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# Estrogen receptor alpha polymorphism and susceptibility to uterine leiomyoma

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ABSTRACT

Uterine leiomyoma is the most frequent pelvic tumor found in female genital tract. Some studies have suggested an association between single nucleotide polymorphisms (SNPs) in estrogen receptors genes with susceptibility in developing uterine leiomyoma. In this work, we estimated the frequency of two SNPs: one located in the intron 1 (rs9322331) and other in the exon 1 (rs17847075) of the estrogen receptor α (ESR1) gene in 125 women with uterine leiomyoma and 125 healthy women. To do this we used a PCR–RFLP method with *Msp*I and *Hae*III restriction enzymes to respectively detect C/T SNPs in the intron 1 and in the exon 1 of ESR1. To our knowledge this is the first study aimed to investigate the association of ESR1 SNPs with the risk of developing uterine leiomyoma in Brazilian women. Our results showed that the allele frequencies of the exon 1 and the intron 1 of the ESR1 gene did not differ between cases and controls ($P = 0.325$ and 0.175, respectively). Furthermore, our findings provided little support for the association of these SNPs on ESR1 with leiomyoma. However, we found that the SNP in the intron 1 of the ESR1 gene was underrepresented in the Brazilian female population.



## 1. Introduction

Uterine leiomyomas, also known as fibroids or myomas are one of the most frequent solid pelvic tumors in women. It is estimated that one in four women during reproductive period will develop this kind of benign neoplasia [1,2]. Leiomyomas are the primary indication for hysterectomy, accounting for over 200,000 surgical interventions each year in the United States [3,4]. Therefore, leiomyoma is considered one of the main public health problems [5].

Estrogen has been reported to be one important risk factor for leiomyomas' development. In this regard, leiomyomas' dependency on estrogen is clearly recognized by the fact that they do not occur before menarche and can increase in size during pregnancy. Conversely, they reduce after ovariectomy, in the menopause, or during gonadotropin-releasing hormone agonist therapy [4–6]. On the other hand, leiomyomas' growth is variable in women with regular menstrual cycles and even among nodules in the same uterus [7].

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The effect of estrogen on leiomyomas' growth and development is mediated by two ligand-inducible transcription factors that regulate the target gene expression: (i) estrogen receptor α (ESR1) and (ii) estrogen receptor β (ESR2) [8,9]. Differences in receptor α and β messenger RNA (mRNA) levels have also been reported, thus, higher concentration were found in uterine leiomyomas compared with autologous myometrium [10]. By this reason, leiomyomatous tissue may be characterized by increased levels of estrogen receptor mRNA higher than that seen in normal myometrial tissue [11–13]. This feature could represent a major predisposing factor for development and growth of leiomyomas.

It has been hypothesized that single nucleotide polymorphisms (SNP) on estrogen receptor genes might correlate with leiomyomas' development. In this regard, the best-studied polymorphisms on ESR1 are two SNPs in the intron 1, commonly detected by RFLP assays with the *Pvu*II (rs2234693) and *Xba*I (rs9340799) restriction enzymes [14–17] plus a dinucleotide repeat (TA*n*) upstream of the ESR1 [18] gene. The later study found an association between TA12 and TA13 dinucleotide repeats with increased risk of leiomyoma in Asiatic women [18]. However, the same polymorphism was not correlated to a higher risk of developing leiomyoma in Italian Caucasian women [16,17]. Because of these discrepancies we therefore speculated whether this polymorphism was indeed a risk factor to developing leiomyoma or the association was biased due to the genetic background of the population. Thus, we explored these possibilities through the study of single nucleotide polymorphisms (SNP) on exon 1 and intron 1 of the ESR1 gene in a highly heterogeneous population from southeast of Brazil [19]. To do this, we evaluated ESR1 allele variants and their potential association with uterine leiomyoma. We used the *Msp*I (C to T substitution, Ser → Ser, refSNP ID: rs1784705) and *Hae*III (C to T substitution, refSNP ID: rs9322331) RFLPs that are located, respectively, in the exon 1 and the intron 1 of the estrogen receptor α gene. We used uterine biopsies from women with leiomyoma and compared the allele and genotype frequencies with healthy women.

## 2. Patients and methods

### 2.1. Patients

A case-control study was conducted in 250 eligible women where the frequencies of polymorphisms of the ESR1 gene were compared. Patients were categorized as follow: (i) 125 women with surgical and histological confirmation of leiomyoma and (ii) 125 normal control women. The 125 cases with leiomyoma (mean age 43.9 ± 7.3 years) were admitted and treated at Perola Byington Hospital in São Paulo, Brazil, from 2003 to 2004.

To provide the control-group we also included 125 women (mean age 56.9 ± 7.4 years) without any evidence of leiomyoma, according to ultrasound exam. All the participants were ethnically classified as white or non-white according to self-reported ethnicity. The main characteristics of the women included in this study are shown in Table 1.

Informed consent was obtained from all participants prior to their inclusion in the study, and the Ethics Committees of the Perola Byington Hospital and the Federal University of São Paulo approved all procedures.

### 2.2. DNA extraction

Sample pieces of the adjacent normal uterine tissue (1 $cm^3$) were collected from women with leiomyoma after hysterectomy or myomectomy. These samples were frozen in liquid $N_2$ and stored at −80 °C until use. For DNA extraction, pieces of 50 mg were incubated overnight at 50 °C in 500 μl of digestion buffer containing proteinase K (Tris–HCl, 10 mM, pH 8; EDTA, 0.25 mM, pH 8; SDS, 0.25%; NaCl, 100 mM; proteinase K, 200 μg). After incubation the enzyme was heat inactivated at 70 °C, for 15 min. Then the lysate was deposited into a column (GFX™ Genomic Blood Purification Kit, GE, Healthcare, Nova Jersey, EUA). All reactions were performed according to manufacturer's specifications.

For the control group, whole blood samples were collected in tubes containing EDTA and kept at 4 °C before extraction. Genomic DNA was isolated by using the GFX™ Genomic Blood Purification Kit (GE, Healthcare, Nova Jersey, EUA), according to manufacturer's instructions.

### 2.3. MspI genotyping

The amplifications for the exon 1 of the ESR1 gene were performed using primers previously published [20] (Table 2). Positive controls with known genotypes and negative controls (reaction mixtures without DNA templates) were also included in each reaction. PCR reactions had a final volume of 30 μl, containing 50–200 ng of genomic DNA, 10 pmol of each primer, 15 μl of PCR Mix (final concentration: 1× reaction buffer pH 8.5, 1.5 mM $MgCl_2$, 200 μM of each dNTP and 0.75 U of *Taq* DNA polymerase; Promega, Madison, USA). The

**Table 1 – Descriptive characteristics of leiomyoma and control groups**

| Variable | Category | Groups | |
|---|---|---|---|
| | | Cases ($n$ = 125) | Controls ($n$ = 125) |
| Age (years) (mean ± S.D.) | | 43.9 (±7.3) | 56.9 (±7.4) |
| UV ($cm^3$) (mean ± S.D.) | | 418.8 (±293.3) | 37.7 (±21.6) |
| Parity (mean ± S.D.) | | 3.3 (±3.6) | 2.1 (±1.8) |
| Ethnicity | White | 63 (50.4%) | 97 (77.6%) |
| | Non-white | 62 (49.6%) | 28 (22.4%) |

UV: uterine volume.