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Factor V Leiden, Prothrombin G20210A and antibodies against phospholipids in recurrent spontaneous abortion

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Recurrent idiopathic pregnancy loss is multifactorial, involving acquired (immunologic) and/or inherited factors, such as point mutations in factor (F)V G1691A/R506Q (FV-Leiden), and prothrombin (PRT; G20210A) genes [1]. Mounting evidences point to a link between autoimmunity and idiopathic pregnancy loss [2], as specific antiphospholipid (PL) autoantibodies including anticardiolipin antibodies (ACL [3]); and lupus anticoagulants (LA; [4]), described as major risk factor for thromboembolism [4], were detected in the sera of aborting women [5].

FV Leiden/R506Q and prothrombin (PRT) G20210A are G(r)A transition at nucleotide 1691 and 20210 in the FV and PRT genes, respectively, and are significant causes of inherited thrombophilia [6,7]. The link between poor obstetrical outcome and high anti-PL levels [2] or FV-Leiden and PRT G20210A point mutations [8–10] was suggested by some [11], and disputed by others [12]. In view of the lack of data on North African population, in particular Tunisia, on the role of anti-PL antibodies and FV Leiden and PRT G20210A mutation on recurrent pregnancy loss, this study addresses their prevalence in 146 recurrent abortion women and 99 age-matched controls.

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From 2002 to 2003, 146 women (mean age 29.0 ± 6.1) with three or more unexplained consecutive pregnancy loss, and 99 healthy women (mean age 28.9 ± 5.3 years) with uncomplicated pregnancies were recruited. Exclusion criteria included induced abortions, systemic disease, uterine structural abnormalities, and personal or family history of thrombosis. Patients and controls were asked to fill a questionnaire detailing personal data, pregnancy status, and risk factors, and to sign an informed consent form. The study was conducted after all institutional ethics requirements were met.

Screening for LA was detected using dilute Russell Viper Venom Time (dRVVT), and ACL (IgG) was assayed using a standardized ELISA (Dynatech labs, Chantilly, VA, USA). Results were expressed in GPL units; levels lower than 13.30 GPL U mL^{-1} were considered normal and positive from this threshold level. Total genomic DNA was isolated from peripheral blood leukocytes by the phenol-chloroform method. FV Leiden and PRT G20210A mutation were detected by PCR-RFLP using M*nl* I [6] and H*ind* III [7] digestion for FV Leiden and PRT G20210A, respectively. In addition, both FV Leiden and PRT G20210A mutations were simultaneously analyzed by multiplex allele specific amplification (PCR-ASA). Statistical analysis was performed on SPSS v 11.5 statistics software. Mann–Whitney non-parametric *U*-test and Pearson chi-square test were used to assess intergroup significance. In addition, the odds ratios (OR) and 95% confidence intervals (CI) were calculated. Statistical significance was set at $P < 0.05$.

Both patients and control subjects had similar education backgrounds, residence, age, alcohol consumption, smoking and use of oral contraceptives. The mean number of live births/

Table 1 Factor V-Leiden mutation among antiphospholipid antibodies-positive patients

			Patients		Controls				
Factor	Status	Genotype[1]	*n*	%	*n*	%	*P*-value[2]	OR	95% CI
LA[3]	Negative	G/G	77	52.74	86	86.87	< 0.001	0.169	0.09–0.34
		G/A	10	6.85	5	5.05	0.761	1.382	0.46–3.83
		A/A	2	1.37	0	0.00	0.656	N/A	N/A
	Positive	G/G	39	26.71	7	7.07	< 0.001	4.790	1.98–10.38
		G/A	14	9.59	1	1.01	0.013	10.394	1.31–39.38
		A/A	4	2.74	0	0.00	0.251	N/A	N/A
APL[3]	Normal	G/G	85	58.22	87	87.88	< 0.001	0.192	0.10–0.39
		G/A	14	9.59	6	6.06	0.452	1.644	0.60–4.12
		A/A	4	2.74	0	0.00	0.251	N/A	N/A
	Elevated	G/G	31	21.23	6	6.06	0.002	4.178	1.62–9.53
		G/A	10	6.85	0	0.00	0.020	N/A	N/A
		A/A	2	1.37	0	0.00	0.656	N/A	N/A

[1]: G/G: wild-type factor V gene; G/A: heterozygous factor V Leiden mutation; A/A: homozygous factor V Leiden mutation.; [2]: Pearson's χ^2 test; [3]: APL assayed by ELISA; LA determined by dRVVT assay; N/A: not applicable.

woman was significantly higher among controls ($P < 0.001$), patients reporting 537 pregnancy losses episodes in the first and second trimesters. Of the subjects tested, 30 patients (20.55%) and six controls (6.06%) were positive for FV-Leiden ($P = 0.003$, OR $= 4.01$); six of the 30 FV Leiden positive patients were homozygous (A/A) and 24 were heterozygous (G/A) carriers, giving an overall allele frequency of 0.123 ± 0.038. In contrast, all of the six FV Leiden positive control subjects were heterozygous carriers, with an allele frequency of 0.03 ± 0.024. In addition, four of the 146 patients (2.74%) and four of the 99 controls (4.04%) were carriers of the PRT G20210A mutation ($P = 0.84$, OR $= 0.67$), all in the heterozygous state (G/A), with allele frequencies of 0.013 ± 0.014 and 0.02 ± 0.019 for patients and controls, respectively. While positive LA and APL were strongly associated with recurrent abortion, LA-positive cases had a higher prevalence of the G/A FV-Leiden genotype ($P = 0.013$) than LA positive controls (Table 1). Similarly, among APL IgG-positive patients, a higher prevalence of the heterozygous FV Leiden genotype was seen compared to corresponding controls (Table 1).

A clear association of FV Leiden, but not of PRT G20210A, mutation with nonearly recurrent pregnancy losses was seen among Tunisian patients, together with association between positive anti-PL antibodies and recurrent pregnancy loss, the clinical risk being increased in presence of FV Leiden. Maternal hypercoagulability leading to recurrent abortion also involves thrombophilic disorders, as confirmed here on FV Leiden. While this was in agreement with findings in which higher prevalence of FV Leiden was reported for women with primary recurrent miscarriages [9,13], others failed to demonstrate any such link [8,10,12,14], probably due the limited numbers of subjects [12,14], to bias in patient selection [10] and ethnic heterogeneity among patients [8,14].

The interrelationship between positive antibody response and FV Leiden in precipitating heightened APCR remains to be seen. In this study, a positive LA, with or without ACL, was associated with a heightened APCR, and hence higher abortion rates in second trimester. Similar rates were also seen in LA-positive women who were carriers for FV Leiden, thus questioning whether enhanced APCR due to LA or FV Leiden are associated or independent events. Whereas the link between anti-PL antibodies and APCR and pregnancy outcomes was reported [12,15], the mechanism of APCR resistance due to anti-PL independently of FV Leiden remains to be determined, and may be exacted at the level of interference with protein C activation [16], which by reducing APC levels, constitutes an additional thrombotic risk factor [16].

The high prevalence of FV Leiden among healthy Tunisians as compared to neighboring Algeria [17] or Morocco [18] where FV Leiden is virtually absent, can be explained by ethnic diversification of the Tunisians brought about by the admixture of native Berbers with ancient (Carthagian/Lebanese) and modern (Ottoman) ethnicities. This prompts the recommendation of screening for FV Leiden before pregnancy, when thinking about instituting anticoagulant therapy during pregnancy, in women with a previous unexplained late pregnancy loss or with a positive history of unexplained venous thrombosis.

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Neonatal bleeding and decreased plasma fibrinogen levels in mice modeled after the dysfibrinogen Vlissingen/Frankfurt IV

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Fibrinogen is a 340 kDa glycoprotein that consists of three pairs of polypeptide chains (Aα, Bβ, and γ)$_2$ joined by 29 intra- and interchain disulfide bonds (for review, see [1]). The polypeptide chains are encoded by three separate genes, which are expressed in hepatocytes [2–5]. The chains are synthesized separately, assembled into fibrinogen in the endoplasmic reticulum, and secreted into the circulation. Mutations in the fibrinogen genes cause afibrinogenenemia, dysfibrinogenemia or hypodysfibrinogenemia where the encoded protein is absent, present or present at lowered levels in the circulation, respectively. Dysfibrinogens are usually asymptomatic although 25% are associated with bleeding and 20% with thrombosis [6], http://www.geht.org/databaseang/fibrinogen/). The dysfibrinogen Vlissingen/Frankfurt IV (V/FIV) was identified in a family whose affected individuals experienced life-threatening thrombosis before the age of 40. These patients were heterozygous for the genotype that encodes an in-frame deletion of γ chain residues Asn319 and Asp320. Studies with purified plasma fibrinogen V/FIV, a mixture of wild-type and variant molecules, demonstrated impaired polymerization, reduced calcium-binding and reduced ADP-induced platelet aggregation [7–10]. Studies with the analogous recombinant V/FIV fibrinogen, comprised of only variant molecules, demonstrated that polymerization, calcium-binding and ADP-induced platelet aggregation were completely lost [11]. It appears counterintuitive that this variant fibrinogen with such extensive loss of clotting function is associated with thrombosis. We have suggested that the presence of heterodimers, where individual fibrinogen molecules contain one normal γ chain and one V/FIV γ chain, are a necessary component of the thrombosis in this family [10].

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