

Venous Thrombosis with Both Heterozygous Factor V Leiden (R507Q) and Factor II (G20210A) Mutations

FERIYL BHAJEE, BRENDA JORDAN, DOMINIQUE J. PEPPER,
RODNEY LEACOCK, WILLIAM A. ROCK JR.

ABSTRACT

Both hereditary and acquired factors increase the risk of venous thromboembolism, thus the clinical management of affected patients involves evaluation of genetic factors that predispose to hypercoagulability. Factor V Leiden (R507Q) and factor II (prothrombin) mutation (G20210A) are the two most common inherited hypercoagulability disorders among populations of European origin. Both factor V Leiden and factor II mutation (G20210A) represent gain-of-function mutations: factor V Leiden causes resistance to activated protein C, and factor II mutation (G20210A) results in higher levels of plasma prothrombin. Herein, we present an uncommon case of combined factor V Leiden mutation (R507Q) and factor II mutation (G20210A), and discuss the prevalence and features of each entity, as well as their role in the clinical management of affected patients.

ABBREVIATIONS

EDTA—ethylenediaminetetraacetic acid, VTE—venous thromboembolism, APC—activated protein C,

INDEX TERMS

factor V Leiden, factor II mutation (G20210A), prothrombin, warfarin

Clin Lab Sci 2012;25(4):199

Feriyil Bhaijee, MD, Dept. of Pathology, University of Mississippi Medical Center, Jackson MS

Brenda Jordan, MD, Dept. of Pathology, University of Mississippi Medical Center, Jackson MS

Dominique J. Pepper, MD, Dept. of Medicine, University of Mississippi Medical Center, Jackson MS

Rodney Leacock, MD, Dept. of Neurology, University of Mississippi Medical Center, Jackson MS

William A. Rock Jr., MD, Dept. of Pathology, University of Mississippi Medical Center, Jackson MS

Address for Correspondence: William A. Rock Jr., MD, Dept. of Pathology, University of Mississippi Medical Center, 2500 North State Street, Jackson MS 39216, 601 853-4424, wfcc42@gmail.com

Introduction

The clinical and laboratory assessment of thrombosis risk is essential in the management of individuals with either congenital or acquired thrombotic risk factors. Acute intervention protocols utilizing heparin or low molecular weight heparin achieve rapid local thrombotic control, while warfarin and newer anti-thrombotic agents are used to prevent subsequent thrombotic events. Herein we describe the case of an individual diagnosed with a heterozygous factor V Leiden (R507Q) a known risk factor for deep vein thrombosis with 15 years of therapy, only to find a second significant undiagnosed risk factor, a heterozygous factor II (G20210A) mutation.

The presence of two or more congenital or acquired thrombotic risk factors would be expected to confer additive risk for thrombotic events. This could be additional justification for long term anticoagulation, taking into consideration the accompanying management issues and bleeding risk.

Medical History

A 59 year-old Caucasian man was admitted to the hospital for management of warfarin therapy for recurrent lower leg deep vein thrombosis that had originally occurred fifteen years ago. The patient was on

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continuous warfarin therapy for repeated deep vein thrombosis events. This was a first time admission to our hospital, and he was unknown to the medical staff. He had a previous diagnosis of the F V Leiden mutation by history.

The admitting physicians requested the comprehensive thrombosis risk panel to confirm the history of a F V Leiden mutation as well as assess for other known deep vein thrombosis risk factors not previously assessed in this patient.

See Table 1 for the comprehensive risk panel available at the time the patient was admitted. A factor V Leiden heterozygous point mutation in the factor V gene, [F5 C.1601G>A(P.ARG534GLN)], also known as factor V Leiden (R507Q) mutation was confirmed. In addition, a factor II (G20210A) heterozygous point mutation [F2 AF478696.1:G.21538G>A], was identified.

Although the patient had been on warfarin, the warfarin effect on the prothrombin time and protein C and S was not present because the patient had received Vitamin K and was partially corrected. In addition, the fibrinogen, factor VIII and C-reactive protein were elevated, consistent with a moderate acute phase hypercoagulable state. The antiphospholipid profile, lupus anticoagulant profile, Anti-thrombin, and protein S (total and free) antigen were all negative. The borderline low protein C antigen reflected the recent warfarin therapy. (Table 1)

METHODS

Methods for factor V Leiden and factor II mutation (G20210A), as well as the complete hypercoagulability assessment, have been described previously.¹

The factor V Leiden and factor II mutation (G20210A) assays were performed with the Invader® DNA assay by Third Wave Technologies, Inc. Madison, Wisconsin. The required specimen is a full adult size EDTA anticoagulated blood sample from which the buffy coat is harvested for the white cell DNA.

DISCUSSION

Venous thromboembolism (VTE) results from various interactions between genetic and environmental risk factors. Hereditary factors predisposing to VTE include factor V Leiden, factor II mutation (G20210A), Anti-

thrombin, protein C, and protein S (total & free) deficiencies. Of these, F V Leiden and Factor II Mutation (G20210A) are the most prevalent genetic risk factors, and both represent gain-of-function mutations: F V Leiden leads to activated protein C resistance,² and factor II mutation (G20210A) results in higher levels of plasma prothrombin.³ In 2003, the US Food and Drug Administration approved the first DNA-based laboratory tests for the detection of F V Leiden and factor II mutation (G20210A).⁴ Testing for these two mutations is now offered throughout the US.

Table 1. Hypercoagulability Panel

	Patient	Reference Range
White Cell Count	11.2 X10 ³ /μL	4.0-11.0 X10 ³ /μL
Hematocrit	36%	42-49%
Platelet count	102 X10 ³ /μL	130-400 X10 ³ /μL
Prothrombin time	12.0 sec	9.3-12.7 sec
Activated PTT	27.8 sec	23.9-33.1 sec
Fibrinogen	521 mg/dL	180-400 mg/dL
F VII	95%	50-150%
F VIII	151%	50-150%
Anti-thrombin III	96%	70-140%
Protein C antigen	42%*	>49%
Protein S (total) antigen	81%	>53%
Protein S (free) antigen	62%	>48%
Lupus Anticoagulant Panel		
aPTT: Actin® FSL activator	27.8 sec	23.9-33.1 sec
aPTT: Silica dioxide activator	32.1 sec	25.9-45.0 sec
Prothrombin time (Inovin®)	12.0 sec	9.3-12.7 sec
Dilute Russell Viper Venom Time	Negative	Negative
Antiphospholipid Panel		
Anticardiolipin:		
IgG	3.4 GPL	0-22 GPL
IgM	5.1 MPL	0-11 GPL
IgA	5.9 APL	0-22 APL
Anti-β-2-glycoprotein-1		
IgG	<1.0 GBU	0-20 GBU
IgM	4.6 MBU	0-20 MBU
IgA	5.8 ABU	0-20 ABU
Anti-Prothrombin		
IgG	<1.0 G UNITS	0-20 G UNITS
IgM	3.1 M UNITS	0-20 M UNITS
Activated Protein C Resistance	1.9 ratio	>2.3 ratio
Factor V Leiden mutation	positive heterozygous	not detected
Factor II (20210) mutation	positive heterozygous	not detected
MTHFR C677T mutation+	positive heterozygous	not detected
C-Reactive Protein	13.6 mg/dL	0.0-0.49 mg/dL
Homocysteine	1.03 mg/L	0.69-2.08 mg/L

* Reflects recent warfarin therapy

+ No known thrombosis risk associated with this heterozygous mutation

F V Leiden is the most common inherited hypercoagulability disorder among populations of European descent. The disorder is named after the city of Leiden in the Netherlands, where it was first identified in 1994.² F V Leiden is an autosomal dominant mutation which results from a single nucleotide polymorphism in exon 10 of the *factor V* gene. This point mutation involves substitution of a glutamine for an arginine at either position 1691 or 1746, and also affects the amino acid position for the Leiden variant (506 or 534). Due to non-standard nomenclature, the F V Leiden mutation may be referred to as G1691A, c.1601G>A, c.1746G>A, p.Arg534Gln, Arg506Gln, R506Q, etc. This mutation eliminates one of the three activated protein C (APC) cleavage sites, resulting in activated protein C (APC) resistance, which prevents efficient inactivation of factor V. Continued factor V activation leads to overproduction of thrombin, excess fibrin generation, and increased clotting.⁵ In addition to F V Leiden, three other factor V mutations with activated protein C resistance have been described, but these are extremely rare and their clinical significance requires further investigation.⁶⁻⁹

F V Leiden has been associated with VTE¹⁰ and pregnancy loss.¹¹ Factor V Leiden has been associated uncommonly but significantly with cerebrovascular events alone¹² or via paradoxical embolism,¹³ and myocardial infarctions.¹⁴ Homozygosity for the mutated allele confers increased risk compared to heterozygosity. Heterozygous individuals (i.e. with a single mutant allele) have a 5- to 10- fold increased risk of VTE, while homozygosity (i.e. two mutant alleles) confers a 15- to 80- fold increased risk of VTE.^{15,16,17}

Haplotype analysis of factor V gene polymorphisms suggests that the F V Leiden mutation arose in a single Caucasoid ancestor approximately 21,000-34,000 years ago.¹⁸ The frequency of F V Leiden in Europe varies widely, from 2% in Italy and Spain to up to 15% in Swedish populations. In the US, a single F V Leiden allele is present in about 5% of white populations, 2.2% of Hispanic populations, and 1.2% of African American populations.¹⁹ This mutation is not found in native African and Southeast Asian populations.¹⁸ (Table 2)

Lindqvist *et al.* postulate that the F V Leiden mutation in Caucasian populations may represent a selective advantage related to an increased risk of life-threatening hemorrhage postpartum.²⁰ This theory is based on the

observations that heterozygous F V Leiden mutation reduces intrapartum blood loss²⁰ and ameliorates the bleeding tendency associated with hemophilia A.²¹ Moreover, the risk of VTE in heterozygous individuals is relatively low in the absence of other risk factors, especially prior to reproductive age.²²

Our patient exhibited an acute phase hypercoagulable state in addition to the factor II mutation (G20210A) and the factor V Leiden mutation. There is the observation and suggestion that F V Leiden may confer a survival advantage in patients with severe sepsis.^{23,24} Recent studies have contradicted the purported survival advantage.^{25,26,27} It remains to be seen if this sepsis survival is substantiated. Our patient did not have an infection or sepsis.

Factor II, also known as prothrombin, is a vitamin K-dependent clotting factor in the blood coagulation cascade. Factor II is cleaved by factor Xa to form thrombin, an active serine protease. The gene encoding factor II is located on chromosome 11 in the region of the centromere.²⁸ This gene consists of 14 exons and 24 kilobases of DNA, which encode a signal region, a propeptide region, a glutamic acid domain, 2 kringle regions, and a catalytic domain. In the presence of vitamin K, the enzyme gamma-glutamyl carboxylase converts the N-terminal glutamic acid residues to gamma-carboxyglutamic acid residues, which facilitate the binding of factor II to platelet membrane phospholipids.

The factor II mutation (G20210A) was first reported in 1996 as a familial cause of VTE.³ This mutation involves substitution of an adenine for a guanine at position 20210 within the 3' untranslated region of the factor II gene.²⁹ This substitution alters the polyadenylation site of the gene, leading to increased mRNA synthesis and subsequent increased protein expression, resulting in elevated plasma factor II levels and an increased risk of thrombosis.^{3,30} Individuals with a single F II mutation (G20210A) [heterozygotes] have a 30% increase in plasma factor II, and those with double G20210A mutations (homozygotes) have a 70% increase.^{3,30}

The factor II mutation (G20210A) is the second most common inherited risk factor for VTE, and occurs in approximately 2% of European populations, with high-

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Table 2. Factor V Leiden and Factor II G20210A mutations

Authors	Study site; characteristics	Participants	Prevalence
Factor V Leiden			
Martinelli et al. 1997	Italy; symptomatic non-fatal pulmonary embolism	106	12%
	Italy; deep venous thrombosis	106	23%
	Italy; healthy controls	212	3%
Martinelli et al. 1998	Italy; cerebral vein thrombosis	40	15%
	Italy; deep venous thrombosis	80	19%
	Italy; healthy controls	120	3%
Martinelli et al. 2000	Italy; woman with late fetal loss	67	7%
	Italy; women with normal pregnancies	232	3%
Eroglu et al. (meta-analysis) 2000	Turkey; venous thrombo-embolism	1202	23%
	Turkey; healthy controls	1283	8%
Bavikatty et al. 2000	Mid-western America; patients with thrombophilia	264	17%
Wahlender et al. 2002	Europe; elective orthopedic hip or knee surgery	1600	6%
Dizon-Townson et al. 2005	USA; singleton pregnancy, no history of thromboembolism	4885	3%
Eid et al. 2005	Jordan; patients with thrombophilia	594	26%
Ho et al. 2006	Australia; first episode of venous thromboembolism	3104	21%
Mazoyer et al. 2009	France; no thrombotic history	6154	4%
Serrano et al. 2011	Portugal; recurrent miscarriage	100	5%
	Portugal; no history of pregnancy loss	100	5%
Factor II G20210A mutation			
Martinelli et al. 1998	Italy; cerebral vein thrombosis	40	20%
	Italy; deep venous thrombosis	80	18%
	Italy; healthy controls	120	3%
Bavikatty et al. 2000	Mid-western America; patients with thrombophilia	264	7%
Martinelli et al. 2000	Italy; woman with late fetal loss	67	9%
Wahlender et al. 2002	Europe; elective orthopedic hip or knee surgery	1600	6%
Eid et al. 2005	Jordan; patients with thrombophilia	594	6%
Ho et al. 2006	Australia; first episode of venous thromboembolism	2903	10%
Mazoyer et al. 2009	France; no thrombotic history	6154	3%
Serrano et al. 2011	Portugal; recurrent miscarriage	100	3%
	Portugal; no history of pregnancy loss	100	1%
Combined Factor V Leiden and Factor II G20210A mutations			
Bavikatty et al. 2000	Mid-western America; patients with thrombophilia	8/264	3%
Heller et al. 2000 neonates and infants	Germany; abdominal venous thrombosis in	2/65	3%

Table 3. Incidence of Factor V Leiden R507Q and Factor II G20210A mutations among healthy controls

	USA Caucasian	USA African- American	USA Hispanic	USA Asian/ Native Hawaiian	Sweden European	France European	Italy European	Africa African	Asia Asian/ Pacific Islanders
Factor V Leiden R507Q ^{18,19,39-45}	5%	1.2%	2.2%	*	15%	4%	3%	*	*
Factor II G20210A ^{33,39-42,44}	1.1%	0.3%	1.1%	*	3%	3%	3%	*	*
Combined incidence in patients with thrombophilia ⁴⁵⁻⁴⁹						3%			

* extremely uncommon, exact prevalence unknown

er frequency in those of southern European descent compared to those of northern European descent.^{31,32} In the US, this mutation is present in 1.1% of non-hispanic whites and Mexican Americans and in 0.3% of

African Americans.³³ It is rarely seen in Asians or Africans. The factor II mutation (G20210A) confers a 2- to 3- fold increased risk of thrombosis.^{3,34} Asymptomatic individuals with the factor II mutation

(G20210A) have a similar incidence (0.6%) of VTE as asymptomatic individuals with a single F V Leiden allele (heterozygotes).³⁵ Factor II mutation (G20210A) is also associated with an increased risk of ischemic cerebrovascular events in men younger than 60 years of age³⁶ and recurrent pregnancy loss (Tables 2 and 3).³⁷

The prevalence of F V Leiden and factor II mutation (G20210A) varies among countries, according to the presence or absence of thrombophilia. The prevalence of F V Leiden among healthy controls in Italy, Turkey, Portugal, Europe, the United States of America, and France varies between 3 and 8%.³⁸⁻⁴⁴ Among patients with thrombophilia from these same countries, the prevalence of F V Leiden is higher – between 5 and 23%.^{38-40,43,44,45} In Jordan and Australia, the prevalence of F V Leiden among patients with thrombophilia may reach 26% and 21%, respectively.^{46, 47}

The prevalence of factor II mutation (G20210A) among healthy controls in Italy, Portugal, Europe, and France varies between 1 and 6%.^{39-42,44} Among patients with thrombophilia from these same countries, the prevalence of factor II mutation (G20210A) is higher – between 3 and 20% (Table 3).^{39,40,44,46,47}

Among patients with thrombophilia, current assessments suggest up to 3% of patients will have combined F V Leiden and factor II mutation (G20210A) (Table 4).^{46,48} Combined F V Leiden and factor II mutation (G20210A) could increase the risk of primary or recurrent VTE.⁴⁹⁻⁵²

Based on a meta-analysis of 46 articles, Segal *et al.* report that both heterozygosity and homozygosity for F V Leiden in probands are predictive of recurrent VTE compared to individuals without F V Leiden (Table 3).⁴ Heterozygosity and homozygosity for F V Leiden also predict VTE in family members compared with family members of adults without F V Leiden. Heterozygosity for factor II mutation (G20210A), however, is not predictive of recurrent VTE in affected individuals. Also, there is insufficient evidence regarding the value of factor II (G20210A) homozygosity for recurrent VTE and the risk of VTE in family members of individuals with the factor II mutation (G20210A). While there is high grade evidence that anticoagulation reduces recurrent VTE events in individuals with either mutation, there is only low grade evidence that this risk

reduction is similar to that in individuals with a history of VTE in the absence of these mutations. Segal *et al.* conclude that patients with F V Leiden are at increased risk of recurrent VTE compared with patients without F V Leiden, but it is unknown whether testing for F V Leiden or factor II mutation (G20210A) improves outcomes in adults with VTE or in family members of individuals with a mutation.⁴

Table 4. Odds ratios for recurrent VTE among individuals with FVL or Factor II G20210A (probands) and first VTE among family members of probands⁴

	Overall odds ratio (95% CI)
Recurrent VTE among probands	
FVL heterozygosity	1.56 (1.14-2.12)
FVL homozygosity	2.65 (1.18-5.97)
Factor II G20210A heterozygosity	1.45 (0.96-2.21)
Factor II G20210A homozygosity	Insufficient evidence
First VTE among family members	
Family members with FVL heterozygosity	3.49 (2.46-4.96)
Family members with FVL homozygosity	17.84 (7.98-39.89)

Testing for factor V Leiden and the factor II G20210A mutation should be considered in any patient who has a thrombotic event in the absence of recognized risk factors. Additionally, testing for factor V Leiden should be considered in any patient of European descent with a thrombotic event before the age of 45 years, or with a family history of VTE.

Observational studies suggest that patients with a VTE and hereditary thrombophilia have only a slightly increased risk of recurrent VTE.⁵³ Furthermore, testing for thrombophilia after the first VTE episode does not appear to reduce the risk of recurrence. However, testing patients with VTE for thrombophilia may alter clinical management, as well as facilitate identification of asymptomatic family members who might benefit from risk reduction measures. Affected females, for example, should avoid oral contraception, if possible, because of the additive risk of VTE or ischemic stroke.^{54,55} Other benefits of thrombophilia testing include ruling out underlying congenital or acquired risk factors for thrombosis and informing the patient and family members about necessary lifestyle changes and pregnancy concerns.

Oral anticoagulation for 3-6 months following the first episode of VTE is the current standard of care.⁵⁶ To

date, there is insufficient evidence to justify routine prolonged anticoagulation in patients with thrombophilia, as the complications of therapy may outweigh the benefits.⁵³ Continued anticoagulation therapy would be considered for patients who have damaged venous valves in the lower leg or other trauma to the lower leg veins that would predispose to decreased blood flow or turbulence especially with a history of repeat episodes of deep vein thrombosis or pulmonary embolism.

The homozygous forms of protein C, factor V Leiden (R507Q) and factor II (G20210A) have been described in young children and young adults with bad outcomes. In our case of a double heterozygous F V Leiden and F II (G20210A) mutation, intervention with warfarin was successful for fifteen years from age 44 to 59 yrs. This suggests that a double heterozygous for F V Leiden and factor II mutation (G20210A) may not necessarily represent as much of an increased risk as does the homozygous form of either factor mutation. The numbers are very small and further studies of this uncommon combination population are indicated.

REFERENCES

- Lobrano A, Blanchard K, Rock W, Johnson W, Schmiegl R, Borman K, et al. Assessing Thrombosis Risk in Patients with Idiopathic, Diabetic, and Postsurgical Gastroparesis. *Adv Ther* 2006;23:750-8.
- Bertina RM, Koeleman BP, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. *Nature* 1994;369:64-7.
- Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. *Blood* 1996;88:3698-703.
- Segal JB, Brotman DJ, Necochea AJ, et al. Predictive value of factor V Leiden and prothrombin G20210A in adults with venous thromboembolism and in family members of those with a mutation: a systematic review. *JAMA* 2009;301:2472-85.
- De Stefano V, Leone G. Resistance to activated protein C due to mutated factor V as a novel cause of inherited thrombophilia. *Haematologica* 1995;80:344-56.
- Lunghi B, Iacoviello L, Gemmati D, et al. Detection of new polymorphic markers in the factor V gene: association with factor V levels in plasma. *Thromb Haemost* 1996;75:45-8.
- Franco RF, Maffei FH, Lourenco D, et al. Factor V Arg306→Thr (factor V Cambridge) and factor V Arg306→Gly mutations in venous thrombotic disease. *Br J Haematol* 1998;103:888-90.
- van der Neut KM, Dirven RJ, Tans G, et al. The activated protein C (APC)-resistant phenotype of APC cleavage site mutants of recombinant factor V in a reconstituted plasma model. *Blood Coag Fibrinolysis* 2002;13:207-15.
- Mumford AD, McVey JH, Morse CV, et al. Factor V I1359T: a novel mutation associated with thrombosis and resistance to activated protein C. *Br J Haematol* 2003;123:496-501.
- Martinelli I. Risk factors in venous thromboembolism. *Thromb Haemost* 2001;86:395-403.
- Rai R, Backos M, Elgaddal S, et al. Factor V Leiden and recurrent miscarriage-prospective outcome of untreated pregnancies. *Hum Reprod*. 2002;17:442-5
- Kujovich JL. Factor V Leiden Thrombophilia, NCBI Bookshelf ID:NBK1368 PMID: 20301542, Updated: March 9, 2010
- Karttunen V, Hiltunen L, Rasi V et al. Factor V Leiden and prothrombin gene mutation may predispose to paradoxical embolism in subjects with patent foramen ovale. *Blood Coagul. Fibrinolysis* 2003;14:261-8
- Middendorf K, Gohring P, Huehns TY, et al. Prevalence of resistance against activated protein C resulting from factor V Leiden is significantly increased in myocardial infarction: investigation of 507 patients with myocardial infarction. *Am Heart J*. 2004;147:897-904
- Rosendaal FR, Koster T, Vandenbroucke JP, Reitsma PH, High Risk of Thrombosis in Patients Homozygous for Factor V Leiden (Activated Protein C Resistance). *Blood*: 1995;85:1504-8
- Van Der Meer FJ, Koster T, Vandenbroucke JP. et al. The Leiden Thrombophilia Study (LETS) *Thromb Haemost* 1998;78:631-5
- Price DT, Ridker PM, Factor V Leiden mutation and the risks for thromboembolic disease; a clinical perspective. *Ann. Intern Med* 1997;127:895-903
- Zivelin A, Griffin JH, Xu X, et al. A single genetic origin for a common Caucasian risk factor for venous thrombosis. *Blood* 1997;89:397-402.
- 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.
- Lindqvist PG, Svensson PJ, Dahlback B, et al. Factor V Q506 mutation (activated protein C resistance) associated with reduced intrapartum blood loss – a possible evolutionary selection mechanism. *Thromb Haemost* 1998;79:69-73.
- Nichols WC, Amano K, Cacheris PM, et al. Moderation of hemophilia A phenotype by the factor V R506Q mutation. *Blood* 1996;88:1183-7.
- Koeleman BP, Reitsma PH, Bertina RM. Familial thrombophilia: a complex genetic disorder. *Semin Hematol* 1997;34:256-64.
- Kerlin BA, Yan SB, Isermann BH, et al. Survival advantage associated with heterozygous factor V Leiden mutation in patients with severe sepsis and in mouse endotoxemia. *Blood* 2003;102:3085-92.
- Elmas E, Suvajac N, Jilma B., et al. Factor V Leiden mutation enhances fibrin formation and dissolution in vivo in a human endotoxemia model. *Blood* 2010;116:801-5
- Yan SB, Nelson DR. Effect of factor V Leiden polymorphism in severe sepsis and on treatment with recombinant human activated protein C. *Crit Care Med* 2004;32(5Suppl):S239-46.
- Benfield TL, Dahl M, Nordestgaard BG, Tybjaerg-Hansen A. Influence of the factor V Leiden mutation on infectious disease susceptibility and outcome: a population-based study. *J Infect*

CLINICAL PRACTICE

- Dis 2006;192:1851-7.
27. Benfield T, Ejraes K, Juul K, et al. Influence of factor V Leiden on susceptibility to and outcome from critical illness: a genetic association study. *Crit. Care* 2010;14:R28.
 28. Degen SJ. The prothrombin gene and its liver-specific expression. *Semin Thromb Hemost* 1992;18(2):230-42.
 29. Pollak ES, Lam HS, Russell JE. The G20210A mutation does not affect the stability of prothrombin mRNA in vivo. *Blood* Jul 1 2002;100:359-62.
 30. Ceelie H, Spaargaren-van Riel CC, Bertina RM, Vos HL. G20210A is a functional mutation in the prothrombin gene; effect on protein levels and 3'-end formation. *J Thromb Haemost* 2004;2:119-27.
 31. Rosendaal FR, Doggen CJ, Zivelin A, et al. Geographic distribution of the 20210 G to A prothrombin variant. *Thromb Haemost* 1998;79:706-8.
 32. Atasay B, Arsan S, Gunlemez A, Kemahli S, Akar N. Factor V Leiden and prothrombin gene 20210A variant in neonatal thromboembolism and in healthy neonates and adults: a study in a single center. *Pediatr Hematol Oncol* 2003;20:627-34.
 33. Chang MH, Lindegren ML, Butler MA, et al. CDC/NCI NHANES III Genomics Working Group. Prevalence in the United States of selected candidate gene variants: Third National Health and Nutrition Examination Survey, 1991-1994. *Am J Epidemiol* 2009;169:54-66.
 34. Margaglione M, Brancaccio V, Giuliani N, et al. Increased risk for venous thrombosis in carriers of the prothrombin G-->A20210 gene variant. *Ann Intern Med* 1998;129:89-93.
 35. Middeldorp S, Meinardi JR, van Theinen JV, et al. The incidence of venous thromboembolism in relatives of patients with the prothrombin 20210A mutation. *Thromb Haemost* 1999;82:277.
 36. Lalouschek W, Schillinger M, Hsieh K, et al. Matched case-control study on factor V Leiden and the prothrombin G20210A mutation in patients with ischemic stroke/transient ischemic attack up to the age of 60 years. *Stroke* 2005;36:1405-9.
 37. Bagheri M, Rad IA, Nanbakhsh F. Factor V Leiden G1691A and factor II G20210A point mutations and pregnancy in North-West of Iran. *Arch Gynecol Obstet* 2011 Jul 20. [Epub ahead of print]
 38. Martinelli I, Cattaneo M, Panzeri D, Mannucci PM. Low prevalence of factor V:Q506 in 41 patients with isolated pulmonary embolism. *Thromb Haemost* 1997;77:440-3.
 39. Martinelli I, Sacchi E, Landi G, Taioli E, Duca F, Mannucci PM. High risk of cerebral-vein thrombosis in carriers of a prothrombin-gene mutation and in users of oral contraceptives. *N Engl J Med* 1998;338:1793-7.
 40. Martinelli I, Taioli E, Cetin I, et al. Mutations in coagulation factors in women with unexplained late fetal loss. *N Engl J Med* 2000;343:1015-8.
 41. Wähländer K, Larson G, Lindahl TL, et al. Factor V Leiden (G1691A) and prothrombin gene G20210A mutations as potential risk factors for venous thromboembolism after total hip or total knee replacement surgery. *Thromb Haemost* 2002;87:580-5.
 42. Mazoyer E, Ripoll L, Gueguen R, et al. FITENAT Study Group. Prevalence of factor V Leiden and prothrombin G20210A mutation in a large French population selected for nonthrombotic history: geographical and age distribution. *Blood Coagul Fibrinolysis* 2009;20:503-10.
 43. Eroglu A, Sertkaya D, Akar N. The Role of Factor V Leiden in Adult Patients With Venous Thromboembolism: A Meta-Analysis of Published Studies From Turkey. *Clin Appl Thromb Hemost* 2011 Jul 6. [Epub in press]
 44. Serrano F, Lima ML, Lopes C, Almeida JP, Branco Factor V Leiden and prothrombin G20210A in Portuguese women with recurrent miscarriage: is it worthwhile to investigate? *J Arch Gynecol Obstet* 2011 Jan 23. [Epub in press]
 45. Dizon-Townson D, Miller C, Sibai B, et al. The relationship of the factor V Leiden mutation and pregnancy outcomes for mother and fetus. *Obstet Gynecol* 2005;106:517-24.
 46. Bavikatty NR, Killeen AA, Akel N, Normolle D, Schmaier AH. Association of the prothrombin G20210A mutation with factor V Leiden in a midwestern American population. *Am J Clin Pathol* 2000;114:272-5.
 47. Ho WK, Hankey GJ, Quinlan DJ, Eikelboom JW. Risk of recurrent venous thromboembolism in patients with common thrombophilia: a systematic review. *Arch Intern Med* 2006;166:729-36.
 48. Eid SS, Shubeilat T. Prevalence of factor V Leiden, prothrombin G20210A, and MTHFR G677A among 594 thrombotic Jordanian patients. *Blood Coagul Fibrinolysis* 2005;16:417-21.
 49. Heller C, Schobess R, Kurnik K, et al. Abdominal venous thrombosis in neonates and infants: role of prothrombotic risk factors - a multicentre case-control study. For the Childhood Thrombophilia Study Group. *Br J Haematol* 2000;111:534-9.
 50. Makris M, Preston FE, Beauchamp NJ, et al. Co-inheritance of the 20210A allele of the prothrombin gene increases the risk of thrombosis in subjects with familial thrombophilia. *Thromb Haemost* 1997;78:1426-9.
 51. De Stefano V, Martinelli I, Mannucci PM, et al. The risk of recurrent deep vein thrombosis among heterozygous carriers of both factor V Leiden and the G20210A prothrombin mutation. *N Engl J Med* 1999;341:801-6.
 52. Margaglione M, D'Andrea G, Colaizzo D, et al. Co-existence of factor V Leiden and factor II A20210 mutations and recurrent venous thromboembolism. *Thromb Haemost* 1999;82:1583-7.
 53. Middeldorp S. Evidence-based approach to thrombophilia testing. *J Thromb Thrombolysis* 2011;31:275-81.
 54. Martinelli I, Battaglioli T, Bucciarelli P, Passamonti SM, Mannucci PM. Risk factors and recurrence rate of primary deep vein thrombosis of the upper extremities. *Circulation* 2004;110:566-70.
 55. Martinelli I, Battaglioli T, Burgo I, Domenico SD, Mannucci PM. Oral contraceptive use, thrombophilia and their interaction in young women with ischemic stroke. *Haematologica* 2006;91:844-7.
 56. Buller HR, Agnelli G, Hull RD, et al. Antithrombotic therapy for venous thromboembolic disease. The seventh ACCP conference on antithrombotic and thrombolytic therapy. *Chest* 2004;126:401S-28S.