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Factor V Leiden and factor II G20210A mutations in patients with recurrent abortion

S.S.Souza[1], R.A.Ferriani[1,5], A.G.Pontes[3], M.A.Zago[2,4] and R.F.Franco[2,4]

[1]Sector of Human Reproduction, Department of Gynecology and Obstetrics and [2]Department of Clinical Medicine, Faculty of Medicine of Ribeirão Preto, 14049-900 Ribeirão Preto, SP, [3]Department of Gynecology and Obstetrics, Faculty of Medicine of Botucatu, SP, and [4]Blood Center of Ribeirão Preto (FUNDHERP), SP, Brazil

[5]To whom correspondence should be addressed. E-mail: raferria@fmrp.usp.br

Recurrent abortion (RA) represents an intriguing problem in obstetric practice in which genetic and acquired factors may play a role. In the present investigation we sought to assess the possibility that inherited thrombophilia might determine the risk of RA. We therefore investigated the prevalence of two genetic abnormalities frequently associated with venous thrombosis [factor V Leiden (FVL) and factor II G20210A] in 56 patients with primary or secondary abortion and in 384 healthy control women. Polymerase chain reaction amplification followed by digestion with the restriction enzymes *Mnl*I and *Hind*III was used to define the *FVL* and *FII G20210A* genotypes respectively. *FVL* was found in 4/56 patients (7.1%) and in 6/384 controls (1.6%), yielding an odds ratio (OR) for RA related to *FVL* of 4.9 [95% confidence interval (CI): 1.3–17.8]. *FII G20210A* was detected in 2/56 (3.6%) patients and in 4/384 (1%) controls (OR for RA: 3.5, CI: 0.6–19.7). In conclusion, *FVL* and *FII G20210A* mutations in patients with RA were more prevalent in comparison with controls. These data support a role for both mutations as determinants of the risk of RA and strengthen the notion that thrombophilia plays a role in this clinical entity.

Key words: factor V Leiden/factor II G20210A/recurrent abortion/risk factor/thrombophilia

Introduction

Recurrent abortion (RA), classically defined as three or more spontaneous fetal losses before the 20th week of pregnancy (Stirrat, 1992), is an intriguing problem in obstetric practice. Several aetiological factors and therapeutic regimens have been proposed. However, little evidence for a single causal factor or an effective treatment is currently available.

During pregnancy, changes in blood coagulation may play a role in the occurrence of abortion, since haemostatic disorders may result in obstruction of placental bed vessels (Stirling *et al*., 1984). Hence, women with thrombophilia, i.e. presenting an increased trend, usually genetic, to thrombosis, may theoretically be at higher risk for fetal loss.

Several inherited abnormalities, especially of the coagulation system, are closely associated with increased predisposition to thrombophilia. For instance, genetic deficiencies of the coagulation inhibitors antithrombin (AT), protein C (PC) and protein S (PS) are rare but well-established risk factors for thrombophilia (Seligsohn and Zivelin, 1997). In 1993 a new abnormality involved in the aetiology of thrombophilia was reported: it is characterized by resistance of the coagulation factor V to neutralization mediated by PC (a phenotype known as activated protein C resistance, APCR) (Dählback *et al*., 1993). In most cases, the genetic basis of APCR is a point mutation in the coagulation factor V, a G to A transition at nucleotide position 1691, which results in the $Arg^{506}\rightarrow$Gln amino acid substitution (Bertina *et al*., 1994). This mutated factor V, factor V Leiden (FVL), is associated with a hypercoagulable state and increased susceptibility for venous thrombosis (Bertina *et al*., 1994).

In 1996 an additional factor involved in the aetiology of thrombophilia was reported: a G to A transition at nucleotide position 20210, in the 3′-untranslated region of the coagulation factor II (prothrombin) gene (*FII G20210A*). *FII G20210A* is associated with higher plasma prothrombin concentrations, augmented thrombin generation and increased risk of venous and (possibly) arterial thrombotic disease (Poort *et al*., 1996; Franco *et al*., 1999a). In contrast to the rarity of the genetic defects in the AT, PC and PS, *FVL* and *FII G20210A* are prevalent in several Caucasian populations, and are considered to be the two most common genetic defects involved in the aetiology of thrombophilia (Mandel *el al*., 1996; Poort *et al*., 1996; Franco *et al*., 1998).

To verify whether inherited thrombophilia may determine the risk of RA, we evaluated the prevalence of *FVL* and *FII G20210A* in a sample of 56 patients with RA and in 384 healthy control women.

Materials and methods

We studied 56 consecutive patients (mean age 29.6 years, range 19–39) with a history of three or more fetal losses admitted for investigation at the Infertility Outpatient Clinics of the University Hospital of Ribeirão Preto, University of São Paulo, Brazil, between January 1995 and December 1998. Included were 46 patients with primary abortion and 10 patients with secondary abortion (the latter having had a successful pregnancy before the sequence of abortions), with defined or undefined causes. None of the patients enrolled had a history of vascular disease. The control group consisted of 384 healthy women from the general population (mean age 24.3 years,

Table I. Factor V Leiden (*FVL*) mutation in patients with recurrent abortion (RA) and in controls

	Patients	Controls (n = 384)	OR (CI)
RA (n = 56)			
Non-carrier	52 (92.9)	378 (98.4)	1.0[a]
Carrier	4 (7.1)	6 (1.6)	4.9 (1.3–17.7)
Primary RA (n = 46)			
Non-carrier	42 (91.3)	378 (98.4)	1.0[a]
Carrier	4 (8.7)	6 (1.6)	6.0 (1.6–22.1)

[a]Reference category (OR = 1.0).
'Non-carrier' refers to the wild-type genotype; 'carrier' refers to the presence of the *FVL* mutation.
Values in parentheses are percentages.
OR = odds ratio; CI = 95% confidence interval.

Table II. *FII G20210A* mutation in patients with recurrent abortion (RA) and in controls

	Patients	Controls (n = 384)	OR (CI)
RA (n = 56)			
Non-carrier	54 (96.4)	380 (98.9)	1.0[a]
Carrier	2 (3.6)	4 (1.1)	3.5 (0.6–19.7)
Primary RA (n = 46)			
Non-carrier	44 (95.6)	380 (98.9)	1.0[a]
Carrier	2 (4.3)	4 (1.1)	4.3 (0.8–24.2)

[a]Reference category (OR = 1.0).
'Non-carrier' refers to the wild-type genotype; 'carrier' refers to the presence of the *FII G20210A* mutation (only heterozygotes were observed).
Values in parentheses are percentages.
OR = odds ratio; CI = 95% confidence interval.

range 15–52) with no history of vascular disease, recruited at the local Blood Centre, in the same geographical area.

Peripheral blood was collected into tubes containing EDTA and genomic DNA was extracted by standard methods (Miller *et al*., 1988). Genomic DNA was amplified by polymerase chain reaction (PCR) using primers previously reported (Bertina *et al*., 1994; Poort *et al*., 1996). Restriction digestion with *Mnl*I and *Hind*III enzymes was employed to determine the *FVL* and *FII G20210A* genotypes respectively (Bertina *et al*., 1994; Poort *et al*., 1996).

Odds ratios (OR) as a measure of the relative risk of RA and 95% confidence intervals (CI) were calculated by standard methods (Woolf, 1955).

Results

The results for the analysis of the prevalence of the *FVL* mutation in patients and controls are given in Table I. The mutation was detected in four (all heterozygotes) out of 56 patients (carrier frequency, 7.1%; allele frequency, 0.036) and in six (all heterozygotes) out of 384 controls (carrier frequency, 1.6%; allele frequency, 0.008). These data yielded an OR for RA related to *FVL* of 4.9 (1.3–17.7). All *FVL* carriers in the patient group had had a diagnosis of primary RA, yielding an OR for primary RA of 6.0 (CI: 1.6–22.1).

Table II summarizes the results for the analysis of *FII G20210A* in patients with RA and controls. *FII G20210A* was found in two (heterozygotes) out of 56 patients (carrier frequency 3.6%; allele frequency 0.017) and in four (all heterozygotes) out of 384 controls (carrier frequency 1%; allele frequency 0.005). These data resulted in an OR for AR related to *FII G20210A* of 3.5 (CI: 0.6–19.7). Similarly to the *FVL* observations, the two heterozygotes in the patient group had had a diagnosis of primary abortion, yielding an OR for primary RA of 4.3 (CI: 0.8–24.2).

Table III. General characteristics of patients with recurrent abortion: relation with *FVL* and *FII G20210A* genotypes

	Patients (n = 56)	*FVL* carriers (n = 4)	*FII G20210A* carriers (n = 2)
Age (years; mean)	29.6	28.7	24.5
Type of abortion			
Primary	46	5	2
Secondary	10	0	0
No. of abortions			
3	25	2	1
4	19	1	1
>4	12	1	0
Defined causes[a]			
Anatomical	17	2	0
Hormonal	6	0	1
Chromosomal	5	0	0
Autoimmune	2	1	0
No apparent cause	28	1	1

[a]A patient may present with more than one cause.

Table III lists general characteristics of the patients investigated, taking into account the *FVL* and *FII G20210A* carriership status. In three out of four *FVL* carrier patients, an additional acquired factor for RA was also identified (endometriosis, synechiae and anticardiolipin antibody positive), whereas in one *FVL* carrier patient no additional apparent cause for the fetal losses was detected. One of the two *FII G20210A* heterozygous patients also had a hormonal cause for RA (luteal insufficiency), whereas in the other heterozygous patient a cause for RA was not found.

Discussion

It has been suggested that genetic thrombophilia may contribute to the occurrence of RA. In fact, the description of *FVL* as a genetic factor involved in the aetiology of thrombosis has stimulated the investigation of this genetic abnormality as a risk factor for RA. Several studies have been reported, some of them supporting this hypothesis (Preston *et al*., 1996; Rai *et al*., 1996; Brenner *et al*., 1997; Grandone *et al*., 1997; Younis *et al*., 1997; Ridker *et al*., 1998) and others detecting no association (Balasch *et al*., 1997; Dizon-Townson *et al*., 1997; Pauer *et al*., 1998). The issue therefore remains unclear, and the differences between the reports may reflect differences in the selection of patients and controls, and a differential contribution of this genetic abnormality determining the occurrence of RA in different patient subgroups.

The *FVL* mutation was detected at a significantly higher frequency among patients with RA in comparison with control women in the present investigation. Specifically, the findings suggest that *FVL* carriership is associated with a 4.9-fold increase in the risk of RA. With respect to the investigation of *FII G20210A*, the prevalence in the patient group was also higher than in the controls (3.6 versus 1% respectively). These

data suggest that this important genetic cause of thrombophilia may also be a risk factor for RA in so far as women carrying the factor II variant allele present a 3.5-fold increase in the risk of fetal loss. Since all *FVL* and *FII G20210A* carriers in the patient group had had a diagnosis of primary abortion, the specific estimated risk for primary RA was 6.0 (CI: 1.6–22.1) for *FVL* and 4.3 (CI: 0.8–24.2) for the *FII G20210A* mutation. The estimated risks were calculated on the basis of small numbers of carriers in the patient and control groups, and therefore the 95% CI were wide and in the case of *FII G20210A* included unity. Hence, these data require confirmation in large, especially prospective, studies. On the other hand, it should be noted that the prevalence data on both mutations in the control group agree well with previous studies conducted in our laboratory to verify the frequency of *FVL* and *FII G20210A* in the Brazilian population (Franco *et al*., 1998, 1999b). Therefore, the frequencies in patients with RA (7.1% for *FVL* and 3.6% for *FII G20210A*) are notably higher than those usually found in the Brazilian general population.

In the present study, patients with RA included some without an apparent cause and some with a cause that might explain the abortions (Table III). Considering RA to be a multifactorial disease, the exclusion of patients due to the identification of a single cause does not rule out the possibility that these patients have other factors contributing to the genesis of abortion. We found that 10.7% (6/56) of patients with RA carried a genetic thrombophilic factor, i.e. four of them carried *FVL* and two carried *FII G20210A*. In four of these carriers, an additional cause was previously identified (Table III).

To the best of our knowledge, the present study represents the first investigation of genetic factors related to thrombophilia as potential risk factors for RA in the Brazilian population. Our data support the hypothesis that both *FVL* and *FII G20210A* are genetic factors that increase susceptibility to fetal loss and add further to the notion that thrombophilia plays a role in the pathophysiology of RA. This information contributes to the understanding of RA as a multifactorial disease in which genetic and acquired factors may coexist, favouring the occurrence of the clinical event. Finally, if the present findings are confirmed in larger studies, discussions concerning screening for *FVL* and *FII G20210A* in patients with RA and the use of anticoagulation strategies in the management of this clinical entity in carriers of the two mutations will be encouraged.

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