American Journal of Hematology 80:12–19 (2005)

Association Between Adverse Pregnancy Outcomes and Maternal Factor V G1691A (Leiden) and Prothrombin G20210A Genotypes in Women With a History of Recurrent Idiopathic Miscarriages

Touhami Mahjoub,[1] Nabil Mtiraoui,[1] Hala Tamim,[2] Sondes Hizem,[1] Ramzi R. Finan,[3] Brahim Nsiri,[1] and Wassim Y. Almawi[4]*

[1] Research unit of Haematological and Autoimmune Diseases, Faculty of Pharmacy, Monastir, Center University, Tunisia
[2] Faculty of Health Sciences, American University of Beirut, Lebanon
[3] Faculté de Médecine, Université St. Joseph, Beirut, Lebanon
[4] Al-Jawhara Center for Molecular Medicine and Genetics, Arabian Gulf University, Manama, Bahrain

Thrombophilia was implicated in the development of pregnancy complications, including recurrent idiopathic pregnancy loss, and is aggravated in women who are carriers of factor V G1691A (FV Leiden) and prothrombin (PRT) G20210A single-nucleotide polymorphisms (SNPs). Previous studies examined the role of FV-Leiden and PRT G20210A in recurrent pregnancy loss with conflicting results. Here we examined the prevalence of FV Leiden and PRT G20210A SNPs, in 200 women with 3 or more consecutive early (n = 87), late (n = 41), or early–late (n = 72) recurrent pregnancy losses, and 200 age-matched fertile parous control women. APC resistance (APCR) was detected functionally (measuring the activated clotting time triggered by activated factor X in presence of a fixed amount of purified APC), and FV-Leiden and PRT G20210A genotypes were assessed by PCR. The frequency of the mutant FV (0.1400 vs. 0.0276; $P < 0.001$) but not PRT 20210 (0.0100 vs. 0.0225; $P = 0.159$) allele was higher in patients than controls, respectively. APC resistance with factor V Leiden was seen in 27% of patients compared to 11.5% of controls, while APC resistance without factor V Leiden was seen in 12.5% of patients compared to 9.5% of controls. Regression analysis demonstrated that the significant predictors for early abortion was FV Leiden; those for late abortion were oral contraceptive, APCR, and FV Leiden; and predictors for early–late abortions were oral contraceptives, obesity, FV Leiden, and smoking. APC resistance and FV Leiden, as well as combination of both, are common thrombotic defects seen in women with idiopathic recurrent pregnancy loss, thus testing for these is recommended in women who have experienced recurrent miscarriages. Am. J. Hematol. 80:12–19, 2005. © 2005 Wiley-Liss, Inc.

Key words: abortion; APC resistance; factor V Leiden; miscarriage; PCR; prothrombin G20210A mutation

INTRODUCTION

Pregnancy is associated with altered blood coagulation, manifested as increases in clotting factors (I, II, VII, VIII, IX, and XII), coupled with a decrease in protein S levels and a significant reduction in the activity of activated protein C (APC) [1,2]. While these changes may be beneficial in minimizing intrapartum blood loss, they reportedly confer an added risk of thromboembolism during pregnancy and post-

*Correspondence to: Wassim Y. Almawi, Ph.D., Al-Jawhara Center for Molecular Medicine, Genetics & Inherited Diseases, College of Medicine & Medical Sciences, Arabian Gulf University, P.O. Box 22979, Manama, Bahrain.E-mail: wyalmawi@yahoo.co.uk

Received for publication 28 December 2004; Accepted 25 January 2005

Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/ajh.20419

partum [1]. Hypercoagulation represents a major risk factor of severe pregnancy complications [3], which include pre-eclampsia and idiopathic recurrent abortions [4].

Venous thromboembolism (VTE) is the leading cause of morbidity and mortality in pregnancy and the postpartum period in developed countries, which, if left untreated, may progress to pulmonary embolism with a high mortality rate [5]. VTE in pregnancy is multifactorial, and both acquired (smoking, oral contraceptives use, obesity, advanced age, hyperemesis, infection, or systemic disease) [6,7] and inherited (mutations in blood coagulation factors) risk factors reportedly cooperate in precipitating idiopathic recurrent fetal loss [4,8]. Among the inherited risk factors are single-nucleotide polymorphisms (SNPs): factor V G1691A/R506Q mutation (FV Leiden) [4,9], prothrombin (PRT) G20210A [4,9], and the C677T and A1298C in methylene tetrahydrofolate reductase (MTHFR) gene [10,11].

FV Leiden is the most common cause of primary and recurrent VTE [12], accounting for 95% of cases of APC resistance (APCR) [13]. Caused by a point mutation in factor V gene by which arginine is substituted for glutamine at position 506 (APC cleavage site of factor Va), FV Leiden renders factor Va resistant to degradation by APC, hence precipitating a thromboembolic state [14]. The PRT G20210A SNP, a G→A substitution at position 20210 in the 3′-untranslated region of the PRT gene [15], induces high plasma PRT levels [15]. Both FV Leiden and PRT G20210A are the most significant causes of inherited thrombophilia, because altered factor V action and/or enhanced PRT accumulation, by enhancing thrombin generation, promote blood coagulation, and precipitate a thromboembolic state [13].

An association between FV Leiden and PRT G20210A SNPs and recurrent idiopathic miscarriages was proposed [4,9]. Women with a history of VTE during pregnancy or the puerperium had a higher prevalence of FV Leiden [16], and increased prevalence of FV Leiden was reported in women who had severe complications of pregnancy, including placental abruption [17,18], fetal death, severe pre-eclampsia [18,19], or intrauterine growth restriction [20]. Furthermore, 20–50% of women who had late pregnancy losses were carriers of FV Leiden compared with less than 5% of control parous women. Others failed to demonstrate any association between FV Leiden or PRT G20210A SNPs with recurrent idiopathic miscarriage [21–23], as similar carrier frequencies for either SNP were seen in patients and controls. Herein we investigate the relationship between recurrent miscarriages and FV Leiden and prothrombin G20210A mutations in 200 women with 3 or consecutive miscarriages of unknown etiology, compared to 200 parous women with uncomplicated pregnancies and deliveries.

MATERIALS AND METHODS

Study Subjects

This was a retrospective case-control study conducted in the maternity service of Hôpital Farhat Hached in Sousse, central Tunisia, in 2002–2004. The cases were 200 women of age 18–43 years (mean 28.68 ± 5.61 years) with 3 or more unexplained consecutive pregnancy losses from 5 to 30 weeks' gestation (Table I).

TABLE I. Characteristics of Study Participants

	Patients[a]	Controls[a]	*P*	OR	95% CI
Age (years)	28.68 ± 5.61	28.24 ± 5.51	0.434[b]		
Regional (Sahel: North: South: Center)	49:50:41:60	42:43:43:72	0.531[c]		
Smokers	9/191	9/191	1.000[c]	1.000	0.389–2.574
Education			<0.001[c]		
Intermediate	41	69			
Secondary	81	92			
University	78	39			
Alcohol	5	5	1.000[c]	1.000	0.285–3.509
BMI[d]	25.78 ± 4.01	24.62 ± 3.75	0.360[b]	1.159	0.395–1.923
Oral contraceptives	51	46	0.641[c]	1.146	0.725–1.811
APCR	54	23	<0.001[c]	2.846	1.667–4.860
Pregnancy outcomes					
Live	0.51 ± 0.72	3.81 ± 1.40	<0.001[b]	3.300	3.081–3.519
Early loss	2.29 ± 1.30	0.02 ± 0.14	<0.001[b]	2.270	2.088–2.452
Late loss	1.34 ± 1.30	0.04 ± 0.184	<0.001[b]	1.305	1.122–1.488

[a]Study subjects comprised 200 patients and 200 controls.
[b]Fisher's exact test.
[c]Pearson's chi-square test.
[d]BMI, body mass index, calculated according to BMI = weight (kg) ÷ height $(m)^2$.

Pregnancy losses were classified as early (5–12 weeks) and late (13–30 weeks). Exclusion criteria included induced abortions, infections, systemic disease, uterine structural abnormalities, and personal or family history of thrombosis. Age-matched controls comprised 200 healthy women, 19–39 years of age (mean 28.24 ± 5.51), with uncomplicated pregnancies (Table I). Participants were interviewed regarding risk factors for VTE, including smoking and oral contraceptive use, and other demographic data (age, educational status, area of residence, number and outcome of pregnancies, and risk factors) were collected from all participants. Cases and controls were then asked to sign an informed consent form, and the study was conducted after all institutional ethics requirements were met. Blood samples were obtained from participants for plasma and DNA preparation 8–12 weeks after the last pregnancy.

Genomic DNA Extraction

Total genomic DNA was isolated from the leukocyte-rich interphase layer of EDTA anti-coagulated blood by the standard phenol–chloroform method and was dissolved in nuclease-free water and stored at 4°C pending assay.

Genotype Analysis

FV Leiden and PRT G20210A mutations were detected by PCR-restriction fragment length polymorphism (PCR-RFLP). A typical PCR mixture comprised genomic DNA (50–200 ng), $MgCl_2$ (1.5 mM), sense and anti-sense oligonucleotide primers (10 pmol), dNTP mixture (10 mM concentration per each dNTP; Promega, Madison, WI), and *Taq* DNA polymerase (2.5 U/reaction; Promega). PCR products were digested by *Mnl*I for FV Leiden [14] and *Hin*dIII for PRT G20210A [15] genotype analysis, respectively (both enzymes were obtained from New England Biolabs, Beverly, MA). DNA bands were separated by ethidium bromide-stained agarose (2% w/v) gel electrophoresis. In addition, both FV Leiden and PRT G20210A mutations were simultaneously analyzed by multiplex allele-specific amplification (ASA-PCR multiplex) as previously described [24]. Both assays gave 100% concordance rates.

APC Resistance Assay

The plasma anticoagulant response to APC was determined based on measuring the activated clotting time (ACT) triggered by activated factor X in presence of a fixed amount of purified APC, and was performed on a 1:1 mixture of sample diluted to 1:5 in factor V-deficient plasma (Accelirimat®, BioMerieux, Paris, France). Normalized APC-sensitive ratio (nAPC-SR) was calculated as sample/control APC-SR ratio, and nAPC-SR values below 0.9 were considered to be APC-resistant (APCR).

Statistical Analysis

Allelic frequencies were calculated by gene-counting method. Linkage analysis, the nonrandom association between FV Leiden and PRT G20210A as defined by the delta (Δ') coefficient, was calculated using the HLAStat 2000 software, and the frequencies of the most frequent haplotypes were determined by the maximum likelihood method. The Mann–Whitney non-parametric *U*-test and the Pearson chi-square test were used to assess inter-group significance, using SPSS v. 12.0.1 statistics software which also calculated the odds ratios (OR) and 95% confidence intervals (CI). Statistical significance was set at $P < 0.05$.

RESULTS

Patients and Controls

Characteristics of the patients and controls are given in Table I. Both patients and control subjects had similar ages, body mass index, and regional distribution, with comparable numbers of smokers, alcohol consumers, and oral contraceptive (OC) users in both groups. While the pregnancy rate was similar between patients and controls, the mean number of live births per woman was significantly higher among controls, patients reporting 726 pregnancy losses in the first and second trimesters compared with 11 single-case pregnancy losses among controls (4 in early pregnancy and 7 in late pregnancy).

FV Leiden and PRT G20210A Genotype Analysis

The frequency of the FV Leiden mutant ("A") allele was significantly higher among patients than controls ($P = 0.001$, OR = 5.426; Table II). Higher frequencies of the G/A (20.0% vs. 5.5%) and the A/A (4.0% vs. 0.0%) genotypes of FV Leiden were seen in patients versus controls, respectively (Table II). In contrast, the frequency of the PRT mutant ("A") allele was comparable between patients and controls ($P = 0.4616$, OR = 0.433); all PRT G20210A carriers were in the heterozygous state (G/A) (Table II). The most frequent FV Leiden–PRT G20210A haplotype seen in patients was 1691A–20210G ($P < 0.001$, OR = 5.638), while a higher prevalence of the 1691G–20210G haplotype was seen among controls than patients ($P < 0.001$; OR = 0.298; Table III). The double mutant (1691A–20210A) haplotype was absent from both patients and controls.

TABLE II. Factor V Leiden and Prothrombin G20210A Genotype and Allele Distribution

	Genotype			Allele	
	G/G	G/A	A/A	G	A
Factor V Leiden					
Controls[a]	189 (94.5)	11 (5.5)	0 (0.0)	0.9725 ± 0.0082	0.0275 ± 0.0082
Patients[a]	152 (76.0)	40 (20.0)	8 (4.0)	0.8600 ± 0.0173	0.1400 ± 0.0173
P value		<0.001[b]			<0.001[c]
OR (95% CI)					5.426 (2.724–10.808)
PRT G20210A					
Controls	191 (95.5)	9 (4.5)	0 (0.0)	0.9775 ± 0.0074	0.0225 ± 0.0074
Patients	196 (98.0)	4 (2.0)	0 (0.0)	0.9900 ± 0.0050	0.0100 ± 0.0050
P value		0.129[b]			0.4616[c]
OR (95% CI)					0.433 (0.131–1.430)

[a]A total of 200 patients and 200 control subjects were analyzed. Allele frequency was determined as follows: frequency = number of an allele ÷ total number of alleles per every group.
[b]Pearson's chi-square test.
[c]Determined by Fisher's exact test.

TABLE III. Factor V Leiden and Prothrombin G20210A Haplotype Distribution*

FV Leiden/PRT G20210A	Patients[a]	Controls[a]	*P*[b]	χ^2	OR	95% CI
1691G/20210G	0.8525	0.951	<0.001	21.125	0.298	0.18–0.51
1691G/20210**A**	0.0100	0.0219	0.263	1.251	0.439	0.15–1.45
1691**A**/20210G	0.1375	0.0269	<0.001	30.534	5.638	2.84–10.44
1691**A**/20210**A**	0.0000	0.000	N/A	N/A	N/A	N/A

*Determined by the maximum-likelihood method.
[a]A total of 200 patients and 200 controls were included.
[b]Yates' chi-square test.

APC Resistance

Insofar as APCR secondary to FV Leiden was associated with pregnancy failure and pre-eclampsia, and as the degree of APCR among nonpregnant women is predictive of venous thrombosis risk, we examined the distribution of FV Leiden genotypes among APC-sensitive and APCR patients and controls. Patients were subdivided into those with early, late, or combined early–late pregnancy losses. A significantly higher proportion of APCR-positive cases was seen in women experiencing late ($P = 0.01$) and early–late ($P < 0.001$), but not early ($P = 0.21$) recurrent abortions (Table IV).

TABLE IV. APC Resistance Among Factor V Genotype Carriers

		Controls[b]	Patients[b]			
APCR[a]	FV-Leiden	None	Early	Late	Early–Late	Total[d]
	G/G	170 (96.0)[c]	66 (90.4)	25 (83.3)	36 (83.7)	297 (92.0)
Negative	G/**A**	7 (4.0)	6 (8.2)	5 (16.7)	5 (11.6)	23 (7.1)
	A/**A**	0 (0.0)	1 (1.4)	0 (0.0)	2 (4.7)	3 (0.9)
Total[d]		177 (100.0)	73 (100.0)	30 (100.0)	43 (100.0)	323 (100.0)
	G/G	19 (82.6)	10 (71.4)	8 (72.7)	7 (24.1)	44 (57.1)
Positive	G/**A**	4 (17.4)	4 (28.6)	3 (27.3)	17 (58.6)	28 (36.4)
	A/**A**	0 (0.0)	0 (0.0)	0 (0.0)	5 (17.2)	5 (6.5)
Total[d]		23 (100.0)	14 (100.0)	11 (100.0)	29 (100.0)	77 (100.0)

[a]APC resistance was determined according to sample/control normalized APC-sensitive ratio (nAPC-SR); nAPC-SR < 0.9 were considered APC-resistant.
[b]A total of 200 patients and 200 controls were included.
[c]Numbers in parentheses indicate percent of patients within each category.
[d]Total number of subjects within each category.

TABLE V. Associated Risk Factors Among Factor V Leiden Carriers*

Risk factor	Status	Controls	Patients			P[b]
		None[a]	Early[a]	Late[a]	Early–Late[a]	
Age (years)	<20	2/14	0/15	1/3	1/6	0.568
	21–25	0/61	4/24	3/13	6/16	
	26–30	2/47	3/21	1/11	8/20	
	31–35	5/55	4/29	3/12	11/19	
	36–40	2/23	0/7	0/2	2/8	
	>40	0/0	0/1	0/0	1/3	
Smoking	Yes	0/9	0/2	0/1	1/6	0.789
Oral contraceptives	Yes	2/46	2/13	4/16	3/22	0.089
	No	9/154	9/74	4/25	26/50	0.002
Obesity[c]	Normal	7/109	4/45	6/23	12/29	0.361
	Overweight	4/74	4/29	1/14	12/28	
	Obese	0/17	3/13	1/3	5/15	

*Includes both heterozygous (G/A) and homozygous (A/A) carriers.
[a]Indicate number of FV Leiden carriers/number of subjects in specific group.
[b]Determined by Pearson's chi-square test.
[c]Body weight determined according to body mass index (BMI): normal, ≤25; overweight, 25–30; obese, >30.

While APCR due to FV Leiden was significantly higher among patients (14.5% vs. 2.0%), APCR without FV Leiden was seen in 12.5% of patients compared with 9.5% of controls (Table IV).

Associated Risk Factors Among FV Leiden Carriers

FV Leiden carriers were subdivided according to age categories, smokers, OC users, and obesity. A high prevalence of FV Leiden was seen in all stages of pregnancy losses and was most pronounced in the 21–35 years of age categories (Table V). Similarly, a higher prevalence of FV Leiden was seen in overweight and obese patients in late and combined early–late pregnancy losses (Table V). Use of oral contraceptives was an added risk factor to FV Leiden in late pregnancy (25.0%), moreso than with early–late (13.63%) or with early pregnancy loss (15.38%), as compared to controls (4.35%) (Table V).

Risk Factors for Pregnancy Losses in FV Leiden Carriers

Predictors of early, late, and early–late abortions were determined by performing three stepwise logistic regression models with the independent variables considered being age, smoking, obesity, OC, FV Leiden, and PRT G20210A (Table VI). Factor V Leiden was the only selected variable associated with having an early abortion, where the odds of having early abortion was 2 times greater among those who have FV Leiden as compared to those who do not (OR = 2.2, 95% CI 1.0–5.5). Oral contraceptive (OC) use and FV Leiden were significantly associated with increased odds of having a late abortion; adjusting for OC use, the odds of having a late abortion was 4 times higher for those with factor V as compared to those without factor V. The variables selected for the third model (the dependent variable being combined abortions as compared to no abortions) were smoking, OC, obesity, and factor V. The odds of having combined abortions was higher for smokers and OC users and increased with increase in BMI. Adjusting for smoking, obesity, and OC, the odds of having combined abortion was 12 times higher for those with factor V as compared to those with no factor V.

TABLE VI. Regression Analysis

Variable	Stage of pregnancy loss		
	Early	Late	Early + late
Smoking	—	—	3.4 (1.0–10.8)
Oral contraceptives	—	2.1 (1.0–4.5)[a]	2.5 (1.2–5.0)
Obesity	—		
Normal	—	—	1
Over-weight	—	—	1.4 (0.7–2.9)
Obese	—	—	4.5 (1.8–11.3)
Factor V Leiden	−2.5 (1.0–5.9)	3.4 (1.2–9.4)	16.5 (7.2–38.0)
APCR	—	2.4 (1.0–5.7)	—

[a]OR (95% CI).

DISCUSSION

Insofar as thrombosis was implicated with recurrent idiopathic miscarriages and later pregnancy com-

plications [25–29], we investigated the association of FV Leiden and PRT G20210A mutations, together with APC resistance, in women with pregnancy loss where routine investigations could not pinpoint an exact cause. Patients and controls were matched for a number of factors, including age, smoking, and use of oral contraceptives, and it was clear that patients with recurrent idiopathic miscarriages had a higher prevalence of hereditary thrombophilia (FV Leiden but not PRT G20210A) than did control parous women. Of significance was the finding that the frequency of congenital (due to FV Leiden) and acquired APCR was significantly higher among women with late miscarriage compared to controls.

Pregnancy losses were divided into early (5–12 weeks), late (13–30 weeks), and combined early–late. A progressive increase in risk of pregnancy failure was seen in FV Leiden carriers (compared with 5.5% carrier rate for controls) in early (12.64%), late (19.51%), and combined early–late (40.28%) pregnancy losses. This was in accordance with earlier studies [4,26,28,30], which reported on the association of FV Leiden with recurrent pregnancy loss moreso with late than for first trimester abortions. Others failed to demonstrate any link between FV Leiden and recurrent miscarriages [21,31–33], possibly due to limited numbers of subjects [21,33], to bias in patient selection [32], or to ethnic heterogeneity among the patients [31,33].

APC resistance develops during normal pregnancy [34,35] and is enhanced in women who are APC resistant prior to pregnancy. Results from the current study demonstrated that APC resistance due to FV Leiden, and also independent of FV Leiden (25/74 for patients and 19/23 for controls), was significantly higher among patients with early or late pregnancy loss (54/200; 27%) compared to parous controls (23/200; 11.5%). The majority of APC resistance cases were seen in combined early–late pregnancy loss (29/72; 40.3%) as compared to early pregnancy loss (14/87; 16.09%). This was reminiscent of an earlier study in which APCR was similar among women with early miscarriage compared with controls [26] and necessitates re-evaluation of the role of APCR in pregnancy loss in terms of stage of abortion.

Both FV Leiden and APCR were major risk factors for late more than early pregnancy losses, in agreement with previous reports that showed no difference in the prevalence of APCR between early aborters and controls [25,26,28,36]. Whereas late pregnancy loss due to APCR was reported here and elsewhere [37,38], others failed to establish a link with abortion [39]. Previous studies on the involvement of a particular SNP or prothrombotic anomaly with obstetric complication (preeclampsia, recurrent idiopathic pregnancy loss, or intrauterine growth restriction) were based largely on comparing the prevalence of a single SNP between women with normal pregnancies and those with recurrent pregnancy failures. Insofar as the presence of these SNPs is found among otherwise healthy parous women, not all women who carry a prothrombotic SNP will suffer a pregnancy loss. This prompts the conclusion that it is the concerted participation of multiple prothrombotic defects that places women at greatest risk.

The cause of the APCR without FV Leiden remains speculative at this stage and may potentially result from inherited and acquired factors. While it may be possible, at least in principle, that this was due to other factor V mutations including R306Q/Cambridge [40] and the HR2 haplotype [41], their very low prevalences argue against a significant role (if any) for these factor V polymorphisms in the patient population studied here. Other candidates for APCR independent of FV Leiden may include pregnancy [34], high concentrations of other coagulation factors [42,43], OC use [44], and anti-phospholipid antibodies (aPL) [45]. In our study, OC use and aPL do not appear to play a major role in APCR, because there was no difference in the prevalence of women taking the contraceptive pill in the patient (54/200) and control (46/200) groups ($P = 0.641$); in a previous communication, we found no association between aPL and APCR among this miscarriage population [46].

The frequency of the FV Leiden allele among control subjects was higher than that reported for neighboring Algeria [47] or Morocco [48] where FV Leiden is virtually absent, which may be explained by the admixture of Berbers (native Tunisians) with ancient [49] and modern ethnicities. APC resistance caused by the FV Leiden mutation is a strong risk factor for venous thrombosis. Our study shows that a reduced sensitivity for APC in the absence of the FV Leiden mutation is also associated with an increased risk of venous thrombosis Because APCR is associated not only with recurrent pregnancy loss but also with maternal thrombotic events [50], we recommend screening of women who plan pregnancy for the existence of APC resistance, as suggested also elsewhere [51].

REFERENCES

1. Sugimura M, Kobayashi T, Kanayama N, Terao T. Detection of decreased response to activated protein C during pregnancy by an endogenous thrombin potential-based assay. Semin Thromb Hemost 1999;25:497–502.
2. Vodnik T, Ignjatovic S, Majkic-Singh N. Changes in the plasma levels of protein C system parameters in pregnancy. Scand J Clin Lab Invest 2003;63:481–488.
3. Alfirevic Z, Roberts D, Martlew V. How strong is the association between maternal thrombophilia and adverse pregnancy outcome? A systematic review. Eur J Obstet Gynecol Reprod Biol 2002;101:6–14.

4. Finan RR, Tamim H, Ameen G, Sharida HE, Rashid M, Almawi WY. Prevalence of factor V G1691A and prothrombin G20210A gene mutations in a recurrent miscarriage population. Am J Hematol 2002;71;300–305.
5. Stone SE, Morris TA. Pulmonary embolism and pregnancy. Crit Care Clin 2004;20:661–677.
6. Samuelsson E, Hagg S. Incidence of venous thromboembolism in young Swedish women and possibly preventable cases among combined oral contraceptive users. Acta Obstet Gynecol Scand 2004;83:674–681.
7. Thorneycroft IH, Goldzieher JW. Venous thromboembolism. A review. J Reprod Med 2003;48:911–920.
8. Stein PD, Hull RD, Kayali F, et al. Venous thromboembolism in pregnancy: 21-year trends. Am J Med 2004;117:121–125.
9. Martinelli I, Taioli E, Cetin I, et al. Mutations in coagulation factors in women with unexplained late fetal loss. N Engl J Med 2000;343:1015–1018.
10. Isotalo PA, Wells GA, Donnelly JG. Neonatal and fetal methylenetetrahydrofolate reductase genetic polymorphisms: an examination of C677T and A1298C mutations. Am J Hum Genet 2000; 67:986–990.
11. Unfried G, Griesmacher A, Weismuller W, Nagele F, Huber JC, Tempfer CB. The C677T polymorphism of the methylenetetrahydrofolate reductase gene and idiopathic recurrent miscarriage. Obstet Gynecol 2002;99:614–619.
12. Lensen R, Rosendaal F, Vandenbroucke J, Bertina R. Factor V Leiden: the venous thrombotic risk in thrombophilic families. Br J Haematol 2000;110:939–945.
13. Dahlbäck B. Resistance to activated protein C as risk factor for thrombosis; molecular mechanism, laboratory investigation and clinical management. Semin Haematol 1997;34:217–234.
14. Bertina RM, Koeleman BPC, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;369:64–67.
15. 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;88:3698–3703.
16. Harvey D, Lowe GM. Factor V Leiden: association with venous thromboembolism in pregnancy and screening issues. Br J Biomed Sci 2004;61:157–164.
17. Prochazka M, Happach C, Marsal K, Dahlback B, Lindqvist PG. Factor V Leiden in pregnancies complicated by placental abruption. Br J Obstet Gynaecol 2003;110:462–466.
18. Agorastos T, Karavida A, Lambropoulos A, et al. Factor V Leiden and prothrombin G20210A mutations in pregnancies with adverse outcome. J Matern Fetal Neonatal Med 2002; 12:267–273.
19. Vefring H, Lie RT, ODegard R, Mansoor MA, Nilsen ST. Maternal and fetal variants of genetic thrombophilias and the risk of preeclampsia. Epidemiology 2004;15:317–322.
20. Verspyck E, Borg JY, Le Cam-Duchez V, et al. Thrombophilia and fetal growth restriction. Eur J Obstet Gynecol Reprod Biol 2004;113:36–40.
21. Dizon-Townson DS, Kinney S, Branch DW, Ward K. The factor V Leiden mutation is not a common cause of recurrent miscarriage. J Reprod Immunol 1997;34:217–223.
22. Pauer HU, Neesen J, Hinney B. Factor V Leiden and its relevance in patients with recurrent abortions. Am J Obstet Gynecol 1998; 178:629.
23. Alfirevic Z, Mousa HA, Martlew V, Briscoe L, Perezcasol M, Toh CH. Postnatal screening for thrombophilia in women with severe pregnancy complications. Obstet Gynecol 2001;97:753–759.
24. Hezard N, Cornillet-Lefebvre P, Gillot L, Potron G, Nguyen P. Multiplex ASA PCR for a simultaneous determination of factor V Leiden gene, GA 20210 prothrombin gene and CT 677 MTHFR gene mutations. Thromb Haemost 1998;79: 1054–1055.
25. Preston FE, Rosendaal FR, Walker ID, et al. Increased fetal loss in women with heritable thrombophilia. Lancet 1996; 348:913–915.
26. Rai R, Regan L, Chitolie A, Donald JG, Cohen H. Placental thrombosis and second trimester miscarriage in association with activated protein C resistance. Br J Obstet Gynaecol 1996;103: 842–846.
27. Younis JS, Ohel G, Brenner B, Ben-Ami M. Familial thrombophilia—the scientific rationale for thrombophylaxis in recurrent pregnancy loss? Hum Reprod 1997;12:1389–1390.
28. Brenner B, Mandel H, Lanir N, et al. Activated protein C resistance can be associated with recurrent fetal loss. Br J Haematol 1997;97:551–554.
29. Kupferminc MJ, Peri H, Zwang E, Yaron Y, Wolman I, Eldor A. High prevalence of the prothrombin gene mutation in women with 0intrauterine growth retardation, abruptio placentae and second trimester loss. Acta Obstet Gynecol Scand 2000;79:963–967.
30. Grandone E, Margaglione M, Colaizzo D, et al. Factor V Leiden, C→T MTHFR polymorphism and genetic susceptibility to preeclampsia. Thromb Haemost 1997;77:1052–1054.
31. Howard C, Ophira S, Daniel S, Rim D, Nurith R, Aida I. Prevalence of genetic markers for thrombophilia in recurrent pregnancy loss. Human Reprod 2002;6:1633–1637.
32. Pauer HU, Voigt-Tschirschwitz T, Hinney B, et al. Analyses of three common thrombophilic gene mutations in German women with recurrent abortions. Acta Obstet Gynecol Scand 2003;82: 942–947.
33. Wramsby ML, Sten-Linder M, Bremme K. Primary habitual abortions are associated with high frequency of factor V Leiden mutation. Fertil Steril 2000;74:987–991.
34. Cumming AM, Tait RC, Fildes S, Yoong A, Keeney S, Hay CR. Development of resistance to activated protein C during pregnancy. Br J Haematol 1995;90:725–727.
35. Clark P, Brennand J, Conkie JA, McCall F, Greer IA, Walker ID. Activated protein C sensitivity, protein C, protein S and coagulation in normal pregnancy. Thromb Haemost 1998;79: 1166–1170.
36. Balasch J, Reverter JC, Fabregues F, et al. First-trimester repeated abortion is not associated with activated protein C resistance. Hum Reprod 1997;12:1094–1097.
37. Younis JS, Brenner B, Ohel G, Tal J, Lanir N, Ben-Ami M. Activated protein C resistance and factor V Leiden mutation can be associated with first-as well as second-trimester recurrent pregnancy loss. Am J Reprod Immunol 2000;43:31–35.
38. Sarig G, Younis JS, Hoffman R, Lanir N, Blumenfeld Z, Brenner B. Thrombophilia is common in women with idiopathic pregnancy loss and is associated with late pregnancy wastage. Fertil Steril 2002;77:342–347.
39. Carp H, Salomon O, Seidman D, Dardik R, Rosenberg N, Inbal A. Prevalence of genetic markers for thrombophilia in recurrent pregnancy loss. Hum Reprod 2002;17:1633–1637.
40. Williamson D, Brown K, Luddington R, Baglin C, Baglin T. Factor V Cambridge: a new mutation (Arg306→Thr) associated with resistance to activated protein C. Blood 1998;91:1140–1144.
41. Bernardi F, Faioni EM, Castoldi E, et al. A factor V genetic component differing from factor V R506Q contributes to the activated protein C resistance phenotype. Blood 1997;90:1552–1557.
42. Laffan M, Tuddenham E. Thrombophilia: an expanding group of genetic defects that predispose to thrombosis. Mol Med Today 1997;3:303–309.
43. Kamphuisen PW, Rosendaal FR, Eikenboom JC, Bos R, Bertina RM. Factor V antigen levels and venous thrombosis: risk profile, interaction with factor V Leiden, and relation with factor VIII antigen levels. Arterioscler Thromb Vasc Biol 2000; 20: 1382–1386.
44. Olivieri O, Friso S, Manzato F, et al. Resistance to activated protein C in healthy women taking oral contraceptives. Br J Haematol 1995;91:465–470.

45. Aznar J, Villa P, Espana F, Estelles A, Grancha S, Falco C. Activated protein C resistance phenotype in patients with antiphospholipid antibodies. J Lab Clin Med 1997;130:202–208.
46. Mtiraoui N, Borgi L, Gris JC, Almawi WY, Mahjoub T. Factor V Leiden, prothrombin G20210A and antibodies against phospholipids in recurrent spontaneous abortion. J Thromb Haemost 2004; 2:1482–1484.
47. Chafa O, Reghis A, Aubert A, Fischer AM. Prevalence of the FVQ506 (factor V Leiden) mutation in the normal and thrombophilic Algerian population. Br J Haematol 1997; 97:688–689.
48. Mathonnet F, Nadifi S, Serazin-Leroy V, Dakouane M, Giudicelli Y. Absence of factor V Leiden mutation and low prothrombin G 20210 A mutation prevalence in a healthy Moroccan population. Thromb Haemost 2002;88:1073–1074.
49. Irani-Hakime N, Tamim H, Kreidy R, Almawi WY. The prevalence of factor V R506Q mutation-Leiden among apparently healthy Lebanese. Am J Hematol 2000;65:45–49.
50. Dahlback B, Zoller B, Hillarp A. Inherited resistance to activated protein C caused by presence of the FV: Q506 allele as a basis of venous thrombosis. Haemostasis 1996;26:301–314.
51. Rouse DJ, Goldenberg RL, Wenstrom KD. Antenatal screening for factor V Leiden mutation: a critical appraisal. Obstet Gynecol 1997;90:848–851.