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Tyr204Phe and Val34Leu polymorphisms in two Brazilian ethnic groups and in patients with recurrent miscarriages

This study investigated the prevalence of Tyr204Phe and Val34Leu polymorphisms in two Brazilian ethnic groups (171 Caucasians and 27 Blacks, and 117 men and 81 women) and in patients with recurrent miscarriages (RM) (86 women: 53 Caucasians and 33 Blacks). Study groups were matched to control groups by race and age. The prevalence of these polymorphisms did not differ between patients with RM and controls or between Caucasian and blacks, suggesting that these polymorphisms cannot be considered a risk factor for RM. (Fertil Steril® 2004;82:1455–7. ©2004 by American Society for Reproductive Medicine.)

Blood coagulation factor XIII (FXIII) is a transglutaminase that plays an important role in the final stage of blood clotting, where it catalyses the formation of covalent bonds between fibrin monomers to produce clot stabilization and resistance to fibrinolysis (1). Inherited homozygous FXIII deficiency results in serious bleeding and a high risk of miscarriage in deficient females (2).

Polymorphic sites related to FXIII activity have been described in the gene for the A-subunit of FXIII, including an A→T substitution in codon 204 of exon 5, which codes for a Tyr→Phe (FXIII Tyr204Phe), and a G→T point mutation in codon 34 of exon 2, which codes for a Val→Leu (FXIII Val34Leu) (3).

The allele Leu34 has been associated with a protective effect on myocardial infarction and deep vein thrombosis, and these results were corroborated by studies performed in the Brazilian population (3–6). These polymorphisms were previously determined in patients with recurrent miscarriages (RM), and the allele Phe204 was more prevalent in these patients (7).

Considering the importance of determining risk factors for RM, the aim of this study was to investigate the prevalence of Tyr204Phe and Val34Leu polymorphisms in Brazilian patients with RM.

The women with RM were recruited from those who attended at the Recurrent Miscarriage Outpatient Clinic in the Department of Gynecology of the Faculty of Medical Sciences at UNICAMP. The case-control study lasted from January 1999 to August 2000 and involved 86 women (53 whites and 33 blacks) with RM. Their median age was 30.4 years (range, 19–40 years). Recurrent miscarriage was diagnosed when women had three or more RMs accompanied by vaginal elimination of a fetus weighing less than 0.5 kg, with or without vital signs, and/or a gestational age under 20 weeks.

Thrombophilia was previously investigated in these women by the determination of protein C, protein S, antithrombin, factor V:Q^{506}, G20210A mutation in the prothrombin gene, and C677T in the methylene tetrahydrofolate reductase (MTHFR) gene. Only those patients homozygous for the C677T mutation were considered thrombophilic carriers for this risk factor. Antiphospholipid syndrome was investigated by detection of lupic anticoagulant (Russell viper venom and caulim clotting time) and IgM and IgG anticardiolipin antibodies.

The control group was matched to the patients with RM by race and age. The subjects were attended at the Outpatient Clinic of Family Planning in the Department of Gynecology of the Faculty of Medical Sciences, UNICAMP. The criteria for inclusion were one or more successful pregnancies in which the child weighed more than 2.5 kg and did not die in the first 28 days of life. The exclusion criteria included a history of unknown fetal deaths, thromboembolic diseases, hypertension or problems during past pregnancies, and low birth weight.

The ethnic group consisted of 171 Caucasians and 27 Blacks, and 117 men and 81 women, with a median age of 32 years (range, 20–49 years). All participants were workers at the Hematology Hemotherapy Center of UNICAMP. All subjects consented to participate in the study, which was approved by the Ethics Committee of the Faculty of Medicine at UNICAMP.

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TABLE 1

Factor XIII Tyr204Phe and Val34Leu genotypes in patients with recurrent miscarriage (RM) and controls.

	Tyr204Phe			Val34Leu		
Case	A/A	A/T	T/T	G/G	G/T	T/T
Women with RM (n = 86)	84 (97.7)	2 (2.3)	0	53 (61.6)	30 (34.9)	3 (3.5)
Healthy women (n = 86)	83 (96.5)	3 (3.5)	0	55 (63.9)	27 (31.4)	4 (4.65)

Note: Percentages are in parentheses.

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Blood samples were obtained between 6 months and 2 years after the last childbirth. Venous blood was collected into EDTA-containing tubes after an overnight (12 hours) fast, and genomic DNA was extracted from peripheral leukocytes by the chloroform/phenol method.

FXIII VAL34LEU

Polymerase chain reaction (PCR) was performed as described elsewhere, and the products were subjected to digestion with Mse I (4).

FXIII TYR204PHE

Polymerase chain reaction was performed as previously reported (2), and the products were denatured and subjected to nonradioactive single-strand conformational polymorphism (SSCP). The sequences of the PCR products that revealed different mobility shifts in SSCP were determined by direct sequencing.

The prevalence of the alleles and genotypes among the patients, controls, and ethnic groups were calculated and compared by the χ^2 test or Fisher's exact test for small samples with the values predicted for an assumed Hardy-Weinberg equilibrium. $P \leq .05$ was considered statistically significant. The odds ratio (OR) was used as a measure of the risk of RM in association with the polymorphism genotypes for Val34Leu and Tyr204Phe. The variability in sampling associated with the estimated OR was assessed by two-sided 95% confidence intervals (CI), which were calculated by the Cornfield method. An OR (95% CI) >1 was considered significant.

Distributions of genotypes of the Tyr204Phe and Val34Leu polymorphisms in patients and controls are shown in Table 1. The prevalence of the FXIII Tyr204Phe genotype did not differ between the patients with RM and controls ($P > .05$). No homozygosity was found for the T allele at codon 204.

Factor XIII Val34Val (G/G) was more common in the healthy women group, but this difference was not statistically significant ($P > .05$). As the patients and controls were previously studied for hereditary thrombophilia and antiphospholipid syndrome, we analyzed the prevalence of the polymorphisms in FXIII after excluding the women with this diagnosis (Table 2). Even after the exclusion of 23 women with RM and 13 controls with a diagnosis of thrombophilia, there was no difference in the prevalence of the polymorphisms between patients and controls.

Of the 23 women with RM with thrombophilia, 12 were homozygous for the MTHFR mutation and 50% were heterozygous for the Val34Leu polymorphism. However, statistically this prevalence was not different from patients with RM without the MTHFR mutation. The prevalence of FXIII polymorphisms between men and women of Black and Caucasian origin was not different statistically.

Miscarriages probably occur because of acquired and/or genetic factors. A number of contributing factors are now well established, including corpus luteum insufficiency, chromosomal abnormalities, anatomical uterine defects, diabetes, thyroid dysfunction, arterial hypertension, and immunological alterations. An increasing number of studies have also addressed the role of hemostasis disorders in miscarriages.

Recently, Anwar et al. (7) showed that heterozygosity for Phe at residue 204 was significantly more common in a group of 35 women with RM (14.3% in patients vs. 2.3% in controls). Although the Phe 204 polymorphism gives rise to almost normal FXIII specific activity when assaying by fibrin cross-linking, these investigators suggested that FXIII could cross-link substrates other than fibrin during its role in the maintenance of pregnancy. Indeed, three-dimensional analysis showed an alteration in the final FXIII structure in the presence of the Phe 204 polymorphism. This variation is very rare and was found in one out of 64 healthy patients in the Caucasian population (1.66%) (2).

Our data demonstrated that FXIII Tyr204Phe was a rare polymorphism in the normal Brazilian population: of 196 individuals of

TABLE 2

Factor XIII Val34Leu and factor XIII Tyr204Phe genotypes in patients and controls, excluding those with acquired or hereditary thrombophilia.

	Tyr204Phe			Val34Leu		
Case	A/A	A/T	T/T	G/G	G/T	T/T
Women with recurrent miscarriage (n = 64)	62 (96.9)	2 (3.1)	0	39 (60.93)	22 (34.37)	3 (4.68)
Healthy women (n = 75)	73 (97.33)	2 (2.66)	0	46 (61.33)	24 (32)	5 (6.66)

Note: Percentages are in parentheses.

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two ethnic groups, only two white men were heterozygous for this mutation. We did not find a higher prevalence of this polymorphism in patients with RM when compared with controls. It is relevant to consider the number of individuals studied, including patients and controls.

Possible factors that could account for the discrepancies between our results and those of Anwar et al. (7) include the criteria for patient selection. Although the Brazilian population has a high ethnic diversity, with a large proportion of Blacks, it is unlikely that this could have influenced the results since the controls were matched to the patients by race.

To avoid confounding factors associated with RM, we excluded women with acquired or hereditary thrombophilia in the group who suffered RM and in the control group to verify any difference between the prevalence of FXIII polymorphisms in each group (Table 2). As expected, the number of individuals excluded was higher in patients with RM, but there was no difference between the prevalence of the polymorphisms in the groups after the exclusion.

Our results indicate that despite the initial promise of polymorphisms as a risk factor for diagnosing RM, further studies are required to assess its true usefulness. We found no difference in the prevalence of the Val34Leu polymorphism between patients with RM and healthy women, although it was more common in the controls. In this respect, our results corroborate those of Anwar et al. (7) and Dossenbach-Glaninger et al. (8). We also investigated whether the compound carrier status of FXIII polymorphisms and a thrombophilia diagnosis could increase the risk of early pregnancy loss, but we observed no statistically relevant association.

The search for genetic abnormalities that lead to an increased risk of RM is interesting, but on the basis of this heterogeneous distribution, caution is necessary before making any statements on the relationship between a polymorphism and a disease.

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