# The progesterone receptor gene polymorphism, PROGINS, may be a factor related to the development of uterine fibroids

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**Objective:** To assess the possible association between the polymorphic allele of the progesterone receptor gene, named PROGINS, and uterine leiomyomas.
**Design:** Case–control study.
**Setting:** Department of Gynecology. Teaching hospital.
**Patient(s):** One hundred twenty-two premenopausal women with fibroids and 125 postmenopausal controls not presenting the disease.
**Intervention(s):** The subjects were classified as White or non-White (Black and Mulatto) and the progesterone receptor genotyping was performed, with DNA extracted from uterus in cases and from peripheric blood in controls and submitted to polymerase chain reaction (PCR) and agarose gel electrophoresis.
**Main Outcome Measure(s):** The presence of the PROGINS allele was recorded, and its frequency as well as the genotypic distribution among cases and controls were compared according to race.
**Result(s):** PROGINS-positive genotypes (heterozygous or mutant homozygous) were found in 19% of White and 11% of non-White women, and allelic frequency of PROGINS in the groups was 10.4% and 6.2%, respectively. Comparing patients and controls, we observed a significant difference among non-White women, both regarding presence of PROGINS-positive genotypes (4.9% vs. 25%, respectively), and PROGINS allele frequency (3.3% vs. 12.5%, respectively). There was no significant difference in PROGINS-positive genotypes among White cases and controls (16.4% vs. 20.6%, respectively), and in their allelic frequency (8.2% vs. 11.9%, respectively). The odds ratio showed reduced risk of fibroids related to PROGINS-positive genotypes in non-White women (odds ratio = 0.16, 95% confidence interval: 0.04–0.66), but not among White subjects (odds ratio = 0.76, 95% confidence interval: 0.33–1.74).
**Conclusion(s):** The PROGINS polymorphism revealed to be protective in terms of uterine fibroids in Brazilian non-White women. (Fertil Steril® 2007;87:1116–21. ©2007 by American Society for Reproductive Medicine.)

**Key Words:** Uterine fibroids, uterine leiomyoma, risk factors, sexual steroids receptors, progesterone receptor, polymorphism, PROGINS

Uterine fibroid is the most frequent benign neoplasm of the female reproductive system in the premenopause, and affects at least 30% of those aged over 30 years (1). It has a higher prevalence in Black individuals, and is more frequent among first-degree relatives in families with two or more affected members (2, 3). Although estrogen is considered a major tumor promoter, there is evidence indicating the role played by progesterone, through interaction with its cell receptors, positively modulating the mitotic activity, propagating somatic mutations, and stimulating tumor growth (4, 5). There is a single progesterone receptor (PR) gene in humans, located in the long arm of chromosome 11, in bands 22–23 (11q22–23), and it is responsible for translation of two protein isoforms, PR-A and PR-B (6).

Recently, some genotypic variations were described, highlighting the PROGINS polymorphism, which is a mutation identified in the PR gene consisting of a 306 base pairs (bp) insertion of *PV/HS-1 Alu* subfamily in intron G, between exons 7 and 8—codifying region of hormone binding

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domain—a silent mutation in exon 5 (C→T) and a single amino acid change from exon 4 (Val→Leu). Such insertion would lead to anomalous transcription and codification of an alternative exon 8, with consequent loss of hormone binding capacity and receptor activity in response to progesterone (7–9). The presence of this polymorphism was previously compared among 21 different populations, and its prevalence varied from zero in two African groups (!Kung and Nguni) to 22% in the Greek Cypriot group, with an average PROGINS allele frequency of 11% and an average heterozigosity of 18.8% (10). Hence, the presence of the PROGINS allele as a possible risk or protection factor regarding uterine leiomyomas was evaluated in Brazilian women.

## MATERIALS AND METHODS

### Patients

We carried out a case-control study, which was approved by the Institutional Review Board of the *Universidade Federal de São Paulo/Escola Paulista de Medicina* (*UNIFESP/EPM*) [Medical School]. All the participants signed an informed consent form.

The case group was composed of 122 premenopausal women with diagnosis of symptomatic uterine fibroid (confirmed by histopathology) and indication of surgical treatment, seen at a teaching hospital in the City of São Paulo/Brazil, from 2002 to 2004. The mean age was 43.9 ± 7.3 years, and the mean uterine volume on ultrasonography was 429.8 ± 293.4 $cm^3$. For the control group, 125 postmenopausal volunteers seen during the same period at the same hospital were recruited. All were clinically and ultrasonographically evaluated, ruling out the presence of fibroids. The mean age was 56.9 ± 7.4 years, with menopause occurring at 48.1 ± 5.1 years, and the mean uterine volume on ultrasound was 38.1 ± 21.7 $cm^3$. As to race, the subjects were classified as white or non-White—a group comprising Blacks and Mulattos. The term non-White refers to the African inheritance present in the composition of the Brazilian population and to the intense racial miscegenation characteristic of our country that hinders a precise differentiation among ethnic groups based on their physical features (11).

### Methods

In the case group, a 1 $cm^3$ fragment of the uterine tissue was removed during hysterectomy or myomectomy, and preserved at −80°C up to DNA extraction. To this end, a portion of the extracted tissue was placed in 100 μL of proteinase K digestion buffer, and this material was incubated at 50°C, for 12 hours. Later, proteinase K was inactivated for 15 minutes at 70°C and 500 μL of GFX kit lysis buffer (Amersham Biosciences Piscataway, NJ) was added. The lysate obtained was centrifuged at 5.4 g/4°C (Eppendorf model 5804 R) for 1 minute in a chromatographic column (silica). After two washing and centrifugation stages with ethanol-containing buffers, the DNA was diluted in 100 μL of milli-Q water, preheated at 70°C.

The purified DNA was stored at −80°C up for further use. In the control group, 5 mL of blood were withdrawn by peripheral venous puncture, using a vacutainer with anticoagulant solution (EDTA). Immediately after, the genomic DNA was extracted using the GFX kit (Amersham Biosciences) by adding 500 μL of lysis buffer to 100 μL of blood. The same procedures described above for the case group were later performed. The amount of DNA was measured by purified DNA aliquot spectrophotometry (260 nm) (Spectronic, model Genesys 5). The polymerase chain reaction (PCR) was carried out using oligonucleotides (primers) for β-globin, which was considered as positive control to verify quality of DNA and efficacy of amplification (12).

The following primers were used in the PCR of the progesterone receptor gene for amplification and detection of PROGINS—sense: 5′ GGC AGA AAG CAA AAT AAA AAG A 3′ and antisense: 5′ AAA GTA TTT TCT TGC TAA ATG TC 3′ (8, 13). Two microliters (100 ng) of total DNA were used in a final 25 μL reaction volume containing 0.4 pmol/μL of each primer and 22 μL of mix Promega (20 mM of Tris–HCl pH 8.4, 50 mM of KCL, 5 mM of $MgCl_2$, 200 μM of dNTPs, and 1.25 U of *Taq* DNA polymerase). The reactions were incubated in a thermocycler (GeneAmp PCR System 9700, Perkin Elmer, Norwalk, CT) in the following conditions: denaturation at 94°C for 5 minutes, followed by 40 cycles at 94°C for 1 minute (denaturation), 55°C for 1 minute (oligonucleotid hybridization), and 72°C for 1 minute (polymerization), finally incubated at 72°C for 5 minutes. The amplification products were applied in agarose gel 2%, stained with ethidium bromide (1 μg/mL), and submitted to electrophoresis for 20 minutes at 100 volts, in a horizontal cube containing TBE buffer 1×.

Detection was made by visualization of PCR products in agarose gel in an ultraviolet light transiluminator. They could be of two different sizes, depending on the presence of *Alu PV/HS-1* insertion of 306 bp into intron G, between exons 7 and 8 (PROGINS). If the progesterone receptor gene did not present the insertion, a 149-bp fragment would be obtained, representing the wild-type allele (T1); however, if the gene had the insertion, the fragment would have 455 bp, corresponding to the mutant or polymorphic allele (T2) (8, 14).

### Statistics

The PROGINS allele frequency and its genotypic distribution were compared among White and non-White women and between the case and control groups according to race. Because there were few mutant homozygous in our series, the PROGINS-positive genotypes (heterozygous and mutant homozygous) were analyzed together in the comparison with the PROGINS-negative genotype (wild homozygous). Hence, the chi-square frequency test was applied, with a significance level of 5% ($P<.05$). The risk to the disease was estimated by odds ratio (OR), using the software SPSS, version 11.0, and the confidence interval (CI) established was 95% (95% CI). Finally, based on the found PROGINS