E J, Bonar R, Sioufi J et al. Multilaboratory testing of thrombophilia: current and past practice in Australasia as assessed through the Royal College of Pathologists of Australasia Quality Assurance Program for Hematology. Semin Thromb Hemost 2005;31(1):49-58
Evaluates only APC resistance

Folsom A R, Cushman M, Tsai M Y et al. Prospective study of the G20210A polymorphism in the prothrombin gene, plasma prothrombin concentration, and incidence of venous thromboembolism. Am J Hematol 2002;71(4):285-90
Case-control or retrospective cohort of probands

Franco R F, Middeldorp S, Meinardi J R et al. Factor XIII Val34Leu and the risk of venous thromboembolism in factor V Leiden carriers. Br J Haematol 2000;111(1):118-21
Other reason

Friedline J A, Ahmad E, Garcia D et al. Combined factor V Leiden and prothrombin genotyping in patients presenting with thromboembolic episodes. Arch Pathol Lab Med 2001;125(1):105-11
No objective confirmation of VTE event

Gadisseur A P, Christiansen S C, VAN D E R et al. The quality of oral anticoagulant therapy and recurrent venous thrombotic events in the Leiden Thrombophilia Study. J Thromb Haemost 2007;5(5):931-6
Does not report results separately for FV Leiden and/or PT 20210A

Gancberg D, Corbisier P, Meeus N et al. Certification of reference materials for detection of the human prothrombin gene G20210A sequence variant. Clin Chem Lab Med 2008;46(4):463-9
Evaluates only DNA extraction methods

Gandrille S, Alhenc-Gelas M, Aiach M. A rapid screening method for the factor V Arg506-->Gln mutation. Blood Coagul Fibrinolysis 95;6(3):245-8
Published before 2000

Gandrille S, Remones V, Aiach M. [Validation of a diagnostic kit for factor V Leiden mutation]. Ann Biol Clin (Paris) 2003;61(6):723-7
Non-English article

Garcia-Fuster, M. J., Forner, M. J., Fernandez, C., Gil, J., Vaya, A., and Maldonado, L., Long-term prospective study of recurrent venous thromboembolism in patients younger than 50 years. Pathophysiol Haemost Thromb. 2005;34(1):6-12
Other reason

Garcia-Hernandez M C, Romero Casanova A, Marco Vera P. [Clinical comments on genetic marker prevalence (factor V Leiden, prothrombin 20210A and homozygous

methylenetetrahydrofolate reductase form. Rev Clin Esp 2007;207(1):26-8
Non-English article

Gardiner C, Cooper P C, Makris M et al. An evaluation of screening tests for defects in the protein C pathway: commercial kits lack sensitivity and specificity. Blood Coagul Fibrinolysis 2002;13(2):155-63
Evaluates only APC resistance

Geisel J, Walz T, Bodis M et al. Fluorescence-based SSCP analysis with automatic allele detection demonstrated for the factor V Leiden mutation. Clin Chem 98;44(9):1986-7
Published before 2000

Ghosh K, Shetty S, Madkaikar M et al. Venous thromboembolism in young patients from western India: a study. Clin Appl Thromb Hemost 2001;7(2):158-65
Case-control or retrospective cohort of probands

Girolami A, Simioni P, Girolami B et al. G to A 20210 prothrombin polymorphism and venous thrombosis: simple association or causal relationship?. Clin Appl Thromb Hemost 2000;6(3):135-8
No original data

Girolami A, Simioni P, Girolami B et al. Low incidence of venous thrombosis in homozygous patients with NT 20210 G to a prothrombin polymorphism. Clin Appl Thromb Hemost 99;5(4):205-7
No original data

Girolami A, Tormene D, Gavasso S et al. Long term use of oral contraceptives without thrombosis in patients with FV Leiden polymorphism: a study of 37 patients (2 homozygous and 35 heterozygous). J Thromb Thrombolysis 2004;17(2):145-9
Other reason

Glynn R J, Ridker P M, Goldhaber S Z et al. Effects of random allocation to vitamin E supplementation on the occurrence of venous thromboembolism: report from the Women's Health Study. Circulation 2007;116(13):1497-503
Doesn't address study question

Golomb M R, Garg B P, Walsh L E et al. Perinatal stroke in baby, prothrombotic gene in mom: does this affect maternal health insurance?. Neurology 2005;65(1):13-6
Other reason

Gomez E, van der, Poel S C et al. Rapid simultaneous screening of factor V Leiden and G20210A prothrombin variant by multiplex polymerase chain reaction on whole blood. Blood 98;91(6):2208-9
Published before 2000

Gonzalez C M, Diaz-Golpe V, Martin S et al. Combination of congenital coagulation disorders: Factor II gene mutation G20210A, Factor V Leiden gene mutation G1691A and protein S deficiency. a family study. Haematologica 2003;88(6):ECR20
Evaluates less than 10 probands or 10 family members

Grand'Maison A, Bates S M, Johnston M et al. "ProC Global": a functional screening test that predicts recurrent venous thromboembolism. Thromb Haemost 2005;93(3):600-4
Evaluates only APC resistance

Gray E, Hawkins J R, Morrison M et al. Establishment of the 1st International Genetic Reference Panel for Factor V Leiden, human gDNA. Thromb Haemost 2006;96(2):215-9
Evaluates less than 10 samples

Greaves M, Baglin T. Laboratory testing for heritable thrombophilia: impact on clinical management of thrombotic disease annotation. Br J Haematol 2000;109(4):699-703
Doesn't address study question

Greengard J S, Xu X, Gandrille S et al. Alternative PCR method for diagnosis of mutation causing activated protein C resistant Gln506-factor V. Thromb Res 95;80(5):441-3
Published before 2000

Guillerm C, Lellouche F, Darnige L et al. Rapid detection of the G1691A mutation of coagulation factor V by PCR-mediated site-directed mutagenesis. Clin Chem 96;42(2):329
Published before 2000

Hanguehard A, Morelle M, Turchetto L et al. Genotyping in routine laboratory for detection of factor V Leiden (1691G>A) and prothrombin (20210G>A) mutations with the Invader(registered trademark) assay technology: EVALUATION EN ROUTINE D'UNE NOUVELLE METHODE POUR LE GENOTYPAGE DE LA MUTATION 1691G>A DU FACTEUR V LEIDEN ET DU VARIANT 20210G>A DU FACTEUR II PAR LA TECHNOLOGIE INVADER(registered trademark). Immuno-Anal. Biol. Spec. 2004;19(2):110-116
Non-English article

Hansson P O, Sorbo J, Eriksson H. Recurrent venous thromboembolism after deep vein thrombosis: incidence and risk factors. Arch Intern Med 2000;160(6):769-74
Doesn't address study question

Happich D, Schwaab R, Hanfland P et al. Allelic discrimination of factor V Leiden using a 5' nuclease assay. Thromb Haemost 99;82(4):1294-6
Published before 2000

Hatzaki A, Anagnostopoulou E, Metaxa-Mariatou V et al. The impact of heterozygosity for the factor V Leiden and factor II G20210A mutations on the risk of thrombosis in Greek patients. Int Angiol 2003;22(1):79-82
Doesn't address study question

Heinemann L A, Dominh T, Assmann A et al. VTE Risk assessment - a prognostic Model: BATER Cohort Study of young women. Thromb J 2005;3(1):5

Other reason

Heldal M, Seem E, Sandset P M et al. Deep vein thrombosis: a 7-year follow-up study. J Intern Med 93;234(1):71-5
Doesn't address study question

Hellmann E A, Leslie N D, Moll S. Knowledge and educational needs of individuals with the factor V Leiden mutation. J Thromb Haemost 2003;1(11):2335-9
Less than 80% of the probands has had VTE

Herrington D M, Vittinghoff E, Howard T D et al. Factor V Leiden, hormone replacement therapy, and risk of venous thromboembolic events in women with coronary disease. Arterioscler Thromb Vasc Biol 2002;22(6):1012-7
Other reason

Hezard N, Cornillet P, Droulle C et al. Factor V Leiden: detection in whole blood by ASA PCR using an additional mismatch in antepenultimate position. Thromb Res 97;88(1):59-66
Published before 2000

Hezard N, Cornillet-Lefebvre P, Gillot L et al. Multiplex ASA PCR for a simultaneous determination of factor V Leiden gene, G-->A 20210 prothrombin gene and C-->T 677 MTHFR gene mutations. Thromb Haemost 98;79(5):1054-5
Published before 2000

Holmberg K, Persson M-L, Uhlen M et al. Pyrosequencing analysis of thrombosis-associated risk markers. Clin. Chem. 2005;51(8):1549-1552
Does not report concordance, discordance, or reproducibility

Hron G, Kollars M, Binder B R et al. Identification of patients at low risk for recurrent venous thromboembolism by measuring thrombin generation. JAMA 2006;296(4):397-402
Other reason

Hudson M, Herr A L, Rauch J et al. The presence of multiple prothrombotic risk factors is associated with a higher risk of thrombosis in individuals with anticardiolipin antibodies. J Rheumatol 2003;30(11):2385-91
Doesn't address study question

Hull R D, Pineo G F, Brant R F et al. Long-term low-molecular-weight heparin versus usual care in proximal-vein thrombosis patients with cancer. Am J Med 2006;119(12):1062-72
Other reason

Hunault M, Marsh-Scott C, Jou C et al. Automated detection of the factor V Leiden mutation using the LCx microparticle enzyme immunoassay. Clin Chem 99;45(1):41-6
Published before 2000

Huxtable L M, Tafreshi M J, Ondreyco S M. A protocol for the use of enoxaparin during pregnancy: Results from 85 pregnancies including 13 multiple gestation pregnancies. Clin. Appl. Thromb. Hemost. 2005;11(2):171-181
Doesn't address study question

Ivanov P, Komsa-Penkova R, Kovacheva K et al. Impact of thrombophilic genetic factors on pulmonary embolism: early onset and recurrent incidences. Lung 2008;186(1):27-36
Other reason

Jastrzebska M, Balszan-Kowalska I, Ciechanowicz A et al. [Activated protein C resistance, Leiden mutation, anticoagulant proteins and fibrinogen levels in patients with deep venous thrombosis]. Pol Arch Med Wewn 2003;109(6):579-88
Non-English article

Kearon C, Ginsberg J S, Anderson D R et al. Comparison of 1 month with 3 months of anticoagulation for a first episode of venous thromboembolism associated with a transient risk factor. J Thromb Haemost 2004;2(5):743-9
Other reason

Keenan C, Livingstone W J, White B et al. Prevalence of the prothrombin G20210A mutation in the Irish populations: use of a novel polymerase chain reaction approach. Blood Coagul Fibrinolysis 2000;11(7):669-72
Doesn't address study question

Kirschbaum N E, Foster P A. The polymerase chain reaction with sequence specific primers for the detection of the factor V mutation associated with activated protein C resistance. Thromb Haemost 95;74(3):874-8
Published before 2000

Klein C L, Marki-Zay J, Corbisier P et al. Reference materials (RMs) for analysis of the human factor II (prothrombin) gene G20210A mutation. Clin Chem Lab Med 2005;43(8):862-8
Does not report concordance, discordance, or reproducibility

Klingler K R, Grote S, Holzem G et al. Activated protein C (APC)-resistance: automated detection of the point mutation at position 1691 in the factor V gene. Clin Chem Lab Med 99;37(2):101-7
Published before 2000

Klingler K R, Holzem G, Wielckens K. APC-Resistance: Automated detection of the point mutation at position 1691 in the factor V gene: APC-RESISTENZ: AUTOMATISIERTER NACHWEIS DER PUNKTMUTATION AN DER POSITION 1691 IM FAKTOR V GEN. LaboratoriumsMedizin 99;23(11):606-611
Non-English article

Klingler K R, Junold T, Wielckens K. Activated protein C resistance: automated detection of the factor V Leiden mutation by mismatch hybridization. Clin Chem 99;45(11):1925-31

Appendix D: List of Excluded Articles

Published before 2000

Koizumi M, Morita K, Takagi M et al. SNP genotyping by allele-specific PCR using ENA primers. Nucleic Acids Symp Ser (Oxf) 2005;(49):47-8
Does not report concordance, discordance, or reproducibility

Kornblihtt L I, Heller P G, Correa G et al. Associated thrombophilic defects in essential thrombocythaemia: their relationship with clinical manifestations. Thromb Res 2003;112(3):131-5
Did not study venous thrombosis

Krawczak M, Cooper D N, Schmidtke J. Estimating the efficacy and efficiency of cascade genetic screening. Am J Hum Genet 2001;69(2):361-70
Doesn't address the study question

Krousel-Wood M, Andersson H C, Rice J et al. Physicians' perceived usefulness of and satisfaction with test reports for cystic fibrosis (DeltaF508) and factor V Leiden. Genet Med 2003;5(3):166-71
Doesn't address the study question

Kwiatkowski R W, Lyamichev V, de Arruda M et al. Clinical, genetic, and pharmacogenetic applications of the Invader assay. Mol Diagn 99;4(4):353-64
Published before 2000

Kwon H J, Peiper S C, Kang K A. Fiber optic immunosensors for cardiovascular disease diagnosis: quantification of Protein C, Factor V Leiden, and cardiac Troponin T in plasma. Adv Exp Med Biol 2003;510115-9
Does not report concordance, discordance, or reproducibility

Kyrle P A, Eichinger S. The risk of recurrent venous thromboembolism: the Austrian Study on Recurrent Venous Thromboembolism. Wien Klin Wochenschr 2003;115(13-14):471-4
No original data

Laczkovics C, Grafenhofer H, Kaider A et al. Risk of recurrence after a first venous thromboembolic event in young women. Haematologica 2007;92(9):1201-7
Other reason

Lapham E V, Kozma C, Weiss J O. Genetic discrimination: perspectives of consumers. Science 96;274(5287):621-4
Doesn't address study question

Lechner D, Wiener C, Weltermann A et al. Comparison between idiopathic deep vein thrombosis of the upper and lower extremity regarding risk factors and recurrence. J Thromb Haemost 2008;6(4):1269-74. Epub 2008 Apr 26
Does not report results separately for FV Leiden and/or PT 20210A

Lengyel T, Sasvari-Szekely M, Guttman A. High-throughput genotyping of factor V Leiden mutation by ultrathin-layer agarose gel electrophoresis. J Chromatogr A 99;853(1-2):519-25
Published before 2000

Lensen R P, Bertina R M, de Ronde H et al. Venous thrombotic risk in family members of unselected individuals with factor V Leiden. Thromb Haemost 2000;83(6):817-21
No objective confirmation of VTE event

Lewandowski K, Rozek M, Zawilska K et al. An alternative method for identifying the factor V gene Leiden mutation. Thromb Res 97;85(2):105-13
Published before 2000

Libourel E J, Bank I, Meinardi J R et al. Co-segregation of thrombophilic disorders in factor V Leiden carriers; the contributions of factor VIII, factor XI, thrombin activatable fibrinolysis inhibitor and lipoprotein(a) to the absolute risk of venous thromboembolism. Haematologica 2002;87(10):1068-73
Other reason

Lim W, Eikelboom J W, Ginsberg J S. Inherited thrombophilia and pregnancy associated venous thromboembolism. BMJ 2007;334(7607):1318-21
No original data

Lindqvist P G, Dahlback B. Reactions to awareness of activated protein C resistance carriership: a descriptive study of 270 women. Acta Obstet Gynecol Scand 2003;82(5):467-70
Other reason

Lindqvist P G, Olofsson P, Dahlback B. Use of selective factor V Leiden screening in pregnancy to identify candidates for anticoagulants. Obstet Gynecol 2002;100(2):332-6
Evaluates less than 10 probands or 10 family members

Linfert D R, Rezuke W N, Tsongalis G J. Rapid multiplex analysis for the factor V Leiden and prothrombin G20210A mutations associated with hereditary thrombophilia. Conn Med 98;62(9):519-25
Published before 2000

Lloyd F J, Reyna V F, Whalen P. Accuracy and ambiguity in counseling patients about genetic risk. Arch Intern Med 2001;161(20):2411-3
Doesn't address study question

Lopaciuk S, Bielska-Falda H, Noszczyk W et al. Low molecular weight heparin versus acenocoumarol in the secondary prophylaxis of deep vein thrombosis. Thromb Haemost 99;81(1):26-31
Doesn't address study question

Ludemann P, Nabavi D G, Junker R et al. Factor V Leiden mutation is a risk factor for cerebral venous thrombosis: a case-control study of 55 patients. Stroke 98;29(12):2507-10
Case-control or retrospective cohort of probands

Lutz C T, Foster P A, Noll W W et al. Multicenter evaluation of PCR methods for the detection of factor V Leiden (R506Q) genotypes. Clin Chem 98;44(6 Pt 1):1356-8
Published before 2000

Lyondagger E, Millsondagger A, Phan T et al. Detection and Identification of Base Alterations Within the Region of Factor V Leiden by Fluorescent Melting Curves. Mol Diagn 98;3(4):203-209
Published before 2000

Mahadevan M S, Benson P V. Factor V null mutation affecting the Roche LightCycler factor V Leiden assay. Clin Chem 2005;51(8):1533-5
Evaluates less than 10 samples

Maher C, Crowley D, Cullen C et al. Double fluorescent-amplification refractory mutation detection (dF-ARMS) of the factor V Leiden and prothrombin mutations. Thromb Haemost 99;81(1):76-80
Published before 2000

Makris M, Preston F E, Beauchamp N J et al. Co-inheritance of the 20210A allele of the prothrombin gene increases the risk of thrombosis in subjects with familial thrombophilia. Thromb Haemost 97;78(6):1426-9
Does not report results separately for FV Leiden and/or PT 20210A

Marcucci R, Liotta A A, Cellai A P et al. Increased plasma levels of lipoprotein(a) and the risk of idiopathic and recurrent venous thromboembolism. Am J Med 2003;115(8):601-5
Does not report results separately for FV Leiden and/or PT 20210A

Margaglione M, D'Andrea G, Colaizzo D et al. Coexistence of factor V Leiden and Factor II A20210 mutations and recurrent venous thromboembolism. Thromb Haemost 99;82(6):1583-7
Other reason

Marteau T M, Croyle R T. The new genetics. Psychological responses to genetic testing. BMJ 98;316(7132):693-6
Doesn't address study question

Martinelli I, Battaglioli T, Bucciarelli P et al. Risk factors and recurrence rate of primary deep vein thrombosis of the upper extremities. Circulation 2004;110(5):566-70
Does not report results separately for FV Leiden and/or PT 20210A

Martinelli I, Mannucci P M, De Stefano V et al. Different risks of thrombosis in four coagulation defects associated with inherited thrombophilia: a study of 150 families. Blood 98;92(7):2353-8
Other reason

McColl M D, Walker I D, Greer I A. A mutation in the prothrombin gene contributing to venous thrombosis during pregnancy. Br J Obstet Gynaecol 98;105(8):923-5
Doesn't address study question

McKenzie S B, Clare C N, Smith L A et al. Laboratory test utilization in the diagnosis of hypercoagulability. Clin Lab Sci 2000;13(4):215-21
Less than 80% of the probands has the FV Leiden or PT 20210A mutation

Medina P, Navarro S, Estelles A et al. Influence of the 4600A/G and 4678G/C polymorphisms in the endothelial protein C receptor (EPCR) gene on the risk of venous thromboembolism in carriers of factor V Leiden. Thromb Haemost 2005;94(2):389-94
Case-control or retrospective cohort of probands

Meinardi J R, Middeldorp S, de Kam P J et al. Risk of venous thromboembolism in carriers of factor V Leiden with a concomitant inherited thrombophilic defect: a retrospective analysis. Blood Coagul Fibrinolysis 2001;12(8):713-20
Other reason

Meltzer M E, Lisman T, Doggen C J et al. Synergistic Effects of Hypofibrinolysis and Genetic and Acquired Risk Factors on the Risk of a First Venous Thrombosis. PLoS Med 2008;5(5):e97
Case-control or retrospective cohort of probands

Meyer M, Kutscher G, Vogel G. Simultaneous genotyping for factor V Leiden and prothrombin G20210A variant by a multiplex PCR-SSCP assay on whole blood. Thromb Haemost 99;81(1):162-3
Published before 2000

Minano A, Ordonez A, Espana F et al. AB0 blood group and risk of venous or arterial thrombosis in carriers of factor V Leiden or prothrombin G20210A polymorphisms. Haematologica 2008;93(5):729-34
Case-control or retrospective cohort of probands

Misgav M, Eldor A, Berliner S. [A new mutation in the prothrombin gene (G20210A) and the risk for venous and arterial thromboembolism]. Harefuah 2000;139(1-2):51-6
Non-English article

Mitterer M, Lanthaler A J, Mair W et al. Simultaneous detection of FV Q506 and prothrombin 20210 A variation by allele-specific PCR. Haematologica 99;84(3):204-7
Published before 2000

Moll S, Escobar M. Prothrombin and factor V mutations in women with a history of thrombosis during pregnancy and the puerperium. N Engl J Med 2000;342(26):1996-7
Doesn't address study question

Muriel G, Christine B, Philippe J et al. An even easier method for one-step detection of both FV Leiden and FII G20210A transition. Blood 98;92(9):3478-9
Published before 2000

Murphy K M, Eshleman J R. Simultaneous sequencing of multiple polymerase chain reaction products and combined

polymerase chain reaction with cycle sequencing in single reactions. Am J Pathol 2002;161(1):27-33
Does not report concordance, discordance, or reproducibility

Murphy K M, Berg K D, Eshleman J R. Sequencing of genomic DNA by combined amplification and cycle sequencing reaction. Clin Chem 2005;51(1):35-9
Evaluates only DNA extraction methods

Mustafa S, Mannhalter C, Rintelen C et al. Clinical features of thrombophilia in families with gene defects in protein C or protein S combined with factor V Leiden. Blood Coagul Fibrinolysis 98;9(1):85-9
No objective confirmation of VTE event

Nagy P L, Schrijver I, Zehnder J L. Molecular Diagnosis of Hypercoagulable States. Lab. Med. 2004;35(4):214-221
No original data

Naudziunas A, Miliauskas S. Factor V Leiden and post thromboembolic pulmonary hypertension. Medicina (Kaunas) 2003;39(12):1171-4
Did not study venous thrombosis

Navarro S, Medina P, Mira Y et al. Haplotypes of the EPCR gene, prothrombin levels, and the risk of venous thrombosis in carriers of the prothrombin G20210A mutation. Haematologica 2008;93(6):885-91. Epub 2008 Apr 9
Case-control or retrospective cohort of probands

Neoh S H, Brisco M J, Firgaira F A et al. Rapid detection of the factor V Leiden (1691 G > A) and haemochromatosis (845 G > A) mutation by fluorescence resonance energy transfer (FRET) and real time PCR. J Clin Pathol 99;52(10):766-9
Published before 2000

Noboa S, Le Gal G, Lacut K et al. Family history as a risk factor for venous thromboembolism. Thromb Res 2008;122(5):624-9. Epub 2008 Feb 20
Does not report results separately for FV Leiden and/or PT 20210A

Oguzulgen I K, Demirtas S, Erkekol F O et al. The Role of Plasminogen Activator Inhibitor-1 Polymorphism, Factor-V-Leiden, and Prothrombin-20210 Mutations in Pulmonary Thromboembolism. Clin Appl Thromb Hemost 2007;[Epub ahead of print]
Case-control or retrospective cohort of probands

Oguzulgen I K, Ekim N N, Akar N et al. The role of thrombophilic risk factors in the severity of pulmonary thromboembolism. Eur Respir J 2002;19(4):709-11
Doesn't address study question

Olds R J, Fitches A C, Geary C P. The multigenic basis for venous thrombosis. Br J Haematol 2000;109(3):508-11
No original data

Orum H, Jakobsen M H, Koch T et al. Detection of the factor V Leiden mutation by direct allele-specific hybridization of PCR amplicons to photoimmobilized locked nucleic acids. Clin Chem 99;45(11):1898-905
Published before 2000

Otterson G A, Monahan B P, Harold N et al. Clinical significance of the FV:Q506 mutation in unselected oncology patients. Am J Med 96;101(4):406-12
Evaluates less than 10 probands or 10 family members

Pabinger I, Grafenhofer H, Kaider A et al. Risk of pregnancy-associated recurrent venous thromboembolism in women with a history of venous thrombosis. J Thromb Haemost 2005;3(5):949-54
Other reason

Palareti G, Cosmi B, Legnani C et al. D-dimer testing to determine the duration of anticoagulation therapy. N Engl J Med 2006;355(17):1780-9
Other reason

Palareti G, Legnani C, Cosmi B et al. Poor anticoagulation quality in the first 3 months after unprovoked venous thromboembolism is a risk factor for long-term recurrence. J Thromb Haemost 2005;3(5):955-61
Doesn't address study question

Paolo, G., Marco, C., Silvia, M., Alessandra, V., Fabbio, M., Nicoletta, C., Giorgio, F., Carlo Federico, P., and Sergio, B. Impact of storage conditions on genetic analysis or viral load determination in clinical specimens. Clin Chem Lab Med, 2007;[Epub ahead of print]
Doesn't address study question

Paschoa A F, Guillaumon A T. Impact of screening on thrombophilia for patients with venous thrombosis. Int Angiol 2006;25(1):52-9
Other reason

Patri S, Salmeron S, Caillon M et al. Multiplex PCR for one-step determination of the G20210A variation and the factor V Leiden mutation by denaturing gradient gel electrophoresis (DGGE). Thromb Haemost 99;81(2):313-4
Published before 2000

Patrushev L I, Zykova E S, Kayushin A L et al. New DNA diagnostic system for detection of factor V Leiden. Thromb Res 98;92(6):251-9
Published before 2000

Pecheniuk N M, Marsh N A, Walsh T P et al. Use of first nucleotide change technology to determine the frequency of factor V Leiden in a population of Australian blood donors. Blood Coagul Fibrinolysis 97;8(8):491-5
Published before 2000

Peng F, Triplett D, Barna L. Direct PCR of leukocytes treated with microwave irradiation to detect factor V Leiden mutation. Thromb Res 96;82(2):193-7
Published before 2000

Pernod G, Mossuz P, Polack B. Optimized factor V gene

mutation detection using buffy-coat direct PCR. Biotechniques 97;22(5):837, 840, 841
Published before 2000

Pettila V, Kaaja R, Leinonen P et al. Thromboprophylaxis with low molecular weight heparin (dalteparin) in pregnancy. Thromb Res 99;96(4):275-82
Doesn't address study question

Pini M, Aiello S, Manotti C et al. Low molecular weight heparin versus warfarin in the prevention of recurrences after deep vein thrombosis. Thromb Haemost 94;72(2):191-7
Doesn't address study question

Prandoni P, Lensing A W, Piccioli A et al. Recurrent venous thromboembolism and bleeding complications during anticoagulant treatment in patients with cancer and venous thrombosis. Blood 2002;100(10):3484-8
Doesn't address study question

Prandoni P, Lensing A W, Prins M H et al. Residual venous thrombosis as a predictive factor of recurrent venous thromboembolism. Ann Intern Med 2002;137(12):955-60
Doesn't address study question

Press R D. Thrombophilic mutations impart a high risk of pregnancy-related venous thrombosis. Mol Diagn 2000;5(2):158
Doesn't address study question

Quehenberger P, Handler S, Mannhalter C et al. The Factor V (Leiden) test: evaluation of an assay based on dilute Russell Viper Venom Time for the detection of the Factor V Leiden mutation. Thromb Res 99;96(2):125-33
Published before 2000

Raoul M, Mathonnet F, Peltier J Y et al. An improved method for the detection of the G20210A transition in the prothrombin gene. Thromb Res 97;88(5):441-3
Published before 2000

Reitsma P H, van der, Velden P A et al. Use of the direct RNA amplification technique NASBA to detect factor V Leiden, a point mutation associated with APC resistance. Blood Coagul Fibrinolysis 96;7(6):659-63
Published before 2000

Ren, Y., Rezania, S., and Kang, K. A. Biosensor for diagnosing Factor V Leiden, a single amino acid mutated abnormality of Factor V. Adv Exp Med Biol 2008;614:245-52
Other reason

Ridker P M, Goldhaber S Z, Danielson E et al. Long-term, low-intensity warfarin therapy for the prevention of recurrent venous thromboembolism. N Engl J Med 2003;348(15):1425-34
Other reason

Rintelen C, Mannhalter C, Lechner K et al. No evidence for an increased risk of venous thrombosis in patients with factor V Leiden by the homozygous 677 C to T mutation in the methylenetetrahydrofolate-reductase gene. Blood Coagul Fibrinolysis 99;10(2):101-5
Does not report results separately for FV Leiden and/or PT 20210A

Rintelen C, Pabinger I, Knobl P et al. Probability of recurrence of thrombosis in patients with and without factor V Leiden. Thromb Haemost 96;75(2):229-32
Other reason

Robert A, Aillaud M F, Eschwege V et al. ABO blood group and risk of venous thrombosis in heterozygous carriers of factor V Leiden. Thromb Haemost 2000;83(4):630-1
Doesn't address study question

Rodeghiero F, Tosetto A. The epidemiology of inherited thrombophilia: the VITA Project. Vicenza Thrombophilia and Atherosclerosis Project. Thromb Haemost 97;78(1):636-40
Doesn't address study question

Ruggeri M, Gisslinger H, Tosetto A et al. Factor V Leiden mutation carriership and venous thromboembolism in polycythemia vera and essential thrombocythemia. Am J Hematol 2002;71(1):1-6
Other reason

Russeva M G, Janakiev P J, Kirov S A et al. A simple method for detection of factor V R506Q (Leiden) mutation in dried blood spots. Clin Chim Acta 99;284(1):89-92
Published before 2000

Ryan D, Nuccie B, Arvan D. Non-PCR-dependent detection of the factor V Leiden mutation from genomic DNA using a homogeneous invader microtiter plate assay. Mol Diagn 99;4(2):135-44
Published before 2000

Sabbagh A S, Ibrahim G, Kanaan Z et al. Prevalence of the prothrombin G20210A polymorphism in the Lebanese population: use of a reverse hybridization strip assay approach. Mol Biol Rep 2007;[Epub ahead of print]
Does not report concordance, discordance, or reproducibility

Saffroy R, Lemoine A, Haas P et al. Rapid automated simultaneous screening of (G1691A) Factor V, (G20210A) prothrombin, and (C677T) methylenetetrahydrofolate reductase variants by multiplex PCR using fluorescence scanning technology. Genet Test 2002;6(3):233-6
Does not report concordance, discordance, or reproducibility

Samama M M, Rached R A, Conard J et al. [Pregnancy-associated venous thrombosis in women with hereditary heterozygous factor V Leiden and/or factor II gene mutations]. Bull Acad Natl Med 2004;188(8):1377-93; discussion 1393-6
Non-English article

Samama M-M, Rached R A, Conard J et al. Pregnancy-associated venous thrombosis in women with hereditary heterozygous factor V Leiden and/or factor II gene mutations: RISQUE DE THROMBOSE LIE A LA GROSSESSE CHEZ LES FEMMES PORTEUSES D'UNE MUTATION HETEROZYGOTE DU FACTEUR V LEIDEN, DU FACTEUR II OU DE LEUR ASSOCIATION. Bull. Acad. Natl. Med. 2004;188(8):1377-1396
Non-English article

Santamaria A, Mateo J, Oliver A et al. Risk of thrombosis associated with oral contraceptives of women from 97 families with inherited thrombophilia: high risk of thrombosis in carriers of the G20210A mutation of the prothrombin gene. Haematologica 2001;86(9):965-71
Other reason

Sasvari-Szekely M, Gerstner A, Ronai Z et al. Rapid genotyping of factor V Leiden mutation using single-tube bidirectional allele-specific amplification and automated ultrathin-layer agarose gel electrophoresis. Electrophoresis 2000;21(4):816-21
Does not report concordance, discordance, or reproducibility

Scharnhorst V, Gijselhart-Nivillac N J F, Goorhuis A D et al. Detection of factor V Leiden and factor II mutations by using duplex PCR: DETECTIE VAN DE FACTOR-V-LEIDEN- EN FACTOR-II-MUTATIES MET BEHULP VAN EEN DUPLEX-PCR. Ned. Tijdschr. Klin. Chem. Lab. Geneeskd. 2004;29(1):33-35
Non-English article

Schattner A, Kasher I, Berrebi A. Causes and outcome of deep-vein thrombosis in otherwise-healthy patients under 50 years. QJM 97;90(4):283-7
Does not report results separately for FV Leiden and/or PT 20210A

Schramm W, Heinemann L A, Spannagl M et al. [The Bavarian Thromboembolic Risk Cohort Study (BATER). Study protocol, state of the investigation and first results]. Dtsch Med Wochenschr 2000;125(1-2):2-6
Non-English article

Schroder W, Koesling M, Wulff K et al. Large-scale screening for factor V Leiden mutation in a north-eastern German population. Haemostasis 96;26(5):233-6
Published before 2000

Schulman S, Granqvist S, Holmstrom M et al. The duration of oral anticoagulant therapy after a second episode of venous thromboembolism. The Duration of Anticoagulation Trial Study Group. N Engl J Med 97;336(6):393-8
Doesn't address study question

Schulman S, Lindmarker P, Holmstrom M et al. Post-thrombotic syndrome, recurrence, and death 10 years after the first episode of venous thromboembolism treated with warfarin for 6 weeks or 6 months. J Thromb Haemost 2006;4(4):734-42
Other reason

Schulman S, Wahlander K, Lundstrom T et al. Secondary prevention of venous thromboembolism with the oral direct thrombin inhibitor ximelagatran. N Engl J Med 2003;349(18):1713-21
Doesn't address study question

Scobie G A, Ho S T, Dolan G et al. Development of a rapid DNA screening procedure for the Factor V Leiden mutation. Clin Mol Pathol 96;49(6):M361-M363
Published before 2000

Shrivastava S, Ridker P M, Glynn R J et al. D-dimer, factor VIII coagulant activity, low-intensity warfarin and the risk of recurrent venous thromboembolism. J Thromb Haemost 2006;4(6):1208-14
Doesn't address study question

Simioni P, Prandoni P, Lensing A W et al. The risk of recurrent venous thromboembolism in patients with an Arg506-->Gln mutation in the gene for factor V (factor V Leiden). N Engl J Med 97;336(6):399-403
Other reason

Simkova M, Batorova A, Dostalova K et al. Factor V Leiden in patients with venous thrombosis in Slovak population. Gen Physiol Biophys 2004;23(4):435-42
Doesn't address study question

Sinclair G D, Low S, Poon M C. A hemi-nested, allele specific, whole blood PCR assay for the detection of the factor V Leiden mutation. Thromb Haemost 97;77(6):1154-5
Published before 2000

Sitbon G, Hurtig M, Palotie A et al. A colorimetric minisequencing assay for the mutation in codon 506 of the coagulation factor V gene. Thromb Haemost 97;77(4):701-3
Published before 2000

Snyder T M, McGown L B. Multiplex single strand conformation polymorphism analysis by capillary electrophoresis with on-the-fly fluorescence lifetime detection. Appl Spectrosc 2005;59(3):335-9
Does not report concordance, discordance, or reproducibility

Solem C A, Loftus E V, Tremaine W J et al. Venous thromboembolism in inflammatory bowel disease. Am J Gastroenterol 2004;99(1):97-101
Doesn't address study question

Souto J C, Mateo J, Soria J M et al. Homozygotes for prothrombin gene 20210 A allele in a thrombophilic family without clinical manifestations of venous thromboembolism. Haematologica 99;84(7):627-32
Did not study venous thrombosis

Appendix D: List of Excluded Articles

Sproul A M, Chalmers E A. Multiplex PCR for the detection of the factor V Leiden and prothrombin 20210A mutations. Methods Mol Med 2004;9179-87
Does not report concordance, discordance, or reproducibility

Srur A E, Vargas R C, Salas F S et al. Primary thombophilia. Report of 93 cases and 12 asymptomatic relatives: TROMBOFILIA PRIMARIA: DETECCION Y MANIFESTACION CLINICA EN 105 CASOS. Rev. Med. Chile 2004;132(12):1466-1473
Non-English article

Stegnar M, Peternel P, Uhrin P et al. Fibrinolysis in patients with the 1691 G->A mutation in factor V gene and history of deep vein thrombosis. FIBRINOLYSIS PROTEOLYSIS 97;11(4):201-207
Doesn't address study question

Strobl F J, Hoffman S, Huber S et al. Activated protein C resistance assay performance: improvement by sample dilution with factor V-deficient plasma. Arch Pathol Lab Med 98;122(5):430-3
Published before 2000

Tirado I, Mateo J, Soria J M et al. Contribution of prothrombin 20210A allele and factor V Leiden mutation to thrombosis risk in thrombophilic families with other hemostatic deficiencies. Haematologica 2001;86(11):1200-8
Other reason

Tormene D, Fortuna S, Tognin G et al. The incidence of venous thromboembolism in carriers of antithrombin, protein C or protein S deficiency associated with the HR2 haplotype of factor V: a family cohort study. J Thromb Haemost 2005;3(7):1414-20
Doesn't address study question

Tormene D, Simioni P, Prandoni P et al. The risk of fetal loss in family members of probands with factor V Leiden mutation. Thromb Haemost 99;82(4):1237-9
Published before 2000

Tosetto A, Missiaglia E, Frezzato M et al. The VITA project: prothrombin G20210A mutation and venous thromboembolism in the general population. Thromb Haemost 99;82(5):1395-8
Case-control or retrospective cohort of probands

Toulon P, Lequerrec A, Piquet P et al. Significant influence of the instrument on the result of the ProC Global assay. A multicenter evaluation using lyophilized plasmas and frozen plasma samples from carriers and non-carriers of the factor V Leiden mutation. Thromb Res 2002;107(3-4):181-8
Evaluates only APC resistance

Tran M, Spencer F A. Thromboepidemiology: identifying patients with heritable risk for thrombin-mediated thromboembolic events. Am Heart J 2005;149(1 Suppl):S9-18
No original data

Uitte de, Willige S, De Visser M C et al. Selectin haplotypes and the risk of venous thrombosis: influence of linkage disequilibrium with the factor V Leiden mutation. J Thromb Haemost 2008;6(3):478-85
Case-control or retrospective cohort of probands

Ulvik A, Ren J, Refsum H et al. Simultaneous determination of methylenetetrahydrofolate reductase C677T and factor V G1691A genotypes by mutagenically separated PCR and multiple-injection capillary electrophoresis. Clin Chem 98;44(2):264-9
Published before 2000

van Boven H H, Vandenbroucke J P, Briet E et al. Gene-gene and gene-environment interactions determine risk of thrombosis in families with inherited antithrombin deficiency. Blood 99;94(8):2590-4
Does not report results separately for FV Leiden and/or PT 20210A

van de, Locht L T, Kuypers A W et al. Semi-automated detection of the factor V mutation by allele specific amplification and capillary electrophoresis. Thromb Haemost 95;74(5):1276-9
Published before 2000

van der, Meer F J, Koster T et al. The Leiden Thrombophilia Study (LETS). Thromb Haemost 97;78(1):631-5
No original data

van Hylckama, Vlieg A, Baglin C A et al. Proof of principle of potential clinical utility of multiple SNP analysis for prediction of recurrent venous thrombosis. J Thromb Haemost 2008;6(5):751-4
Other reason

Van Rooijen M, Bremme K. A family history can display a synergistic effect of atherogenic and prothrombotic risk in pregnancy. Acta Obstet Gynecol Scand 2001;80(9):873-4
Doesn't address study question

Vandenbroucke J P, Briet E, Helmerhorst F et al. [Oral contraception increases the chance of venous thrombosis in factor V Leiden mutation]. Ned Tijdschr Geneeskd 95;139(21):1067-71
Non-English article

Varga Elizabeth A, Moll Stephan. Prothrombin 20210 Mutation (Factor II Mutation). Circulation 2004;110(3):e15-18
No original data

Vatan R, Delpeu K, Vergnes C et al. [Clinicobiological study of 100 symptomatic patients with factor V Leiden mutation]. Rev Med Interne 2000;21(5):465-6
Non-English article

Veiga F, Escriba A, Maluenda M P et al. Low molecular weight heparin (enoxaparin) versus oral anticoagulant

therapy (acenocoumarol) in the long-term treatment of deep venous thrombosis in the elderly: a randomized trial. Thromb Haemost 2000;84(4):559-64
Doesn't address study question

Voelkerding K V, Huber S, Strobl F et al. Resistance to Activated Protein C: Comparison of Three Different PCR Methods for Detection FV R506Q. Mol Diagn 96;1(4):297-304
Published before 2000

von Ahsen N, Oellerich M, Schutz E. A method for homogeneous color-compensated genotyping of factor V (G1691A) and methylenetetrahydrofolate reductase (C677T) mutations using real-time multiplex fluorescence PCR. Clin Biochem 2000;33(7):535-9
Does not report concordance, discordance, or reproducibility

von Ahsen N, Oellerich M, Armstrong V W et al. Application of a thermodynamic nearest-neighbor model to estimate nucleic acid stability and optimize probe design: prediction of melting points of multiple mutations of apolipoprotein B-3500 and factor V with a hybridization probe genotyping assay on the LightCycler. Clin Chem 99;45(12):2094-101
Published before 2000

von Ahsen N, Schutz E, Armstrong V W et al. Rapid detection of prothrombotic mutations of prothrombin (G20210A), factor V (G1691A), and methylenetetrahydrofolate reductase (C677T) by real-time fluorescence PCR with the LightCycler. Clin Chem 99;45(5):694-6
Does not report concordance, discordance, or reproducibility

Walz T, Geisel J, Bodis M et al. Fluorescence-based single-strand conformation polymorphism analysis of mutations by capillary electrophoresis. Electrophoresis 2000;21(2):375-9
Does not report concordance, discordance, or reproducibility

Welbourn J T, Maiti S, Paley J. Factor V Leiden detection by polymerase chain reaction-restriction fragment length polymorphism with mutagenic primers in a multiplex reaction with Pro G20210A--a novel technique. Hematology 2003;8(2):73-5
Does not report concordance, discordance, or reproducibility

Weltermann A, Eichinger S, Bialonczyk C et al. The risk of recurrent venous thromboembolism among patients with high factor IX levels. J Thromb Haemost 2003;1(1):28-32
Other reason

White R H, Chan W S, Zhou H et al. Recurrent venous thromboembolism after pregnancy-associated versus unprovoked thromboembolism. Thromb Haemost 2008;100(2):246-52
Doesn't address study question

Wilde J T, O'Sullivan J J, Roper J L et al. A novel ELISA-based primer extension assay for the detection of the factor V Leiden mutation. Br J Haematol 99;106(2):427-30
Published before 2000

Wisotzkey J D, Bell T, Monk J S. Simultaneous polymerase chain reaction restriction fragment length polymorphism identification of the factor V Leiden allele and the prothrombin 20210A mutation. Diagn Mol Pathol 98;7(3):180-3
Published before 2000

Wong T, Nightingale J, Winter M et al. Spontaneous venous thrombosis in inflammatory bowel disease: relevance of factor V Leiden and the prothrombin gene mutation. J Thromb Haemost 2003;1(6):1326-8
Doesn't address study question

Xu X, Bauer K A, Griffin J H. Two multiplex PCR-based DNA assays for the thrombosis risk factors prothrombin G20210A and coagulation factor V G1691A polymorphisms. Thromb Res 99;93(6):265-9
Does not report concordance, discordance, or reproducibility

Yandava C N, Zappulla D C, Korf B R et al. ARMS test for diagnosis of factor VLeiden mutation, a common cause of inherited thrombotic tendency. J Clin Lab Anal 96;10(6):414-7
Published before 2000

Young L, Ockelford P, Milne D et al. Post-treatment residual thrombus increases the risk of recurrent deep vein thrombosis and mortality. J Thromb Haemost 2006;4(9):1919-24
Doesn't address study question

Zehnder J L, Benson R C, Cheng S. A microplate allele-specific oligonucleotide hybridization assay for detection of factor V Leiden. Diagn Mol Pathol 97;6(6):347-52
Published before 2000

Zehnder J, Van Atta R, Jones C et al. Cross-linking hybridization assay for direct detection of factor V Leiden mutation. Clin Chem 97;43(9):1703-8
Published before 2000

Zotz R B, Maruhn-Debowski B, Scharf R E. Mutation in the gene coding for coagulation factor V and resistance to activated protein C: detection of the genetic mutation by oligonucleotide ligation assay using a semi-automated system. Thromb Haemost 96;76(1):53-5
Published before 2000

Impact of Testing for Factor V Leiden (G1691A) and Prothrombin (G20210A) Mutations on Health Outcomes in Adults with a History of Venous Thromboembolic Events and Their Adult Family Members

Feedback Form

Reviewer Name: _____

Please use this form to enter your comments on the sections assigned to you. In addition to reviewing the sections assigned to you, we will accept any additional comments you include.

1	Structured Abstract:
2	Executive Summary :
3	Chapter 1: Introduction : .

Feedback Form

4	Chapter 2: Methods
5	Chapter 3: Results
	Comments concerning description of results: Key Questions #3a *What is the evidence that the presence of FVL alone or prothrombin G20210A alone or the two in combination predicts risk of recurrent VTE in individuals (probands) who have had VTE and predicts the risk of VTE in the probands' family members who have been tested? Does the testing add predictive information beyond clinical data?:*

Impact of Testing for Factor V Leiden (G1691A) and Prothrombin (G20210A) Mutations on Health Outcomes in Adults with a History of Venous Thromboembolic Events and Their Adult Family Members

Feedback Form

	Comments concerning description of results: Key Questions #3b *What is the evidence that demographic or clinical factors modify the relationship between the presence of FVL or prothrombin G20210A and the risk of VTE?:*
	Comments concerning description of results: Key Question #4a *What is the evidence that clinicians manage patients differently based on the results of testing for FVL or prothrombin G20210A? How do clinicians manage anticoagulation of individuals who have had testing compared to those who have not had testing? What other diagnostic tests do clinicians order or not order based on testing results? What recommendations do clinicians make regarding other therapies and exposures based on testing results?:*
	Comments concerning description of results: Key Question #4b *What is the evidence that testing, and the resultant management, reduces VTE related-outcomes or has other benefits in individuals who have had VTE or in the probands' family members who have been tested?:*
	Comments concerning description of results: Key Question #4c *What is the evidence of harms to individuals with VTE or to the probands' family members who are tested for FVL or prothrombin G20210A as a result of testing or as a result of changed management based on the test results?:*

Impact of Testing for Factor V Leiden (G1691A) and Prothrombin (G20210A) Mutations on Health Outcomes in Adults with a History of Venous Thromboembolic Events and Their Adult Family Members

Feedback Form

	Comments concerning description of results: Key Question #4d *What is the evidence that testing for FVL alone, prothrombin G20210A alone, or the two tests in combination is a cost-effective strategy when caring for a patient with VTE or a family member of a proband?:*
6	Chapter 4: Discussion
7	Figures, Tables, and Appendix materials including Evidence Tables: As above
8	Specific comments about text (e.g., typos, unclear sentence)

	Chapter	Page	Line	Comment

Impact of Testing for Factor V Leiden (G1691A) and Prothrombin (G20210A) Mutations on Health Outcomes in Adults with a History of Venous Thromboembolic Events and Their Adult Family Members

Feedback Form

9	General comments:			

Thank you for your feedback. Please **e-mail** to Renee F. Wilson
rwilsob@jhmi.edu
Feedback must be received no later than **February 1, 2009**

Appendix F

Appendix G

Evidence Table 1. Quality of studies addressing Analytic Validity of Tests to Identify Factor V Leiden and Prothrombin G20210A Mutations

Reference List

1. Hessner MJ, Budish MA, Friedman KD. Genotyping of factor V G1691A (Leiden) without the use of PCR by invasive cleavage of oligonucleotide probes. Clin Chem 2000; 46(8 Pt 1):1051-6.
2. Kowalski A, Radu D, Gold B. Colorimetric microwell plate detection of the factor V Leiden mutation. Clin Chem 2000; 46(8 Pt 1):1195-8.
3. Ledford M, Friedman KD, Hessner MJ, Moehlenkamp C, Williams TM, Larson RS. A multi-site study for detection of the factor V (Leiden) mutation from genomic DNA using a homogeneous invader microtiter plate fluorescence resonance energy transfer (FRET) assay. J Mol Diagn 2000; 2(2):97-104.
4. Nauck M, Marz W, Wieland H. Evaluation of the Roche diagnostics LightCycler-Factor V Leiden Mutation Detection Kit and the LightCycler-Prothrombin Mutation Detection Kit. Clin Biochem 2000; 33(3):213-6.
5. Pecheniuk NM, Marsh NA, Walsh TP. Multiple analysis of three common genetic alterations associated with thrombophilia. Blood Coagul Fibrinolysis 2000; 11(2):183-9.
6. Sanders Sevall J. Factor V Leiden genotyping using real-time fluorescent polymerase chain reaction. Mol Cell Probes 2000; 14(4):249-53.
7. Benson JM, Ellingsen D, El-Jamil M *et al*. Factor V Leiden and factor V R2 allele: high-throughput analysis and association with venous thromboembolism. Thromb Haemost 2001; 86(5):1188-92.
8. Crockett AO, Wittwer CT. Fluorescein-labeled oligonucleotides for real-time pcr: using the inherent quenching of deoxyguanosine nucleotides. Anal Biochem 2001; 290(1):89-97.
9. Humeny A, Bonk T, Berkholz A, Wildt L, Becker CM. Genotyping of thrombotic risk factors by MALDI-TOF mass spectrometry. Clin Biochem 2001; 34(7):531-6.
10. Parks SB, Popovich BW, Press RD. Real-time polymerase chain reaction with fluorescent hybridization probes for the detection of prevalent mutations causing common thrombophilic and iron overload phenotypes. Am J Clin Pathol 2001; 115(3):439-47.
11. Potter CG, Liu YT, Rees DC. Factor V Leiden mutation screened by PCR and detected with lanthanide-labeled probes. Genet Test 2001; 5(4):291-7.
12. Reyes AA, Ugozzoli LA, Lowery JD *et al*. Linked linear amplification: a new method for the amplification of DNA. Clin Chem 2001; 47(1):31-40.
13. Rhodes RB, Lewis K, Shultz J *et al*. Analysis of the factor V Leiden mutation using the READIT Assay. Mol Diagn 2001; 6(1):55-61.
14. Tsongalis GJ, Rainey BJ, Hodges KA. READIT: a novel technology used in the interrogation of nucleic acid sequences for single-nucleotide polymorphisms. Exp Mol Pathol 2001; 71(3):222-5.
15. Evans JG, Lee-Tataseo C. Determination of the factor V Leiden single-nucleotide polymorphism in a commercial clinical laboratory by use of NanoChip microelectronic array technology. Clin Chem 2002; 48(9):1406-11.
16. Huang TJ, Liu M, Knight LD, Grody WW, Miller JF, Ho CM. An electrochemical detection scheme for identification of single nucleotide polymorphisms using hairpin-forming probes. Nucleic Acids Res 2002; 30(12):e55.
17. Hung K, Sun X, Ding H, Kalafatis M, Simioni P, Guo B. A matrix-assisted laser desorption/ionization time-of-flight based method for screening the 1691G --> A mutation in the factor V gene. Blood Coagul Fibrinolysis 2002; 13(2):117-22.
18. Ozkan D, Erdem A, Kara P *et al*. Allele-specific genotype detection of factor V Leiden mutation from polymerase chain reaction amplicons based on label-free electrochemical genosensor. Anal Chem 2002; 74(23):5931-6.
19. Alsmadi OA, Bornarth CJ, Song W *et al*. High accuracy genotyping directly from genomic DNA using a rolling circle amplification based assay. BMC Genomics 2003; 4(1):21.
20. Cooper PC, Cooper SM, Smith JM, Kitchen S, Makris M. Evaluation of the Roche LightCycler: a simple and rapid method for direct detection of factor V Leiden and prothrombin G20210A genotypes from blood samples without the need for DNA extraction. Blood Coagul Fibrinolysis 2003; 14(5):499-503.
21. El Housni H, Heimann P, Parma J, Vassart G. Single-nucleotide polymorphism genotyping by melting analysis of dual-labeled probes: examples using factor V Leiden and prothrombin 20210A mutations. Clin Chem 2003; 49(10):1669-72.
22. Moser MJ, Marshall DJ, Grenier JK *et al*. Exploiting the enzymatic recognition of an unnatural base pair to develop a universal genetic analysis system. Clin Chem 2003; 49(3):407-14.
23. Murphy K, Hafez M, Philips J, Yarnell K, Gutshall K, Berg K. Evaluation of temperature gradient capillary electrophoresis for detection of the Factor V Leiden mutation: coincident identification of a novel polymorphism in Factor V. Mol Diagn 2003; 7(1):35-40.
24. Ozsoz M, Erdem A, Kerman K *et al*. Electrochemical genosensor based on colloidal gold nanoparticles for the detection of Factor V Leiden mutation using disposable pencil graphite electrodes. Anal Chem 2003; 75(9):2181-7.
25. Schroell-Metzger B, Dicato M, Bosseler M, Berchem G. Comparison of standard PCR and the LightCycler technique to determine the thrombophilic mutations: an efficiency and cost study. Clin Chem Lab Med 2003; 41(4):482-5.
26. Simundic AM, Topic E, Stefanovic M. Detection of factor V Leiden by PCR-SSCP using GMA precast Elchrom scientific gels. Clin Appl Thromb

Hemost 2003; 9(3):227-31.
27. Vaughn CP, Elenitoba-Johnson KS. Hybridization-induced dequenching of fluorescein-labeled oligonucleotides: a novel strategy for PCR detection and genotyping. Am J Pathol 2003; 163(1):29-35.
28. Behrens M, Lange R. A highly reproducible and economically competitive SNP analysis of several well characterized human mutations. Clin Lab 2004; 50(5-6):305-16.
29. French C, Li C, Strom C *et al*. Detection of the factor V Leiden mutation by a modified photo-cross-linking oligonucleotide hybridization assay. Clin Chem 2004; 50(2):296-305.
30. Liew M, Pryor R, Palais R *et al*. Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons. Clin Chem 2004; 50(7):1156-64.
31. Louis M, Dekairelle AF, Gala JL. Rapid combined genotyping of factor V, prothrombin and methylenetetrahydrofolate reductase single nucleotide polymorphisms using minor groove binding DNA oligonucleotides (MGB probes) and real-time polymerase chain reaction. Clin Chem Lab Med 2004; 42(12):1364-9.
32. Angeline T, Bentley HA, Hawk AB *et al*. Prevalence of the Factor V G1691A and the Factor II/prothrombin G20210A gene polymorphisms among Tamilians. Exp Mol Pathol 2005; 79(1):9-13.
33. Castley A, Higgins M, Ivey J, Mamotte C, Sayer DC, Christiansen FT. Clinical applications of whole-blood PCR with real-time instrumentation. Clin Chem 2005; 51(11):2025-30.
34. Mammo L, Sheereen A, Saour T, Saour J. Comparative study between the Light Cycler and the PCR-restriction fragment length polymorphism in detecting factor V Leiden and factor II 20210G>A mutations. Clin Biochem 2006; 39(7):767-9.
35. Bravo-Osorio M, Bydlowski SP. Detection of methylenetetrahydrofolate reductase (MTHFR) C677T and prothrombin G20210A mutations: second restriction site for digestion control of PCR products. Clin Chim Acta 2000; 301(1-2):219-23.
36. Happich D, Madlener K, Schwaab R, Hanfland P, Potzsch B. Application of the TaqMan-PCR for genotyping of the prothrombin G20210A mutation and of the thermolabile methylenetetrahydrofolate reductase mutation. Thromb Haemost 2000; 84(1):144-5.
37. Meyer M, Kutscher G, Vogel G. The prothrombin nt20210 A allele as a risk factor for venous thromboembolism: detection of heterozygous and homozygous carriers by alternative methods. Thromb Res 2000; 97(5):359-63.
38. Gellings A, Holzem G, Wielckens K, Klingler KR. Prothrombin mutation: Employing the electrochemiluminescence technology of the elecsys(registered trademark) system for the detection of the point mutation at position 20210 in the 3' untranslated region of the prothrombin gene: PROTHROMBIN MUTATION: VERWENDUNG DER ELEKTROCHEMILUMINESZENZTECHNOLOGIE DES ELECSYS(registered trademark) SYSTEMS ZUR DETEKTION DER PUNKTMUTATION AN DER POSITION 20210 IN DER 3' UNTRANSLATIERTEN. LaboratoriumsMedizin 2001; 25(1-2):26-30.
39. Gilchrist A, Solomon N, Erickson D *et al*. Automated detection of the G20210A prothrombin mutation using the LCx microparticle enzyme immunoassay. Clin Chim Acta 2001; 314(1-2):249-54.
40. Hessner MJ, Friedman KD, Voelkerding KV *et al*. Multisite study for genotyping of the factor II (prothrombin) G20210A mutation by the invader assay. Clin Chem 2001; 47(11):2048-50.
41. Huber S, McMaster KJ, Voelkerding KV. Analytical evaluation of primer engineered multiplex polymerase chain reaction-restriction fragment length polymorphism for detection of factor V Leiden and prothrombin G20210A. J Mol Diagn 2000; 2(3):153-7.
42. Leyte A, Smits PH, van Straalen JP, van Doorn LJ, Quint WG. Automated, simultaneous detection of the factor V Leiden and prothrombin (G20210A) variants using multiplex PCR and a line probe assay. Thromb Haemost 2000; 83(2):354-5.
43. van den Bergh FA, van Oeveren-Dybicz AM, Bon MA. Rapid single-tube genotyping of the factor V Leiden and prothrombin mutations by real-time PCR using dual-color detection. Clin Chem 2000; 46(8 Pt 1):1191-5.
44. DelRio-LaFreniere SA, McGlennen RC. Simultaneous allele-specific amplification: a strategy using modified primer-template mismatches for SNP detection--application to prothrombin 20210A (factor II) and factor V Leiden (1691A) gene mutations. Mol Diagn 2001; 6(3):201-9.
45. Endler G, Kyrle PA, Eichinger S, Exner M, Mannhalter C. Multiplexed mutagenically separated PCR: simultaneous single-tube detection of the factor V R506Q (G1691A), the prothrombin G20210A, and the methylenetetrahydrofolate reductase A223V (C677T) variants. Clin Chem 2001; 47(2):333-5.
46. Ameziane N, Lamotte M, Lamoril J *et al*. Combined factor V leiden (G1691A) and prothrombin (G20210A) genotyping by multiplex real-time polymerase chain reaction using fluorescent resonance energy transfer hybridization probes on the Rotor-Gene 2000. Blood Coagul Fibrinolysis 2003; 14(4):421-4.
47. Erali M, Schmidt B, Lyon E, Wittwer C. Evaluation of electronic microarrays for genotyping factor V, factor II, and MTHFR. Clin Chem 2003; 49(5):732-9.
48. Lucotte G, Champenois T. Duplex PCR-RFLP for simultaneous detection of factor V Leiden and prothrombin G20210A. Mol Cell Probes 2003; 17(5):267-9.
49. Neitzel B, Matern C, Holinski-Feder E. Easy, accurate and reliable screening for SNPs by ion pair/reverse phase HPLC: simultaneous detection of factor V G1691A, prothrombin G20210A and methylenetetrahydrofolate

reductase C677T variants. Clin Lab 2003; 49(7-8):313-8.
50. Schrijver I, Lay MJ, Zehnder JL. Diagnostic single nucleotide polymorphism analysis of factor V Leiden and prothrombin 20210G > A. A comparison of the Nanogen Eelectronic Microarray with restriction enzyme digestion and the Roche LightCycler. Am J Clin Pathol 2003; 119(4):490-6.
51. Baris I, Koksal V, Etlik O. Multiplex PCR-RFLP assay for detection of factor V Leiden and prothrombin G20210A. Genet Test 2004; 8(4):381-3.
52. Carmi N, Cohen D, Zvang E, Naparstek E, Deutsch V. Pronto ThromboRisk--a novel primer-extension ELISA based assay for the detection of mutations associated with increased risk for thrombophilia. J Clin Lab Anal 2004; 18(5):259-64.
53. Patnaik M, Dlott JS, Fontaine RN *et al*. Detection of genomic polymorphisms associated with venous thrombosis using the invader biplex assay. J Mol Diagn 2004; 6(2):137-44.
54. Ugozzoli LA, Hamby K. Four-color multiplex 5' nuclease assay for the simultaneous detection of the factor V Leiden and the prothrombin G20210A mutations. Mol Cell Probes 2004; 18(3):161-6.
55. Hobson-Peters J, Toye P. A whole-blood homogeneous assay for the multiplex detection of the factor V G1691A and the prothrombin G20210A mutations. Mol Cell Probes 2005; 19(4):290-7.
56. Koksal V, Baris I, Etlik O. Primer-engineered multiplex PCR-RFLP for detection of MTHFR C677T, prothrombin G20210A and factor V Leiden mutations. Exp Mol Pathol 2007; 83(1):1-3.
57. Lopez M, Giraldo P, Alvarez P *et al*. Multiplex assay for genetic testing of thrombophilia: a method for routine clinical care. J Clin Lab Anal 2007; 21(6):349-55.
58. Hertzberg M, Neville S, Favaloro E, McDonald D. External quality assurance of DNA testing for thrombophilia mutations. Am J Clin Pathol 2005; 123(2):189-93.
59. Jennings I, Kitchen S, Woods TA, Preston FE. Multilaboratory testing in thrombophilia through the United Kingdom National External Quality Assessment Scheme (Blood Coagulation) Quality Assurance Program. Semin Thromb Hemost 2005; 31(1):66-72.
60. Tripodi A, Chantarangkul V, Menegatti M, Tagliabue L, Peyvandi F. Performance of clinical laboratories for DNA analyses to detect thrombophilia mutations. Clin Chem 2005; 51(7):1310-1.

Evidence Table 2. Results of tests for detection of factor V leiden.

Reference List

1. Hertzberg M, Neville S, Favaloro E, McDonald D. External quality assurance of DNA testing for thrombophilia mutations. Am J Clin Pathol 2005; 123(2):189-93.
2. Jennings I, Kitchen S, Woods TA, Preston FE. Multilaboratory testing in thrombophilia through the United Kingdom National External Quality Assessment Scheme (Blood Coagulation) Quality Assurance Program. Semin Thromb Hemost 2005; 31(1):66-72.
3. Angeline T, Bentley HA, Hawk AB *et al*. Prevalence of the Factor V G1691A and the Factor II/prothrombin G20210A gene polymorphisms among Tamilians. Exp Mol Pathol 2005; 79(1):9-13.
5. Nauck M, Marz W, Wieland H. Evaluation of the Roche diagnostics LightCycler-Factor V Leiden Mutation Detection Kit and the LightCycler-Prothrombin Mutation Detection Kit. Clin Biochem 2000; 33(3):213-6.
6. Parks SB, Popovich BW, Press RD. Real-time polymerase chain reaction with fluorescent hybridization probes for the detection of prevalent mutations causing common thrombophilic and iron overload phenotypes. Am J Clin Pathol 2001; 115(3):439-47.
7. Ledford M, Friedman KD, Hessner MJ, Moehlenkamp C, Williams TM, Larson RS. A multi-site study for detection of the factor V (Leiden) mutation from genomic DNA using a homogeneous invader microtiter plate fluorescence resonance energy transfer (FRET) assay. J Mol Diagn 2000; 2(2):97-104.
8. Moser MJ, Marshall DJ, Grenier JK *et al*. Exploiting the enzymatic recognition of an unnatural base pair to develop a universal genetic analysis system. Clin Chem 2003; 49(3):407-14.
9. Castley A, Higgins M, Ivey J, Mamotte C, Sayer DC, Christiansen FT. Clinical applications of whole-blood PCR with real-time instrumentation. Clin Chem 2005; 51(11):2025-30.
10. Hessner MJ, Budish MA, Friedman KD. Genotyping of factor V G1691A (Leiden) without the use of PCR by invasive cleavage of oligonucleotide probes. Clin Chem 2000; 46(8 Pt 1):1051-6.
11. Cooper PC, Cooper SM, Smith JM, Kitchen S, Makris M. Evaluation of the Roche LightCycler: a simple and rapid method for direct detection of factor V Leiden and prothrombin G20210A genotypes from blood samples without the need for DNA extraction. Blood Coagul Fibrinolysis 2003; 14(5):499-503.
12. Schroell-Metzger B, Dicato M, Bosseler M, Berchem G. Comparison of standard PCR and the LightCycler technique to determine the thrombophilic mutations: an efficiency and cost study. Clin Chem Lab Med 2003; 41(4):482-5.
13. Liew M, Pryor R, Palais R *et al*. Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons. Clin Chem 2004; 50(7):1156-64.
14. Sanders Sevall J. Factor V Leiden genotyping using real-time fluorescent polymerase chain reaction. Mol Cell Probes 2000; 14(4):249-53.
15. Behrens M, Lange R. A highly reproducible and economically competitive SNP analysis of several well characterized human mutations. Clin Lab 2004; 50(5-6):305-16.
16. Louis M, Dekairelle AF, Gala JL. Rapid combined genotyping of factor V, prothrombin and methylenetetrahydrofolate reductase single nucleotide polymorphisms using minor groove binding DNA oligonucleotides (MGB probes) and real-time polymerase chain reaction. Clin Chem Lab Med 2004; 42(12):1364-9.
17. Benson JM, Ellingsen D, El-Jamil M *et al*. Factor V Leiden and factor V R2 allele: high-throughput analysis and association with venous thromboembolism. Thromb Haemost 2001; 86(5):1188-92.
18. Murphy K, Hafez M, Philips J, Yarnell K, Gutshall K, Berg K. Evaluation of temperature gradient capillary electrophoresis for detection of the Factor V Leiden mutation: coincident identification of a novel polymorphism in Factor V. Mol Diagn 2003; 7(1):35-40.
19. Ozkan D, Erdem A, Kara P *et al*. Allele-specific genotype detection of factor V Leiden mutation from polymerase chain reaction amplicons based on label-free electrochemical genosensor. Anal Chem 2002; 74(23):5931-6.
20. Huang TJ, Liu M, Knight LD, Grody WW, Miller JF, Ho CM. An electrochemical detection scheme for identification of single nucleotide polymorphisms using hairpin-forming probes. Nucleic Acids Res 2002; 30(12):e55.
21. Ozsoz M, Erdem A, Kerman K *et al*. Electrochemical genosensor based on colloidal gold nanoparticles for the detection of Factor V Leiden mutation using disposable pencil graphite electrodes. Anal Chem 2003; 75(9):2181-7.
22. Humeny A, Bonk T, Berkholz A, Wildt L, Becker CM. Genotyping of thrombotic risk factors by MALDI-TOF mass spectrometry. Clin Biochem 2001; 34(7):531-6.
23. Hung K, Sun X, Ding H, Kalafatis M, Simioni P, Guo B. A matrix-assisted laser desorption/ionization time-of-flight based method for screening the 1691G --> A mutation in the factor V gene. Blood Coagul Fibrinolysis 2002; 13(2):117-22.
25. Pecheniuk NM, Marsh NA, Walsh TP. Multiple analysis of three common genetic alterations associated with thrombophilia. Blood Coagul Fibrinolysis 2000; 11(2):183-9.

26. Potter CG, Liu YT, Rees DC. Factor V Leiden mutation screened by PCR and detected with lanthanide-labeled probes. Genet Test 2001; 5(4):291-7.
27. Mammo L, Sheereen A, Saour T, Saour J. Comparative study between the Light Cycler and the PCR-restriction fragment length polymorphism in detecting factor V Leiden and factor II 20210G>A mutations. Clin Biochem 2006; 39(7):767-9.
28. El Housni H, Heimann P, Parma J, Vassart G. Single-nucleotide polymorphism genotyping by melting analysis of dual-labeled probes: examples using factor V Leiden and prothrombin 20210A mutations. Clin Chem 2003; 49(10):1669-72.
29. Alsmadi OA, Bornarth CJ, Song W *et al*. High accuracy genotyping directly from genomic DNA using a rolling circle amplification based assay. BMC Genomics 2003; 4(1):21.
30. Tsongalis GJ, Rainey BJ, Hodges KA. READIT: a novel technology used in the interrogation of nucleic acid sequences for single-nucleotide polymorphisms. Exp Mol Pathol 2001; 71(3):222-5.
31. Kowalski A, Radu D, Gold B. Colorimetric microwell plate detection of the factor V Leiden mutation. Clin Chem 2000; 46(8 Pt 1):1195-8.
32. Simundic AM, Topic E, Stefanovic M. Detection of factor V Leiden by PCR-SSCP using GMA precast Elchrom scientific gels. Clin Appl Thromb Hemost 2003; 9(3):227-31.
33. Reyes AA, Ugozzoli LA, Lowery JD *et al*. Linked linear amplification: a new method for the amplification of DNA. Clin Chem 2001; 47(1):31-40.
34. Evans JG, Lee-Tataseo C. Determination of the factor V Leiden single-nucleotide polymorphism in a commercial clinical laboratory by use of NanoChip microelectronic array technology. Clin Chem 2002; 48(9):1406-11.
35. Crockett AO, Wittwer CT. Fluorescein-labeled oligonucleotides for real-time pcr: using the inherent quenching of deoxyguanosine nucleotides. Anal Biochem 2001; 290(1):89-97.
36. Vaughn CP, Elenitoba-Johnson KS. Hybridization-induced dequenching of fluorescein-labeled oligonucleotides: a novel strategy for PCR detection and genotyping. Am J Pathol 2003; 163(1):29-35.
37. Tripodi A, Chantarangkul V, Menegatti M, Tagliabue L, Peyvandi F. Performance of clinical laboratories for DNA analyses to detect thrombophilia mutations. Clin Chem 2005; 51(7):1310-1.
38. French C, Li C, Strom C *et al*. Detection of the factor V Leiden mutation by a modified photo-cross-linking oligonucleotide hybridization assay. Clin Chem 2004; 50(2):296-305.
39. Rhodes RB, Lewis K, Shultz J *et al*. Analysis of the factor V Leiden mutation using the READIT Assay. Mol Diagn 2001; 6(1):55-61.

Evidence Table 3. Results of tests for detection of prothrombin G20210A.

Reference List

1. Hertzberg M, Neville S, Favaloro E, McDonald D. External quality assurance of DNA testing for thrombophilia mutations. Am J Clin Pathol 2005; 123(2):189-93.
2. Jennings I, Kitchen S, Woods TA, Preston FE. Multilaboratory testing in thrombophilia through the United Kingdom National External Quality Assessment Scheme (Blood Coagulation) Quality Assurance Program. Semin Thromb Hemost 2005; 31(1):66-72.
3. Angeline T, Bentley HA, Hawk AB *et al*. Prevalence of the Factor V G1691A and the Factor II/prothrombin G20210A gene polymorphisms among Tamilians. Exp Mol Pathol 2005; 79(1):9-13.
4. Bravo-Osorio M, Bydlowski SP. Detection of methylenetetrahydrofolate reductase (MTHFR) C677T and prothrombin G20210A mutations: second restriction site for digestion control of PCR products. Clin Chim Acta 2000; 301(1-2):219-23.
6. Nauck M, Marz W, Wieland H. Evaluation of the Roche diagnostics LightCycler-Factor V Leiden Mutation Detection Kit and the LightCycler-Prothrombin Mutation Detection Kit. Clin Biochem 2000; 33(3):213-6.
7. Parks SB, Popovich BW, Press RD. Real-time polymerase chain reaction with fluorescent hybridization probes for the detection of prevalent mutations causing common thrombophilic and iron overload phenotypes. Am J Clin Pathol 2001; 115(3):439-47.
8. Hessner MJ, Friedman KD, Voelkerding KV *et al*. Multisite study for genotyping of the factor II (prothrombin) G20210A mutation by the invader assay. Clin Chem 2001; 47(11):2048-50.
9. Moser MJ, Marshall DJ, Grenier JK *et al*. Exploiting the enzymatic recognition of an unnatural base pair to develop a universal genetic analysis system. Clin Chem 2003; 49(3):407-14.
10. Castley A, Higgins M, Ivey J, Mamotte C, Sayer DC, Christiansen FT. Clinical applications of whole-blood PCR with real-time instrumentation. Clin Chem 2005; 51(11):2025-30.
11. Cooper PC, Cooper SM, Smith JM, Kitchen S, Makris M. Evaluation of the Roche LightCycler: a simple and rapid method for direct detection of factor V Leiden and prothrombin G20210A genotypes from blood samples without the need for DNA extraction. Blood Coagul Fibrinolysis 2003; 14(5):499-503.
12. Schroell-Metzger B, Dicato M, Bosseler M, Berchem G. Comparison of standard PCR and the LightCycler technique to determine the thrombophilic mutations: an efficiency and cost study. Clin Chem Lab Med 2003; 41(4):482-5.
13. Liew M, Pryor R, Palais R *et al*. Genotyping of single-nucleotide polymorphisms by high-resolution melting of small amplicons. Clin Chem 2004; 50(7):1156-64.
14. Behrens M, Lange R. A highly reproducible and economically competitive SNP analysis of several well characterized human mutations. Clin Lab 2004; 50(5-6):305-16.
15. Louis M, Dekairelle AF, Gala JL. Rapid combined genotyping of factor V, prothrombin and methylenetetrahydrofolate reductase single nucleotide polymorphisms using minor groove binding DNA oligonucleotides (MGB probes) and real-time polymerase chain reaction. Clin Chem Lab Med 2004; 42(12):1364-9.
16. Happich D, Madlener K, Schwaab R, Hanfland P, Potzsch B. Application of the TaqMan-PCR for genotyping of the prothrombin G20210A mutation and of the thermolabile methylenetetrahydrofolate reductase mutation. Thromb Haemost 2000; 84(1):144-5.
17. Gellings A, Holzem G, Wielckens K, Klingler KR. Prothrombin mutation: Employing the electrochemiluminescence technology of the elecsys(registered trademark) system for the detection of the point mutation at position 20210 in the 3' untranslated region of the prothrombin gene: PROTHROMBIN MUTATION: VERWENDUNG DER ELEKTROCHEMILUMINESZENZTECHNOLOGIE DES ELECSYS(registered trademark) SYSTEMS ZUR DETEKTION DER PUNKTMUTATION AN DER POSITION 20210 IN DER 3' UNTRANSLATIERTEN. LaboratoriumsMedizin 2001; 25(1-2):26-30.
18. Humeny A, Bonk T, Berkholz A, Wildt L, Becker CM. Genotyping of thrombotic risk factors by MALDI-TOF mass spectrometry. Clin Biochem 2001; 34(7):531-6.
19. Gilchrist A, Solomon N, Erickson D *et al*. Automated detection of the G20210A prothrombin mutation using the LCx microparticle enzyme immunoassay. Clin Chim Acta 2001; 314(1-2):249-54.
21. Pecheniuk NM, Marsh NA, Walsh TP. Multiple analysis of three common genetic alterations associated with thrombophilia. Blood Coagul Fibrinolysis 2000; 11(2):183-9.
22. Mammo L, Sheereen A, Saour T, Saour J. Comparative study between the Light Cycler and the PCR-restriction fragment length polymorphism in detecting factor V Leiden and factor II 20210G>A mutations. Clin Biochem 2006; 39(7):767-9.
23. El Housni H, Heimann P, Parma J, Vassart G. Single-nucleotide polymorphism genotyping by melting analysis of dual-labeled probes: examples using factor V Leiden and prothrombin 20210A mutations. Clin Chem 2003; 49(10):1669-72.
24. Alsmadi OA, Bornarth CJ, Song W *et al*. High accuracy genotyping directly from genomic DNA using a rolling circle amplification based assay. BMC Genomics 2003; 4(1):21.

25. Tripodi A, Chantarangkul V, Menegatti M, Tagliabue L, Peyvandi F. Performance of clinical laboratories for DNA analyses to detect thrombophilia mutations. Clin Chem 2005; 51(7):1310-1.
26. Meyer M, Kutscher G, Vogel G. The prothrombin nt20210 A allele as a risk factor for venous thromboembolism: detection of heterozygous and homozygous carriers by alternative methods. Thromb Res 2000; 97(5):359-63.

Evidence Table 4. Results of tests for detection of simultaneous mutations.

Reference List

1. Neitzel B, Matern C, Holinski-Feder E. Easy, accurate and reliable screening for SNPs by ion pair/reverse phase HPLC: simultaneous detection of factor V G1691A, prothrombin G20210A and methylenetetrahydrofolate reductase C677T variants. Clin Lab 2003; 49(7-8):313-8.
2. Huber S, McMaster KJ, Voelkerding KV. Analytical evaluation of primer engineered multiplex polymerase chain reaction-restriction fragment length polymorphism for detection of factor V Leiden and prothrombin G20210A. J Mol Diagn 2000; 2(3):153-7.
3. Lucotte G, Champenois T. Duplex PCR-RFLP for simultaneous detection of factor V Leiden and prothrombin G20210A. Mol Cell Probes 2003; 17(5):267-9.
4. Koksal V, Baris I, Etlik O. Primer-engineered multiplex PCR-RFLP for detection of MTHFR C677T, prothrombin G20210A and factor V Leiden mutations. Exp Mol Pathol 2007; 83(1):1-3.
5. Baris I, Koksal V, Etlik O. Multiplex PCR-RFLP assay for detection of factor V Leiden and prothrombin G20210A. Genet Test 2004; 8(4):381-3.
6. DelRio-LaFreniere SA, McGlennen RC. Simultaneous allele-specific amplification: a strategy using modified primer-template mismatches for SNP detection--application to prothrombin 20210A (factor II) and factor V Leiden (1691A) gene mutations. Mol Diagn 2001; 6(3):201-9.
7. Ameziane N, Lamotte M, Lamoril J *et al*. Combined factor V leiden (G1691A) and prothrombin (G20210A) genotyping by multiplex real-time polymerase chain reaction using fluorescent resonance energy transfer hybridization probes on the Rotor-Gene 2000. Blood Coagul Fibrinolysis 2003; 14(4):421-4.
8. Hobson-Peters J, Toye P. A whole-blood homogeneous assay for the multiplex detection of the factor V G1691A and the prothrombin G20210A mutations. Mol Cell Probes 2005; 19(4):290-7.
9. van den Bergh FA, van Oeveren-Dybicz AM, Bon MA. Rapid single-tube genotyping of the factor V Leiden and prothrombin mutations by real-time PCR using dual-color detection. Clin Chem 2000; 46(8 Pt 1):1191-5.
10. Patnaik M, Dlott JS, Fontaine RN *et al*. Detection of genomic polymorphisms associated with venous thrombosis using the invader biplex assay. J Mol Diagn 2004; 6(2):137-44.
11. Carmi N, Cohen D, Zvang E, Naparstek E, Deutsch V. Pronto ThromboRisk--a novel primer-extension ELISA based assay for the detection of mutations associated with increased risk for thrombophilia. J Clin Lab Anal 2004; 18(5):259-64.
12. Lopez M, Giraldo P, Alvarez P *et al*. Multiplex assay for genetic testing of thrombophilia: a method for routine clinical care. J Clin Lab Anal 2007; 21(6):349-55.
13. Ugozzoli LA, Hamby K. Four-color multiplex 5' nuclease assay for the simultaneous detection of the factor V Leiden and the prothrombin G20210A mutations. Mol Cell Probes 2004; 18(3):161-6.
14. Endler G, Kyrle PA, Eichinger S, Exner M, Mannhalter C. Multiplexed mutagenically separated PCR: simultaneous single-tube detection of the factor V R506Q (G1691A), the prothrombin G20210A, and the methylenetetrahydrofolate reductase A223V (C677T) variants. Clin Chem 2001; 47(2):333-5.
15. Leyte A, Smits PH, van Straalen JP, van Doorn LJ, Quint WG. Automated, simultaneous detection of the factor V Leiden and prothrombin (G20210A) variants using multiplex PCR and a line probe assay. Thromb Haemost 2000; 83(2):354-5.
16. Schrijver I, Lay MJ, Zehnder JL. Diagnostic single nucleotide polymorphism analysis of factor V Leiden and prothrombin 20210G > A. A comparison of the Nanogen Eelectronic Microarray with restriction enzyme digestion and the Roche LightCycler. Am J Clin Pathol 2003; 119(4):490-6.
17. Erali M, Schmidt B, Lyon E, Wittwer C. Evaluation of electronic microarrays for genotyping factor V, factor II, and MTHFR. Clin Chem 2003; 49(5):732-9.
18. Verri A, Focher F, Tettamanti G, Grazioli V. Two-step genetic screening of thrombophilia by pyrosequencing. Clin Chem 2005; 51(7):1282-4.

Evidence Table 5. Quality and applicability of studies addressing Factor V Leiden and Recurrent Venous Thrombosis in Probands

Reference List

1. Ridker PM, Miletich JP, Stampfer MJ, Goldhaber SZ, Lindpaintner K, Hennekens CH. Factor V Leiden and risks of recurrent idiopathic venous thromboembolism. Circulation 1995; 92(10):2800-2.
2. Kearon C, Gent M, Hirsh J *et al*. A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. N Engl J Med 1999; 340(12):901-7.
3. Lindmarker P, Schulman S, Sten-Linder M, Wiman B, Egberg N, Johnsson H. The risk of recurrent venous thromboembolism in carriers and non-carriers of the G1691A allele in the coagulation factor V gene and the G20210A allele in the prothrombin gene. DURAC Trial Study Group. Duration of Anticoagulation. Thromb Haemost 1999; 81(5):684-9.
4. Simioni P, Prandoni P, Lensing AW *et al*. Risk for subsequent venous thromboembolic complications in carriers of the prothrombin or the factor V gene mutation with a first episode of deep-vein thrombosis. Blood 2000; 96(10):3329-33.
5. Hoibraaten E, Mowinckel MC, de Ronde H, Bertina RM, Sandset PM. Hormone replacement therapy and acquired resistance to activated protein C: results of a randomized, double-blind, placebo-controlled trial. Br J Haematol 2001; 115(2):415-20.
6. Miles JS, Miletich JP, Goldhaber SZ, Hennekens CH, Ridker PM. G20210A mutation in the prothrombin gene and the risk of recurrent venous thromboembolism. J Am Coll Cardiol 2001; 37(1):215-8.
7. Eichinger S, Weltermann A, Mannhalter C *et al*. The risk of recurrent venous thromboembolism in heterozygous carriers of factor V Leiden and a first spontaneous venous thromboembolism. Arch Intern Med 2002; 162(20):2357-60.
8. Baglin T, Luddington R, Brown K, Baglin C. Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. Lancet 2003; 362(9383):523-6.
9. Is recurrent venous thromboembolism more frequent in homozygous patients for the factor V Leiden mutation than in heterozygous patients? Blood Coagul Fibrinolysis 2003; 14(6):523-9.
   Notes: CORPORATE NAME: Procare Group
10. Palareti G, Legnani C, Cosmi B *et al*. Predictive value of D-dimer test for recurrent venous thromboembolism after anticoagulation withdrawal in subjects with a previous idiopathic event and in carriers of congenital thrombophilia. Circulation 2003; 108(3):313-8.
11. Baarslag HJ, Koopman MM, Hutten BA *et al*. Long-term follow-up of patients with suspected deep vein thrombosis of the upper extremity: survival, risk factors and post-thrombotic syndrome. Eur J Intern Med 2004; 15(8):503-7.
12. Kyrle PA, Minar E, Bialonczyk C, Hirschl M, Weltermann A, Eichinger S. The risk of recurrent venous thromboembolism in men and women. N Engl J Med 2004; 350(25):2558-63.
13. Christiansen SC, Cannegieter SC, Koster T, Vandenbroucke JP, Rosendaal FR. Thrombophilia, clinical factors, and recurrent venous thrombotic events. JAMA 2005; 293(19):2352-61.
14. Mansilha A, Araujo F, Severo M, Sampaio SM, Toledo T, Albuquerque R. Genetic polymorphisms and risk of recurrent deep venous thrombosis in young people: prospective cohort study. Eur J Vasc Endovasc Surg 2005; 30(5):545-9.
15. Santamaria MG, Agnelli G, Taliani MR *et al*. Thrombophilic abnormalities and recurrence of venous thromboembolism in patients treated with standardized anticoagulant treatment. Thromb Res 2005; 116(4):301-6.
   Notes: CORPORATE NAME: Warfarin Optimal Duration Italian Trial (WODIT) Investigators
16. Vossen CY, Walker ID, Svensson P *et al*. Recurrence rate after a first venous thrombosis in patients with familial thrombophilia. Arterioscler Thromb Vasc Biol 2005; 25(9):1992-7.
17. Gonzalez-Porras JR, Garcia-Sanz R, Alberca I *et al*. Risk of recurrent venous thrombosis in patients with G20210A mutation in the prothrombin gene or factor V Leiden mutation. Blood Coagul Fibrinolysis 2006; 17(1):23-8.
18. Hron G, Eichinger S, Weltermann A *et al*. Family history for venous thromboembolism and the risk for recurrence. Am J Med 2006; 119(1):50-3.
19. Wahlander K, Eriksson H, Lundstrom T *et al*. Risk of recurrent venous thromboembolism or bleeding in relation to thrombophilic risk factors in patients receiving ximelagatran or placebo for long-term secondary prevention of venous thromboembolism. Br J Haematol 2006; 133(1):68-77.
   Notes: CORPORATE NAME: THRIVE III Investigators
20. Prandoni P, Noventa F, Ghirarduzzi A *et al*. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. Haematologica 2007; 92(2):199-205.
21. Strandberg K, Svensson PJ, Ohlin AK. Venous thromboembolism in carriers of the Factor V Leiden mutation and in patients without known thrombophilic risk factor; prediction of recurrence and APC-PCI complex concentration and/or soluble thrombomodulin antigen and activity. Thromb Res 2007; 121(2):145-51.
22. Kearon C, Julian JA, Kovacs MJ *et al*. Influence of thrombophilia on risk of

recurrent venous thromboembolism while on warfarin: Results from a randomized trial. Blood 2008.
23. Eichinger S, Minar E, Hirschl M *et al*. The risk of early recurrent venous thromboembolism after oral anticoagulant therapy in patients with the G20210A transition in the prothrombin gene. Thromb Haemost 1999; 81(1):14-7.

Evidence Table 6. Results for Probands Heterozygous for the Factor V Leiden Mutation

Reference List

1. Gonzalez-Porras JR, Garcia-Sanz R, Alberca I *et al*. Risk of recurrent venous thrombosis in patients with G20210A mutation in the prothrombin gene or factor V Leiden mutation. Blood Coagul Fibrinolysis 2006; 17(1):23-8.
2. Wahlander K, Eriksson H, Lundstrom T *et al*. Risk of recurrent venous thromboembolism or bleeding in relation to thrombophilic risk factors in patients receiving ximelagatran or placebo for long-term secondary prevention of venous thromboembolism. Br J Haematol 2006; 133(1):68-77.
Notes: CORPORATE NAME: THRIVE III Investigators
3. Is recurrent venous thromboembolism more frequent in homozygous patients for the factor V Leiden mutation than in heterozygous patients? Blood Coagul Fibrinolysis 2003; 14(6):523-9.
Notes: CORPORATE NAME: Procare Group
4. Palareti G, Legnani C, Cosmi B *et al*. Predictive value of D-dimer test for recurrent venous thromboembolism after anticoagulation withdrawal in subjects with a previous idiopathic event and in carriers of congenital thrombophilia. Circulation 2003; 108(3):313-8.
5. Eichinger S, Weltermann A, Mannhalter C *et al*. The risk of recurrent venous thromboembolism in heterozygous carriers of factor V Leiden and a first spontaneous venous thromboembolism. Arch Intern Med 2002; 162(20):2357-60.
6. Hoibraaten E, Mowinckel MC, de Ronde H, Bertina RM, Sandset PM. Hormone replacement therapy and acquired resistance to activated protein C: results of a randomized, double-blind, placebo-controlled trial. Br J Haematol 2001; 115(2):415-20.
7. Miles JS, Miletich JP, Goldhaber SZ, Hennekens CH, Ridker PM. G20210A mutation in the prothrombin gene and the risk of recurrent venous thromboembolism. J Am Coll Cardiol 2001; 37(1):215-8.
8. Simioni P, Prandoni P, Lensing AW *et al*. Risk for subsequent venous thromboembolic complications in carriers of the prothrombin or the factor V gene mutation with a first episode of deep-vein thrombosis. Blood 2000; 96(10):3329-33.
9. Lindmarker P, Schulman S, Sten-Linder M, Wiman B, Egberg N, Johnsson H. The risk of recurrent venous thromboembolism in carriers and non-carriers of the G1691A allele in the coagulation factor V gene and the G20210A allele in the prothrombin gene. DURAC Trial Study Group. Duration of Anticoagulation. Thromb Haemost 1999; 81(5):684-9.
10. Ridker PM, Miletich JP, Stampfer MJ, Goldhaber SZ, Lindpaintner K, Hennekens CH. Factor V Leiden and risks of recurrent idiopathic venous thromboembolism. Circulation 1995; 92(10):2800-2.
11. Prandoni P, Noventa F, Ghirarduzzi A *et al*. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. Haematologica 2007; 92(2):199-205.
12. Kearon C, Gent M, Hirsh J *et al*. A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. N Engl J Med 1999; 340(12):901-7.
13. Kearon C, Julian JA, Kovacs MJ *et al*. Influence of thrombophilia on risk of recurrent venous thromboembolism while on warfarin: Results from a randomized trial. Blood 2008.
14. Christiansen SC, Cannegieter SC, Koster T, Vandenbroucke JP, Rosendaal FR. Thrombophilia, clinical factors, and recurrent venous thrombotic events. JAMA 2005; 293(19):2352-61.

Evidence Table 7. Results for probands homozygous for Factor V Leiden mutation

Reference List

1. Wahlander K, Eriksson H, Lundstrom T *et al*. Risk of recurrent venous thromboembolism or bleeding in relation to thrombophilic risk factors in patients receiving ximelagatran or placebo for long-term secondary prevention of venous thromboembolism. Br J Haematol 2006; 133(1):68-77.
   Notes: CORPORATE NAME: THRIVE III Investigators
2. Is recurrent venous thromboembolism more frequent in homozygous patients for the factor V Leiden mutation than in heterozygous patients? Blood Coagul Fibrinolysis 2003; 14(6):523-9.
   Notes: CORPORATE NAME: Procare Group
3. Palareti G, Legnani C, Cosmi B *et al*. Predictive value of D-dimer test for recurrent venous thromboembolism after anticoagulation withdrawal in subjects with a previous idiopathic event and in carriers of congenital thrombophilia. Circulation 2003; 108(3):313-8.
4. Hoibraaten E, Mowinckel MC, de Ronde H, Bertina RM, Sandset PM. Hormone replacement therapy and acquired resistance to activated protein C: results of a randomized, double-blind, placebo-controlled trial. Br J Haematol 2001; 115(2):415-20.
5. Lindmarker P, Schulman S, Sten-Linder M, Wiman B, Egberg N, Johnsson H. The risk of recurrent venous thromboembolism in carriers and non-carriers of the G1691A allele in the coagulation factor V gene and the G20210A allele in the prothrombin gene. DURAC Trial Study Group. Duration of Anticoagulation. Thromb Haemost 1999; 81(5):684-9.
6. Christiansen SC, Cannegieter SC, Koster T, Vandenbroucke JP, Rosendaal FR. Thrombophilia, clinical factors, and recurrent venous thrombotic events. JAMA 2005; 293(19):2352-61.
7. Kearon C, Gent M, Hirsh J *et al*. A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. N Engl J Med 1999; 340(12):901-7.
8. Kearon C, Julian JA, Kovacs MJ *et al*. Influence of thrombophilia on risk of recurrent venous thromboembolism while on warfarin: Results from a randomized trial. Blood 2008.

Evidence Table 8. Results for probands with unspecified Factor V Leiden mutations

Reference List

1. Strandberg K, Svensson PJ, Ohlin AK. Venous thromboembolism in carriers of the Factor V Leiden mutation and in patients without known thrombophilic risk factor; prediction of recurrence and APC-PCI complex concentration and/or soluble thrombomodulin antigen and activity. Thromb Res 2007; 121(2):145-51.
2. Hron G, Eichinger S, Weltermann A *et al*. Family history for venous thromboembolism and the risk for recurrence. Am J Med 2006; 119(1):50-3.
3. Mansilha A, Araujo F, Severo M, Sampaio SM, Toledo T, Albuquerque R. Genetic polymorphisms and risk of recurrent deep venous thrombosis in young people: prospective cohort study. Eur J Vasc Endovasc Surg 2005; 30(5):545-9.
4. Santamaria MG, Agnelli G, Taliani MR *et al*. Thrombophilic abnormalities and recurrence of venous thromboembolism in patients treated with standardized anticoagulant treatment. Thromb Res 2005; 116(4):301-6. Notes: CORPORATE NAME: Warfarin Optimal Duration Italian Trial (WODIT) Investigators
5. Vossen CY, Walker ID, Svensson P *et al*. Recurrence rate after a first venous thrombosis in patients with familial thrombophilia. Arterioscler Thromb Vasc Biol 2005; 25(9):1992-7.
6. Baarslag HJ, Koopman MM, Hutten BA *et al*. Long-term follow-up of patients with suspected deep vein thrombosis of the upper extremity: survival, risk factors and post-thrombotic syndrome. Eur J Intern Med 2004; 15(8):503-7.
7. Kyrle PA, Minar E, Bialonczyk C, Hirschl M, Weltermann A, Eichinger S. The risk of recurrent venous thromboembolism in men and women. N Engl J Med 2004; 350(25):2558-63.
8. Baglin T, Luddington R, Brown K, Baglin C. Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. Lancet 2003; 362(9383):523-6.

Evidence Table 9. Results for probands double heterozygous for Factor V Leiden mutation and prothrombin G20210A mutation

Reference List

1. Vossen CY, Walker ID, Svensson P *et al*. Recurrence rate after a first venous thrombosis in patients with familial thrombophilia. Arterioscler Thromb Vasc Biol 2005; 25(9):1992-7.
2. Palareti G, Legnani C, Cosmi B *et al*. Predictive value of D-dimer test for recurrent venous thromboembolism after anticoagulation withdrawal in subjects with a previous idiopathic event and in carriers of congenital thrombophilia. Circulation 2003; 108(3):313-8.
3. Miles JS, Miletich JP, Goldhaber SZ, Hennekens CH, Ridker PM. G20210A mutation in the prothrombin gene and the risk of recurrent venous thromboembolism. J Am Coll Cardiol 2001; 37(1):215-8.
4. Simioni P, Prandoni P, Lensing AW *et al*. Risk for subsequent venous thromboembolic complications in carriers of the prothrombin or the factor V gene mutation with a first episode of deep-vein thrombosis. Blood 2000; 96(10):3329-33.

Evidence Table 10. Results for probands heterozygous for prothrombin G20210A

Reference List

1. Gonzalez-Porras JR, Garcia-Sanz R, Alberca I *et al*. Risk of recurrent venous thrombosis in patients with G20210A mutation in the prothrombin gene or factor V Leiden mutation. Blood Coagul Fibrinolysis 2006; 17(1):23-8.
2. Wahlander K, Eriksson H, Lundstrom T *et al*. Risk of recurrent venous thromboembolism or bleeding in relation to thrombophilic risk factors in patients receiving ximelagatran or placebo for long-term secondary prevention of venous thromboembolism. Br J Haematol 2006; 133(1):68-77.
   Notes: CORPORATE NAME: THRIVE III Investigators
3. Mansilha A, Araujo F, Severo M, Sampaio SM, Toledo T, Albuquerque R. Genetic polymorphisms and risk of recurrent deep venous thrombosis in young people: prospective cohort study. Eur J Vasc Endovasc Surg 2005; 30(5):545-9.
4. Palareti G, Legnani C, Cosmi B *et al*. Predictive value of D-dimer test for recurrent venous thromboembolism after anticoagulation withdrawal in subjects with a previous idiopathic event and in carriers of congenital thrombophilia. Circulation 2003; 108(3):313-8.
5. Miles JS, Miletich JP, Goldhaber SZ, Hennekens CH, Ridker PM. G20210A mutation in the prothrombin gene and the risk of recurrent venous thromboembolism. J Am Coll Cardiol 2001; 37(1):215-8.
6. Simioni P, Prandoni P, Lensing AW *et al*. Risk for subsequent venous thromboembolic complications in carriers of the prothrombin or the factor V gene mutation with a first episode of deep-vein thrombosis. Blood 2000; 96(10):3329-33.
7. Lindmarker P, Schulman S, Sten-Linder M, Wiman B, Egberg N, Johnsson H. The risk of recurrent venous thromboembolism in carriers and non-carriers of the G1691A allele in the coagulation factor V gene and the G20210A allele in the prothrombin gene. DURAC Trial Study Group. Duration of Anticoagulation. Thromb Haemost 1999; 81(5):684-9.
8. Prandoni P, Noventa F, Ghirarduzzi A *et al*. The risk of recurrent venous thromboembolism after discontinuing anticoagulation in patients with acute proximal deep vein thrombosis or pulmonary embolism. A prospective cohort study in 1,626 patients. Haematologica 2007; 92(2):199-205.
9. Kearon C, Gent M, Hirsh J *et al*. A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. N Engl J Med 1999; 340(12):901-7.
10. Kearon C, Julian JA, Kovacs MJ *et al*. Influence of thrombophilia on risk of recurrent venous thromboembolism while on warfarin: Results from a randomized trial. Blood 2008.

Reference List

1. Hron G, Eichinger S, Weltermann A *et al*. Family history for venous thromboembolism and the risk for recurrence. Am J Med 2006; 119(1):50-3.

2. Santamaria MG, Agnelli G, Taliani MR *et al*. Thrombophilic abnormalities and recurrence of venous thromboembolism in patients treated with standardized anticoagulant treatment. Thromb Res 2005; 116(4):301-6.

3. Baarslag HJ, Koopman MM, Hutten BA *et al*. Long-term follow-up of patients with suspected deep vein thrombosis of the upper extremity: survival, risk factors and post-thrombotic syndrome. Eur J Intern Med 2004; 15(8):503-7.

4. Kyrle PA, Minar E, Bialonczyk C, Hirschl M, Weltermann A, Eichinger S. The risk of recurrent venous thromboembolism in men and women. N Engl J Med 2004; 350(25):2558-63.

5. Baglin T, Luddington R, Brown K, Baglin C. Incidence of recurrent venous thromboembolism in relation to clinical and thrombophilic risk factors: prospective cohort study. Lancet 2003; 362(9383):523-6 .

6. Christiansen SC, Cannegieter SC, Koster T, Vandenbroucke JP, Rosendaal FR. Thrombophilia, clinical factors, and recurrent venous thrombotic events. JAMA 2005; 293(19):2352-61.

7. Eichinger S, Minar E, Hirschl M *et al*. The risk of early recurrent venous thromboembolism after oral anticoagulant therapy in patients with the G20210A transition in the prothrombin gene. Thromb Haemost 1999; 81(1):14-7.

Evidence Table 12. Results for probands homozygous for prothrombin G20210A

Reference List

1. Wahlander K, Eriksson H, Lundstrom T *et al*. Risk of recurrent venous thromboembolism or bleeding in relation to thrombophilic risk factors in patients receiving ximelagatran or placebo for long-term secondary prevention of venous thromboembolism. Br J Haematol 2006; 133(1):68-77.
Notes: CORPORATE NAME: THRIVE III Investigators
2. Kearon C, Julian JA, Kovacs MJ *et al*. Influence of thrombophilia on risk of recurrent venous thromboembolism while on warfarin: Results from a randomized trial. Blood 2008.

Evidence Table 13. Quality of studies addressing Factor V Leiden and Prothrombin G20210A predicting events in family members

Reference List

1. Samama MM, Simon D, Horellou MH, Trossaert M, Elalamy I, Conard J. Diagnosis and clinical characteristics of inherited activated protein C resistance. Haemostasis 1996; 26 Suppl 4:315-30.
2. Middeldorp S, Henkens CM, Koopman MM *et al*. The incidence of venous thromboembolism in family members of patients with factor V Leiden mutation and venous thrombosis. Ann Intern Med 1998; 128(1):15-20.
3. Le Cam-Duchez V, Gandrille S, Tregouet D *et al*. Influence of three potential genetic risk factors for thrombosis in 43 families carrying the factor V Arg 506 to Gln mutation. Br J Haematol 1999; 106(4):889-97.
4. Simioni P, Sanson BJ, Prandoni P *et al*. Incidence of venous thromboembolism in families with inherited thrombophilia. Thromb Haemost 1999; 81(2):198-202.
5. Simioni P, Prandoni P, Girolami A. Low rate of venous thromboembolism in asymptomatic relatives of probands with factor V Leiden mutation. Ann Intern Med 1999; 130(6):538.
6. Lensen R, Rosendaal F, Vandenbroucke J, Bertina R. Factor V Leiden: the venous thrombotic risk in thrombophilic families. Br J Haematol 2000; 110(4):939-45.
7. Martinelli I, Bucciarelli P, Margaglione M, De Stefano V, Castaman G, Mannucci PM. The risk of venous thromboembolism in family members with mutations in the genes of factor V or prothrombin or both. Br J Haematol 2000; 111(4):1223-9.
8. Simioni P, Tormene D, Luni S, Caldato M, Girolami A. Clinical and laboratory expression of associated thrombophilic conditions (homozygous/heterozygous factor V Leiden mutation and heterozygous prothrombin variant 20210A) in an Italian family. Blood Coagul Fibrinolysis 2000; 11(4):379-84.
9. Lensen R, Bertina RM, Vandenbroucke JP, Rosendaal FR. High factor VIII levels contribute to the thrombotic risk in families with factor V Leiden. Br J Haematol 2001; 114(2):380-6.
10. Middeldorp S, Meinardi JR, Koopman MM *et al*. A prospective study of asymptomatic carriers of the factor V Leiden mutation to determine the incidence of venous thromboembolism. Ann Intern Med 2001; 135(5):322-7.
11. Middeldorp S, Libourel EJ, Hamulyak K, Van der Meer J, Buller HR. The risk of pregnancy-related venous thromboembolism in women who are homozygous for factor V Leiden. Br J Haematol 2001; 113(2):553-5.
12. Rintelen C, Pabinger I, Bettelheim P *et al*. Impact of the factor II: G20210A variant on the risk of venous thromboembolism in relatives from families with the factor V: R506Q mutation. Eur J Haematol 2001; 67(3):165-9.
13. Meinardi JR, Middeldorp S, de Kam PJ *et al*. The incidence of recurrent venous thromboembolism in carriers of factor V Leiden is related to concomitant thrombophilic disorders. Br J Haematol 2002; 116(3):625-31.
14. Simioni P, Tormene D, Prandoni P *et al*. Incidence of venous thromboembolism in asymptomatic family members who are carriers of factor V Leiden: a prospective cohort study. Blood 2002; 99(6):1938-42.
15. Vossen CY, Conard J, Fontcuberta J *et al*. Familial thrombophilia and lifetime risk of venous thrombosis. J Thromb Haemost 2004; 2(9):1526-32.
16. Vossen CY, Conard J, Fontcuberta J *et al*. Risk of a first venous thrombotic event in carriers of a familial thrombophilic defect. The European Prospective Cohort on Thrombophilia (EPCOT). J Thromb Haemost 2005; 3(3):459-64.
17. Couturaud F, Kearon C, Leroyer C *et al*. Incidence of venous thromboembolism in first-degree relatives of patients with venous thromboembolism who have factor V Leiden. Thromb Haemost 2006; 96(6):744-9.
    Notes: CORPORATE NAME: Groupe d'Etude de la Thrombose de Bretagne Occidentale (G.E.T.B.O)
18. Castaman G, Tosetto A, Cappellari A, Ruggeri M, Rodeghiero F. The A20210 allele in the prothrombin gene enhances the risk of venous thrombosis in carriers of inherited protein S deficiency. Blood Coagul Fibrinolysis 2000; 11(4):321-6.
19. Martinelli I, Legnani C, Bucciarelli P, Grandone E, De Stefano V, Mannucci PM. Risk of pregnancy-related venous thrombosis in carriers of severe inherited thrombophilia. Thromb Haemost 2001; 86(3):800-3.
20. Tormene D, Simioni P, Prandoni P *et al*. Factor V Leiden mutation and the risk of venous thromboembolism in pregnant women. Haematologica 2001; 86(12):1305-9.
21. Tormene D, De Stefano V, Grandone E *et al*. The G20210A prothrombin variant and the risk of venous thromboembolism or fetal loss in pregnant women: a family study. J Thromb Haemost 2007; 5(11):2193-6.
22. Martinelli I, Battaglioli T, De Stefano V *et al*. The risk of first venous thromboembolism during pregnancy and puerperium in double heterozygotes for factor V Leiden and prothrombin G20210A. J Thromb Haemost 2008; 6(3):494-8.
    Notes: CORPORATE NAME: GIT (Gruppo Italiano Trombofilia)

Evidence Table 14. FVL predicting events in family members, study characteristics

Reference List

1. Samama MM, Simon D, Horellou MH, Trossaert M, Elalamy I, Conard J. Diagnosis and clinical characteristics of inherited activated protein C resistance. Haemostasis 1996; 26 Suppl 4:315-30.
2. Middeldorp S, Henkens CM, Koopman MM *et al*. The incidence of venous thromboembolism in family members of patients with factor V Leiden mutation and venous thrombosis. Ann Intern Med 1998; 128(1):15-20.
3. Le Cam-Duchez V, Gandrille S, Tregouet D *et al*. Influence of three potential genetic risk factors for thrombosis in 43 families carrying the factor V Arg 506 to Gln mutation. Br J Haematol 1999; 106(4):889-97.
4. Simioni P, Sanson BJ, Prandoni P *et al*. Incidence of venous thromboembolism in families with inherited thrombophilia. Thromb Haemost 1999; 81(2):198-202.
5. Simioni P, Prandoni P, Girolami A. Low rate of venous thromboembolism in asymptomatic relatives of probands with factor V Leiden mutation. Ann Intern Med 1999; 130(6):538.
6. Lensen R, Rosendaal F, Vandenbroucke J, Bertina R. Factor V Leiden: the venous thrombotic risk in thrombophilic families. Br J Haematol 2000; 110(4):939-45.
7. Martinelli I, Bucciarelli P, Margaglione M, De Stefano V, Castaman G, Mannucci PM. The risk of venous thromboembolism in family members with mutations in the genes of factor V or prothrombin or both. Br J Haematol 2000; 111(4):1223-9.
8. Simioni P, Tormene D, Luni S, Caldato M, Girolami A. Clinical and laboratory expression of associated thrombophilic conditions (homozygous/heterozygous factor V Leiden mutation and heterozygous prothrombin variant 20210A) in an Italian family. Blood Coagul Fibrinolysis 2000; 11(4):379-84.
9. Lensen R, Bertina RM, Vandenbroucke JP, Rosendaal FR. High factor VIII levels contribute to the thrombotic risk in families with factor V Leiden. Br J Haematol 2001; 114(2):380-6.
10. Middeldorp S, Meinardi JR, Koopman MM *et al*. A prospective study of asymptomatic carriers of the factor V Leiden mutation to determine the incidence of venous thromboembolism. Ann Intern Med 2001; 135(5):322-7.
11. Rintelen C, Pabinger I, Bettelheim P *et al*. Impact of the factor II: G20210A variant on the risk of venous thromboembolism in relatives from families with the factor V: R506Q mutation. Eur J Haematol 2001; 67(3):165-9.
12. Meinardi JR, Middeldorp S, de Kam PJ *et al*. The incidence of recurrent venous thromboembolism in carriers of factor V Leiden is related to concomitant thrombophilic disorders. Br J Haematol 2002; 116(3):625-31.
13. Simioni P, Tormene D, Prandoni P *et al*. Incidence of venous thromboembolism in asymptomatic family members who are carriers of factor V Leiden: a prospective cohort study. Blood 2002; 99(6):1938-42.
14. Vossen CY, Conard J, Fontcuberta J *et al*. Familial thrombophilia and lifetime risk of venous thrombosis. J Thromb Haemost 2004; 2(9):1526-32.
15. Vossen CY, Conard J, Fontcuberta J *et al*. Risk of a first venous thrombotic event in carriers of a familial thrombophilic defect. The European Prospective Cohort on Thrombophilia (EPCOT). J Thromb Haemost 2005; 3(3):459-64.
16. Couturaud F, Kearon C, Leroyer C *et al*. Incidence of venous thromboembolism in first-degree relatives of patients with venous thromboembolism who have factor V Leiden. Thromb Haemost 2006; 96(6):744-9.
    Notes: CORPORATE NAME: Groupe d'Etude de la Thrombose de Bretagne Occidentale (G.E.T.B.O)

Evidence Table 15. Results of studies of family members with Factor V Leiden

Reference List

1. Samama MM, Simon D, Horellou MH, Trossaert M, Elalamy I, Conard J. Diagnosis and clinical characteristics of inherited activated protein C resistance. Haemostasis 1996; 26 Suppl 4:315-30.
2. Middeldorp S, Henkens CM, Koopman MM *et al*. The incidence of venous thromboembolism in family members of patients with factor V Leiden mutation and venous thrombosis. Ann Intern Med 1998; 128(1):15-20.
3. Le Cam-Duchez V, Gandrille S, Tregouet D *et al*. Influence of three potential genetic risk factors for thrombosis in 43 families carrying the factor V Arg 506 to Gln mutation. Br J Haematol 1999; 106(4):889-97.
4. Simioni P, Sanson BJ, Prandoni P *et al*. Incidence of venous thromboembolism in families with inherited thrombophilia. Thromb Haemost 1999; 81(2):198-202.
5. Simioni P, Prandoni P, Girolami A. Low rate of venous thromboembolism in asymptomatic relatives of probands with factor V Leiden mutation. Ann Intern Med 1999; 130(6):538.
6. Lensen R, Rosendaal F, Vandenbroucke J, Bertina R. Factor V Leiden: the venous thrombotic risk in thrombophilic families. Br J Haematol 2000; 110(4):939-45.
7. Martinelli I, Bucciarelli P, Margaglione M, De Stefano V, Castaman G, Mannucci PM. The risk of venous thromboembolism in family members with mutations in the genes of factor V or prothrombin or both. Br J Haematol 2000; 111(4):1223-9.
8. Simioni P, Tormene D, Luni S, Caldato M, Girolami A. Clinical and laboratory expression of associated thrombophilic conditions (homozygous/heterozygous factor V Leiden mutation and heterozygous prothrombin variant 20210A) in an Italian family. Blood Coagul Fibrinolysis 2000; 11(4):379-84.
9. Lensen R, Bertina RM, Vandenbroucke JP, Rosendaal FR. High factor VIII levels contribute to the thrombotic risk in families with factor V Leiden. Br J Haematol 2001; 114(2):380-6.
10. Middeldorp S, Meinardi JR, Koopman MM *et al*. A prospective study of asymptomatic carriers of the factor V Leiden mutation to determine the incidence of venous thromboembolism. Ann Intern Med 2001; 135(5):322-7.
11. Rintelen C, Pabinger I, Bettelheim P *et al*. Impact of the factor II: G20210A variant on the risk of venous thromboembolism in relatives from families with the factor V: R506Q mutation. Eur J Haematol 2001; 67(3):165-9.
12. Meinardi JR, Middeldorp S, de Kam PJ *et al*. The incidence of recurrent venous thromboembolism in carriers of factor V Leiden is related to concomitant thrombophilic disorders. Br J Haematol 2002; 116(3):625-31.
13. Simioni P, Tormene D, Prandoni P *et al*. Incidence of venous thromboembolism in asymptomatic family members who are carriers of factor V Leiden: a prospective cohort study. Blood 2002; 99(6):1938-42.
14. Vossen CY, Conard J, Fontcuberta J *et al*. Familial thrombophilia and lifetime risk of venous thrombosis. J Thromb Haemost 2004; 2(9):1526-32.
15. Vossen CY, Conard J, Fontcuberta J *et al*. Risk of a first venous thrombotic event in carriers of a familial thrombophilic defect. The European Prospective Cohort on Thrombophilia (EPCOT). J Thromb Haemost 2005; 3(3):459-64.
16. Couturaud F, Kearon C, Leroyer C *et al*. Incidence of venous thromboembolism in first-degree relatives of patients with venous thromboembolism who have factor V Leiden. Thromb Haemost 2006; 96(6):744-9.
    Notes: CORPORATE NAME: Groupe d'Etude de la Thrombose de Bretagne Occidentale (G.E.T.B.O)

Evidence Table 16. Characteristics of studies of prothrombin G20210A in family members

Reference List

1. Castaman G, Tosetto A, Cappellari A, Ruggeri M, Rodeghiero F. The A20210 allele in the prothrombin gene enhances the risk of venous thrombosis in carriers of inherited protein S deficiency. Blood Coagul Fibrinolysis 2000; 11(4):321-6.
2. Martinelli I, Bucciarelli P, Margaglione M, De Stefano V, Castaman G, Mannucci PM. The risk of venous thromboembolism in family members with mutations in the genes of factor V or prothrombin or both. Br J Haematol 2000; 111(4):1223-9.
3. Simioni P, Tormene D, Luni S, Caldato M, Girolami A. Clinical and laboratory expression of associated thrombophilic conditions (homozygous/heterozygous factor V Leiden mutation and heterozygous prothrombin variant 20210A) in an Italian family. Blood Coagul Fibrinolysis 2000; 11(4):379-84.
4. Rintelen C, Pabinger I, Bettelheim P *et al*. Impact of the factor II: G20210A variant on the risk of venous thromboembolism in relatives from families with the factor V: R506Q mutation. Eur J Haematol 2001; 67(3):165-9.
5. Couturaud F, Kearon C, Leroyer C *et al*. Incidence of venous thromboembolism in first-degree relatives of patients with venous thromboembolism who have factor V Leiden. Thromb Haemost 2006; 96(6):744-9.
   Notes: CORPORATE NAME: Groupe d'Etude de la Thrombose de Bretagne Occidentale (G.E.T.B.O)

Evidence Table 17. Results of studies on prothrombin G20210A predicting events in family members.

Reference List

1. Castaman G, Tosetto A, Cappellari A, Ruggeri M, Rodeghiero F. The A20210 allele in the prothrombin gene enhances the risk of venous thrombosis in carriers of inherited protein S deficiency. Blood Coagul Fibrinolysis 2000; 11(4):321-6.
2. Martinelli I, Bucciarelli P, Margaglione M, De Stefano V, Castaman G, Mannucci PM. The risk of venous thromboembolism in family members with mutations in the genes of factor V or prothrombin or both. Br J Haematol 2000; 111(4):1223-9.
3. Simioni P, Tormene D, Luni S, Caldato M, Girolami A. Clinical and laboratory expression of associated thrombophilic conditions (homozygous/heterozygous factor V Leiden mutation and heterozygous prothrombin variant 20210A) in an Italian family. Blood Coagul Fibrinolysis 2000; 11(4):379-84.
4. Rintelen C, Pabinger I, Bettelheim P *et al*. Impact of the factor II: G20210A variant on the risk of venous thromboembolism in relatives from families with the factor V: R506Q mutation. Eur J Haematol 2001; 67(3):165-9.
5. Couturaud F, Kearon C, Leroyer C *et al*. Incidence of venous thromboembolism in first-degree relatives of patients with venous thromboembolism who have factor V Leiden. Thromb Haemost 2006; 96(6):744-9.
   Notes: CORPORATE NAME: Groupe d'Etude de la Thrombose de Bretagne Occidentale (G.E.T.B.O)

Evidence Table 18. Characteristics of studies of Factor V Leiden and prothrombin G20210A in pregnant family members

Reference List

1. Martinelli I, Legnani C, Bucciarelli P, Grandone E, De Stefano V, Mannucci PM. Risk of pregnancy-related venous thrombosis in carriers of severe inherited thrombophilia. Thromb Haemost 2001; 86(3):800-3.
2. Middeldorp S, Libourel EJ, Hamulyak K, Van der Meer J, Buller HR. The risk of pregnancy-related venous thromboembolism in women who are homozygous for factor V Leiden. Br J Haematol 2001; 113(2):553-5.
3. Tormene D, Simioni P, Prandoni P *et al*. Factor V Leiden mutation and the risk of venous thromboembolism in pregnant women. Haematologica 2001; 86(12):1305-9.
4. Couturaud F, Kearon C, Leroyer C *et al*. Incidence of venous thromboembolism in first-degree relatives of patients with venous thromboembolism who have factor V Leiden. Thromb Haemost 2006; 96(6):744-9.
   Notes: CORPORATE NAME: Groupe d'Etude de la Thrombose de Bretagne Occidentale (G.E.T.B.O)
5. Tormene D, De Stefano V, Grandone E *et al*. The G20210A prothrombin variant and the risk of venous thromboembolism or fetal loss in pregnant women: a family study. J Thromb Haemost 2007; 5(11):2193-6.
6. Martinelli I, Battaglioli T, De Stefano V *et al*. The risk of first venous thromboembolism during pregnancy and puerperium in double heterozygotes for factor V Leiden and prothrombin G20210A. J Thromb Haemost 2008; 6(3):494-8.
   Notes: CORPORATE NAME: GIT (Gruppo Italiano Trombofilia)

Evidence Table 19. Results of studies of Factor V Leiden and prothrombin G20210A in pregnant family members

Reference List

1. Martinelli I, Legnani C, Bucciarelli P, Grandone E, De Stefano V, Mannucci PM. Risk of pregnancy-related venous thrombosis in carriers of severe inherited thrombophilia. Thromb Haemost 2001; 86(3):800-3.
2. Middeldorp S, Libourel EJ, Hamulyak K, Van der Meer J, Buller HR. The risk of pregnancy-related venous thromboembolism in women who are homozygous for factor V Leiden. Br J Haematol 2001; 113(2):553-5.
3. Tormene D, Simioni P, Prandoni P *et al*. Factor V Leiden mutation and the risk of venous thromboembolism in pregnant women. Haematologica 2001; 86(12):1305-9.
4. Couturaud F, Kearon C, Leroyer C *et al*. Incidence of venous thromboembolism in first-degree relatives of patients with venous thromboembolism who have factor V Leiden. Thromb Haemost 2006; 96(6):744-9.
   Notes: CORPORATE NAME: Groupe d'Etude de la Thrombose de Bretagne Occidentale (G.E.T.B.O)
5. Tormene D, De Stefano V, Grandone E *et al*. The G20210A prothrombin variant and the risk of venous thromboembolism or fetal loss in pregnant women: a family study. J Thromb Haemost 2007; 5(11):2193-6.
6. Martinelli I, Battaglioli T, De Stefano V *et al*. The risk of first venous thromboembolism during pregnancy and puerperium in double heterozygotes for factor V Leiden and prothrombin G20210A. J Thromb Haemost 2008; 6(3):494-8.
   Notes: CORPORATE NAME: GIT (Gruppo Italiano Trombofilia)

Evidence Table 20. Quality of studies using survey designs included for effect of testing on clinicians' management or effect of testing and results on other outcomes

Reference List

1. Heshka J, Palleschi C, Wilson B *et al*. Cognitive and Behavioural Effects of Genetic Testing for Thrombophilia. J Genet Couns 2008.
2. Kaptein AA, van Korlaar IM, Cameron LD, Vossen CY, van der Meer FJ, Rosendaal FR. Using the common-sense model to predict risk perception and disease-related worry in individuals at increased risk for venous thrombosis. Health Psychol 2007; 26(6):807-12.
3. Rodger MA, Carrier M, Keely E *et al*. The management of thrombophilia during pregnancy: a Canadian survey. J Obstet Gynaecol Can 2002; 24(12):946-52.
4. Vossen CY, Walker ID, Svensson P *et al*. Recurrence rate after a first venous thrombosis in patients with familial thrombophilia. Arterioscler Thromb Vasc Biol 2005; 25(9):1992-7.
5. Wahlander K, Eriksson H, Lundstrom T *et al*. Risk of recurrent venous thromboembolism or bleeding in relation to thrombophilic risk factors in patients receiving ximelagatran or placebo for long-term secondary prevention of venous thromboembolism. Br J Haematol 2006; 133(1):68-77.
   Notes: CORPORATE NAME: THRIVE III Investigators
6. Kearon C, Julian JA, Kovacs MJ *et al*. Influence of thrombophilia on risk of recurrent venous thromboembolism while on warfarin: Results from a randomized trial. Blood 2008.
7. Ridker PM, Goldhaber SZ, Danielson E *et al*. Long-term, low-intensity warfarin therapy for the prevention of recurrent venous thromboembolism. N Engl J Med 2003; 348(15):1425-34.
   Notes: CORPORATE NAME: PREVENT Investigators
8. Saukko PM, Ellard S, Richards SH, Shepherd MH, Campbell JL. Patients' understanding of genetic susceptibility testing in mainstream medicine: qualitative study on thrombophilia. BMC Health Serv Res 2007; 7:82.
9. Saukko PM, Richards SH, Shepherd MH, Campbell JL. Are genetic tests exceptional? Lessons from a qualitative study on thrombophilia. Soc Sci Med 2006; 63(7):1947-59.
10. Bank I, Scavenius MP, Buller HR, Middeldorp S. Social aspects of genetic testing for factor V Leiden mutation in healthy individuals and their importance for daily practice. Thromb Res 2004; 113(1):7-12.

Evidence Table 21. Study characteristics for studies on effect of testing on clinicians' management and the effect of testing and results on other outcomes

Reference List

1. Rodger MA, Carrier M, Keely E *et al*. The management of thrombophilia during pregnancy: a Canadian survey. J Obstet Gynaecol Can 2002; 24(12):946-52.
2. Bank I, Scavenius MP, Buller HR, Middeldorp S. Social aspects of genetic testing for factor V Leiden mutation in healthy individuals and their importance for daily practice. Thromb Res 2004; 113(1):7-12.
3. Kaptein AA, van Korlaar IM, Cameron LD, Vossen CY, van der Meer FJ, Rosendaal FR. Using the common-sense model to predict risk perception and disease-related worry in individuals at increased risk for venous thrombosis. Health Psychol 2007; 26(6):807-12.
4. Saukko PM, Ellard S, Richards SH, Shepherd MH, Campbell JL. Patients' understanding of genetic susceptibility testing in mainstream medicine: qualitative study on thrombophilia. BMC Health Serv Res 2007; 7:82.
5. Saukko PM, Richards SH, Shepherd MH, Campbell JL. Are genetic tests exceptional? Lessons from a qualitative study on thrombophilia. Soc Sci Med 2006; 63(7):1947-59.
6. Heshka J, Palleschi C, Wilson B *et al*. Cognitive and Behavioural Effects of Genetic Testing for Thrombophilia. J Genet Couns 2008.

Evidence Table 22. Study characteristics for studies on the effect of testing on management

Reference List

1. Vossen CY, Walker ID, Svensson P *et al*. Recurrence rate after a first venous thrombosis in patients with familial thrombophilia. Arterioscler Thromb Vasc Biol 2005; 25(9):1992-7.
2. Wahlander K, Eriksson H, Lundstrom T *et al*. Risk of recurrent venous thromboembolism or bleeding in relation to thrombophilic risk factors in patients receiving ximelagatran or placebo for long-term secondary prevention of venous thromboembolism. Br J Haematol 2006; 133(1):68-77.
   Notes: CORPORATE NAME: THRIVE III Investigators
3. Kearon C, Julian JA, Kovacs MJ *et al*. Influence of thrombophilia on risk of recurrent venous thromboembolism while on warfarin: Results from a randomized trial. Blood 2008.
4. Ridker PM, Goldhaber SZ, Danielson E *et al*. Long-term, low-intensity warfarin therapy for the prevention of recurrent venous thromboembolism. N Engl J Med 2003; 348(15):1425-34.
   Notes: CORPORATE NAME: PREVENT Investigators

Evidence Table 23. Population characteristics for studies addressing other effects of testing

Reference List

1. Vossen CY, Walker ID, Svensson P *et al*. Recurrence rate after a first venous thrombosis in patients with familial thrombophilia. Arterioscler Thromb Vasc Biol 2005; 25(9):1992-7.
2. Wahlander K, Eriksson H, Lundstrom T *et al*. Risk of recurrent venous thromboembolism or bleeding in relation to thrombophilic risk factors in patients receiving ximelagatran or placebo for long-term secondary prevention of venous thromboembolism. Br J Haematol 2006; 133(1):68-77.
   Notes: CORPORATE NAME: THRIVE III Investigators
3. Kearon C, Julian JA, Kovacs MJ *et al*. Influence of thrombophilia on risk of recurrent venous thromboembolism while on warfarin: Results from a randomized trial. Blood 2008.
4. Ridker PM, Goldhaber SZ, Danielson E *et al*. Long-term, low-intensity warfarin therapy for the prevention of recurrent venous thromboembolism. N Engl J Med 2003; 348(15):1425-34.
   Notes: CORPORATE NAME: PREVENT Investigators

Evidence Table 24. Results for articles addressing the effects of testing on VTE outcomes

Reference List

1. Vossen CY, Walker ID, Svensson P *et al*. Recurrence rate after a first venous thrombosis in patients with familial thrombophilia. Arterioscler Thromb Vasc Biol 2005; 25(9):1992-7.
2. Wahlander K, Eriksson H, Lundstrom T *et al*. Risk of recurrent venous thromboembolism or bleeding in relation to thrombophilic risk factors in patients receiving ximelagatran or placebo for long-term secondary prevention of venous thromboembolism. Br J Haematol 2006; 133(1):68-77.
   Notes: CORPORATE NAME: THRIVE III Investigators
3. Kearon C, Julian JA, Kovacs MJ *et al*. Influence of thrombophilia on risk of recurrent venous thromboembolism while on warfarin: Results from a randomized trial. Blood 2008.
4. Ridker PM, Goldhaber SZ, Danielson E *et al*. Long-term, low-intensity warfarin therapy for the prevention of recurrent venous thromboembolism. N Engl J Med 2003; 348(15):1425-34.
   Notes: CORPORATE NAME: PREVENT Investigators

Evidence Table 25. Description of Studies Evaluating the Cost-Effectiveness of Testing for Factor V Leiden or Prothrombin G20210A Mutation

Reference List

1. Sarasin FP, Bounameaux H. Decision analysis model of prolonged oral anticoagulant treatment in factor V Leiden carriers with first episode of deep vein thrombosis. BMJ 1998; 316(7125):95-9.
2. Marchetti M, Quaglini S, Barosi G. Cost-effectiveness of screening and extended anticoagulation for carriers of both factor V Leiden and prothrombin G20210A. QJM 2001; 94(7):365-72.
3. Marchetti M, Pistorio A, Barosi G. Extended anticoagulation for prevention of recurrent venous thromboembolism in carriers of factor V Leiden--cost-effectiveness analysis. Thromb Haemost 2000; 84(5):752-7.
4. Eckman MH, Singh SK, Erban JK, Kao G. Testing for factor V Leiden in patients with pulmonary or venous thromboembolism: a cost-effectiveness analysis. Med Decis Making 2002; 22(2):108-24.
5. Clark P, Twaddle S, Walker ID, Scott L, Greer IA. Cost-effectiveness of screening for the factor V Leiden mutation in pregnant women. Lancet 2002; 359(9321):1919-20.
6. Auerbach AD, Sanders GD, Hambleton J. Cost-effectiveness of testing for hypercoagulability and effects on treatment strategies in patients with deep vein thrombosis. Am J Med 2004; 116(12):816-28.
7. Wu O, Robertson L, Twaddle S *et al*. Screening for thrombophilia in high-risk situations: systematic review and cost-effectiveness analysis. The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) study. Health Technol Assess 2006; 10(11):1-110.

Evidence Table 26. Quality Assessment of the Studies Evaluating the Cost-Effectiveness of Testing for Factor V Leiden or Prothrombin G20210A Mutation

Reference List

1. Sarasin FP, Bounameaux H. Decision analysis model of prolonged oral anticoagulant treatment in factor V Leiden carriers with first episode of deep vein thrombosis. BMJ 1998; 316(7125):95-9.
2. Marchetti M, Quaglini S, Barosi G. Cost-effectiveness of screening and extended anticoagulation for carriers of both factor V Leiden and prothrombin G20210A. QJM 2001; 94(7):365-72.
3. Marchetti M, Pistorio A, Barosi G. Extended anticoagulation for prevention of recurrent venous thromboembolism in carriers of factor V Leiden--cost-effectiveness analysis. Thromb Haemost 2000; 84(5):752-7.
4. Eckman MH, Singh SK, Erban JK, Kao G. Testing for factor V Leiden in patients with pulmonary or venous thromboembolism: a cost-effectiveness analysis. Med Decis Making 2002; 22(2):108-24.
5. Clark P, Twaddle S, Walker ID, Scott L, Greer IA. Cost-effectiveness of screening for the factor V Leiden mutation in pregnant women. Lancet 2002; 359(9321):1919-20.
6. Auerbach AD, Sanders GD, Hambleton J. Cost-effectiveness of testing for hypercoagulability and effects on treatment strategies in patients with deep vein thrombosis. Am J Med 2004; 116(12):816-28.
7. Wu O, Robertson L, Twaddle S *et al*. Screening for thrombophilia in high-risk situations: systematic review and cost-effectiveness analysis. The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) study. Health Technol Assess 2006; 10(11):1-110.

Evidence Table 27. Applicability of the Studies Evaluating the Cost-Effectiveness of Testing for Factor V Leiden or Prothrombin G20210A Mutation to Research Question

Reference List

1. Sarasin FP, Bounameaux H. Decision analysis model of prolonged oral anticoagulant treatment in factor V Leiden carriers with first episode of deep vein thrombosis. BMJ 1998; 316(7125):95-9.
2. Marchetti M, Quaglini S, Barosi G. Cost-effectiveness of screening and extended anticoagulation for carriers of both factor V Leiden and prothrombin G20210A. QJM 2001; 94(7):365-72.
3. Marchetti M, Pistorio A, Barosi G. Extended anticoagulation for prevention of recurrent venous thromboembolism in carriers of factor V Leiden--cost-effectiveness analysis. Thromb Haemost 2000; 84(5):752-7.
4. Eckman MH, Singh SK, Erban JK, Kao G. Testing for factor V Leiden in patients with pulmonary or venous thromboembolism: a cost-effectiveness analysis. Med Decis Making 2002; 22(2):108-24.
5. Clark P, Twaddle S, Walker ID, Scott L, Greer IA. Cost-effectiveness of screening for the factor V Leiden mutation in pregnant women. Lancet 2002; 359(9321):1919-20.
6. Auerbach AD, Sanders GD, Hambleton J. Cost-effectiveness of testing for hypercoagulability and effects on treatment strategies in patients with deep vein thrombosis. Am J Med 2004; 116(12):816-28.
7. Wu O, Robertson L, Twaddle S *et al*. Screening for thrombophilia in high-risk situations: systematic review and cost-effectiveness analysis. The Thrombosis: Risk and Economic Assessment of Thrombophilia Screening (TREATS) study. Health Technol Assess 2006; 10(11):1-110.