The role of MSP I CYP1A1 gene polymorphism in the development of uterine fibroids

The cytochrome P-450 1A1 (CYP1A1) gene plays an important role in the metabolism of estrogen and is therefore a candidate marker for fibroids. In a case-control study, we were unable to demonstrate any association between MSP I CYP1A1 polymorphism and the risk of leiomyoma in Brazilian women. (Fertil Steril® 2010;94:2783–5. ©2010 by American Society for Reproductive Medicine.)

**Key Words:** Leiomyoma, polymorphism, CYP1A1

Uterine leiomyomas are benign smooth-muscle tumors. Symptoms of the high-prevalence uterine leiomyoma include abnormal vaginal bleeding and pelvic pain, which are the main indications for hysterectomy in women of reproductive age (1, 2).

Earlier studies have shown the importance of sex hormones in the development and proliferation of uterine leiomyoma. Estrogen, particularly E2, is directly associated with tumor growth, and is found in higher concentrations in the leiomyoma tumor than in the normal myometrium. Also, the levels of estrogen receptors are higher on the tumor surface than on the original myometrium (3–6).

A series of enzymes are involved in estrogen synthesis and metabolism. Of these enzymes, cytochrome P-450 1A1 (CYP1A1), catechol-O-methyltransferase (COMT), and glutathione S-transferases (GSTs) play an important role in the excretion of estrogen and catechol estrogen (7). Therefore, polymorphisms in these enzymes could be involved in the risk of uterine leiomyomas (8–11).

Estrogens are metabolized to the formation of the catechol estrogens 2-hydroxy-E2 and 2-hydroxy-E1. Subsequent metabolism of catechol estrogens involves O-methylation by COMT and the conjugation of these estrogens by other phase II enzymes. The methylation product 2-methoxy-E2 exhibits an antitumorigenic effect, and its endogenous formation may protect against estrogen-induced cancers in target organs. In contrast to the potentially beneficial roles of the CYP1A1-COMT pathway, the CYP-mediated oxidation of catechol estrogens to semiquinones and quinones has been postulated to be an initiating/promoting factor in estrogen-induced diseases (12–15). Polymorphisms of this enzyme change the concentration of estrogens and their metabolites and could be important in the genesis of uterine leiomyoma.

Individuals carrying the MSP I CYP1A1 allele have increased activity in the respective enzymes’ isofoms, which could contribute to increasing levels of estrogen metabolites (16).

Our current hypothesis focuses on the fact that MSP I CYP1A1 activity could be associated with an increased clearance of E2, leading to a hypoestrogenic milieu that could be conducive to a decreased risk of leiomyoma. Thus, the MSP I CYP1A1 polymorphism would be a candidate marker for fibroids.

Despite the limited data available in Brazil, the frequency of the MSP I CYP1A1 polymorphism was higher among African-Brazilians than among European-Brazilians (adjusted odds ratio 3.19; 95% confidence interval 1.53–6.65) (17).

We carried out a case-control study which was approved by the Institutional Review Board of the Federal University of São Paulo/Escola Paulista de Medicina. Each of the participants signed an informed consent form. A total of 124 patients with a histologic diagnosis of uterine leiomyoma were selected as case subjects, and 215 patients with no evidence of leiomyoma in the ultrasound served as control subjects. Patients in the control group were required to be postmenopausal and declared free of uterine fibroids after clinical and ultrasonographic evaluations. In the control group, 27% of the patients were <50 and 73% were >50 years of age. In the group of patients with leiomyoma, 80% were <50 and 20% were >50 years of age. The participants were separated into 2 groups according to race: White and Nonwhite.

In the case group, a 1-mL fragment of uterine tissue was removed during a hysterectomy or myomectomy and preserved at −80°C until DNA extraction. To this end, a portion of the extracted tissue was placed in 100 µL of proteinase K digestion buffer and 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 (Eppendorf model 5804 R) at 4°C for 1 minute in a chromatographic column (silica). After 2 washing and centrifugation stages with ethanol-containing buffers, the DNA was diluted in 100 μL of milli-Q water, which was preheated at 70°C. The purified DNA was stored at −80°C for further use.

In the control group, 5 mL blood was withdrawn by peripheral venous puncture, using a vacutainer with anticoagulant solution (ethylene-diaminetetraacetic acid). Immediately afterward, the genomic DNA was extracted using the GFX kit (Amersham Biosciences) by adding 500 μL lysis buffer to 100 μL blood. The same procedures described above for the case group were performed again later. The amount of DNA was measured using purified DNA aliquot spectrophotometry (260 nm; Spectronic model Genesys 5). A polymerase amount of DNA was measured using purified DNA aliquot spectrophotometry (260 nm; Spectronic model Genesys 5). A polymerase

The PCR protocol of MSP I CYP1A1 included 11/4-C for 30 seconds, 64.2/4-C for 1 minute, and 72/4-C for 1 minute. The PCR product was subjected to a restriction enzyme in the TA/CG transition in 3’ antisense sequence to extract the base pairs of each allele: 1) 5’-TAG GAG TCT TGT CTC ATG CCT-3’; and 2) 5’-CAG TGA AGA GGT GTA GCC GCT-3’. For wild alleles of the MSP gene, 340 base pairs were found, 340/200/140 in the heterozygous genes and 200/140 in mutated genes. Detection was made by visualization of PCR products in an agarose gel in an ultraviolet light transilluminator.

The allele frequency and genotypic distribution were compared between the case and control groups, according to age, uterine volume, and race. The chi-squared frequency test was applied with a significance level of 5% (P<.05), and the risk of the disease was estimated by odds ratio, using the software SPSS, version 11.0 for Windows (SPSS, Chicago, IL), with the confidence interval established as 95%. Finally, based on the observed allele frequency, we compared the real genotypic distribution with the expected rates in the groups. This distribution, if in Hardy-Weinberg equilibrium, should be close to the following: wild homozygous rate = A1²/heterozygous rate = 2 × A1 × A2/mutant homozygous rate = A2². The purpose of the Hardy-Weinberg calculation is to ensure that the results obtained do not carry a sample or specimen bias, because a representative population that is studied using appropriate methods usually respects the equilibrium.

Polymorphisms of MSP I CYP1A1–positive genotypes (heterozygous and mutant homozygous) were found in 36.5% of the White group and 44.7% of the Nonwhite group. Polymorphisms of MSP I CYP1A1–positive genotypes were found in 41.2% of patients with uterine leiomyoma and in 37.9% of the control group. No significant difference between case and control subjects were observed based on genotype, nor did we find any significant differences based on age, race, or uterine volume (Table 1).

Our results are different from those found by Honna et al. (17) regarding the frequency of the MSP I CYP1A1 among African- and European-Brazilians. We believe that this discrepancy is due to the fact that populations examined in the two studies are not similar; Honna et al. (17) included patients of both genders with lung cancer in their study.

We were unable to demonstrate any association between uterine leiomyomas and the presence of MSP I CYP1A1 in the present case-control study. However, this is one of the first investigations to evaluate the existence of the relationship of leiomyomas with this polymorphism, and further studies of different populations are essential.

### TABLE 1

<table>
<thead>
<tr>
<th>Variable/category</th>
<th>MSP I CYP1A1, n (%)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>Homozygous Mutant Wild</td>
<td>P value</td>
</tr>
<tr>
<td>≤50</td>
<td>54 (33.9) 8 (5.0) 97 (61.0)</td>
<td>.788</td>
</tr>
<tr>
<td>&gt;50</td>
<td>60 (35.5) 6 (3.5) 103 (60.9)</td>
<td>.017</td>
</tr>
<tr>
<td>Race</td>
<td>Homozygous Mutant Wild</td>
<td>P value</td>
</tr>
<tr>
<td>White</td>
<td>73 (32.9) 8 (3.6) 141 (63.5)</td>
<td>.313</td>
</tr>
<tr>
<td>Nonwhite</td>
<td>41 (39.0) 6 (5.7) 58 (55.2)</td>
<td>.298</td>
</tr>
<tr>
<td>Uterine volume (mL)</td>
<td>Homozygous Mutant Wild</td>
<td>P value</td>
</tr>
<tr>
<td>≤93</td>
<td>39 (32.2) 5 (4.1) 77 (63.6)</td>
<td>.632</td>
</tr>
<tr>
<td>&gt;93</td>
<td>39 (35.1) 7 (6.3) 65 (58.6)</td>
<td>.816</td>
</tr>
<tr>
<td>Group</td>
<td>Homozygous Mutant Wild</td>
<td>P value</td>
</tr>
<tr>
<td>Control</td>
<td>74 (34.6) 7 (3.3) 133 (62.1)</td>
<td>.454</td>
</tr>
<tr>
<td>Case</td>
<td>40 (35.1) 7 (6.1) 67 (58.7)</td>
<td>.788</td>
</tr>
</tbody>
</table>


### REFERENCES


