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RESEARCH ARTICLE

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Analysis of estrogen and progesterone receptor gene polymorphisms in leiomyoma

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Background: Leiomyoma, one of the most common benign tumors, causes morbidity during the reproductive years in women. The molecular pathogenesis of the disease is not clear. Leiomyomas are hormone-sensitive tumors affecting around 20%-25% of women. Gene polymorphism studies could be important and explaining in the evaluation of multifactorial diseases such as leiomyoma. Polymorphisms involving genes responsible for the synthesis and signalization of steroid hormones could be used as genetic markers for hormone-related conditions. The purpose of this study was to analyze the effect of ERα-351 *XbaI* A/G, ERα-397 *PvuII* T/C, and progesterone receptor (PGR) PROGINS polymorphisms on the development of leiomyomas.

Material and Methods: In this study, 213 samples (103 leiomyoma patients and 110 healthy controls) participated. The ERα-351 *XbaI* A/G and ERα-397 *PvuII* T/C gene polymorphisms were analyzed using PCR-RFLP method. PGR PROGINS polymorphism was analyzed by PCR method with specific primers.

Results: The genotype distribution and allele frequency of the ERα-351 *XbaI* A/G, ERα-397 *PvuII* T/C, and PGR PROGINS polymorphisms were not statistically different between leiomyoma patient and control groups ($p > 0.05$).

Conclusion: This study reflects that ERα and PGR PROGINS polymorphisms may not be one of the many genetic factors for leiomyoma susceptibility.

KEYWORDS
estrogen, gene polymorphism, leiomyoma, PCR, progesterone receptor

1 | INTRODUCTION

Uterine leiomyomas are one of the most common gynecological conditions in women older than 30 years old, and its incidence is about 30% throughout the world. Despite their high incidence rate, pathophysiology of leiomyoma is still not well understood.[1,2] It has been proposed that genetic tendency, steroid hormone concentration, and fibrotic process which develop through the effect of growth factors, angiogenesis, and chromosome abnormalities are responsible for the development of leiomyomas.[3,4]

Leiomyomas are hormone-sensitive tumors. Estrogen and progesterone hormones and their receptors have a critical role in the formation and developmental processes of leiomyomas. Especially, estrogen plays a promoting role and is effective in leiomyoma proliferation.[5,6] Estrogen binds specific nuclear receptors, which are estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ), and exerts its effects on the target cell.[7] ERα and ERβ mRNA levels are higher in leiomyomas

compared to those in myometrium.[8] ERα is an important intermediary in the signal transduction pathway and also a member of the steroid/thyroid hormone superfamily of nuclear receptors.[9] The ERα gene located on chromosome 6q25 and contains eight exons and greater than 140 kilobases. The gene contains intron 1 polymorphisms *XbaI* and *PvuII*.[9,10] It has been reported that these polymorphisms are associated with a large number of estrogen-related diseases, such as osteoarthritis, breast cancer, and endometriosis. ERα-mutant XbaI * G and PvuII * C alleles may be responsible for increased serum estradiol (E2) production.[10] Another factor that has a critical role in the development of leiomyoma is progesterone.[8] Studies suggested that mitotic activity increased in leiomyomas in the secretory phase of cycle and progesterone levels effects leiomyoma development.[11] There are two forms of progesterone receptor: receptor-A (PGR-A) and receptor-B (PGR-B). PGR-A and PGR-B mRNAs were isolated in leiomyoma and myometrium tissues, and mRNA concentrations are higher in leiomyoma than in neighboring myometrium.[12] The PGR gene is located on chromosome 11q22-23, and several genetic variants were detected. The genetic variations can be listed in as PROGINS, rs10895068, rs561650, and rs608995. These variants are located on the hormone-binding domain encoding sequence of PROGINS gene. The PROGINS include a 306-bp Alu repeat insertion in intron G in the hormone-binding domain-encoding region of the gene.[13-15]

Gene polymorphism studies could be useful and enlightening in multifactorial conditions such as leiomyoma. Polymorphisms of genes involved in synthesis and signalization of steroid hormones could be used as genetic markers in hormone-related conditions.[16,17] The purpose of our study was to analyze the effect of ERα-351 *XbaI* A/G-397 *PvuII* T/C and PGR PROGINS polymorphisms on leiomyoma development.

2 | MATERIAL AND METHOD

2.1 | Study groups

The present study was carried out with patients who applied to Obstetrics and Gynecology Clinic of Gaziosmanpasa University. The study group consisted of 103 female patients with leiomyoma (mean age: 42.08 ± 6.32) and 110 female postmenopausal healthy subjects without leiomyoma (mean age: 53.56 ± 5.1), all of whom live in Tokat, Turkey. The patient group had 103 leiomyoma patients, and the control group had 110 healthy subjects who had taken part in another polymorphism study.[2] All patients were subjected to ultrasonography examination. Patients with leiomyomas needing surgical treatment had laparoscopic myomectomy, hysteroscopic myomectomy, vaginal myomectomy, hysterectomy, and laparotomic myomectomy depending upon the nature of leiomyoma. Materials taken from patients by surgical procedure were sent to pathological examination. Only the patients who were diagnosed with uterine leiomyoma were incorporated in the study, and patients with different diagnosis were excluded. Control group included subjects who did not have leiomyoma or any other pathology based on abdominal and transvaginal ultrasonography. The demographic characteristics of the leiomyoma patients and healthy control are given in Table 1. Blood samples were taken from the patients in the follicular phase. The study

TABLE 1 The demographic features of leiomyoma patients and control groups

Demographic feature	Leiomyoma patients, n = 103	Control groups, n = 110
Age (y)	42.08 ± 6.32	53.56 ± 5.1

TABLE 2 PCR primers, PCR programs, product sizes, and restriction enzymes for ERα-351 *XbaI* A/G, ERα-397 *PvuII* T/C, and PGR PROGINS polymorphisms

Polymorphisms	Primers	RFLP Enzyme	Product size	PCR Program	
ERα-351 *XbaI* A/G	F:5'CTGCCACCCTATCTGT ATCTTTTCCTATTCTCC-3' R-TCTTTCTCTGCCACCCTG GCGTCGATTATCTGA-3'	XbaI	1374 bp	95°C 5 min	
				95°C 50 s 60°C 50 s 72°C 50 s	30 cycles
			A: 1374 bp G: 982 bp+392 bp	72°C 5 min	
ERα-397 PvuII T/C	F:5'CTGCCACCCTATCTGT ATCTTTTCCTATTCTCC-3' R-TCTTTCTCTGCCACCCT GGCGTCGATTATCTGA-3'	Pvu II	1374 bp	95°C 5 min	
				94°C 1 min 62°C 1 min 72°C 1 min	30 cycles
			T:1374 bp C: 937 bp+437 bp	72°C 6 min	
PGR PROGINS	F: 5'-GGCAGAAAGCAAAA TAAAAAGA-3' R: 5'-AAAGTATTTTCTTG CTAAATGTC-3'	—	T1(D) 159	94°C 5 min	
			T2(I) 465	94°C 45 s 55°C 45 s 72°C 45 s	35 cycles
				72°C 3 min	

protocol was approved by the ethics committee of Gaziosmanpasa University Faculty of Medicine. All participants are allowed to use the peripheral blood sampling for this analysis and signed a written informed consent.

2.2 | Genetic analysis

Genomic DNA (gDNA) was isolated from peripheral blood using Invitrogen DNA isolation kit. The ERα-351 *XbaI* A/G and ERα-397 *PvuII* T/C gene polymorphisms were analyzed by a polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) method. PGR PROGINS polymorphism genotypes were analyzed by PCR method with specific primers. PCR was performed in a total volume of 25 μL containing 2 μL gDNA, 0.8 nmole/μL of each primer, 1.5 μL $MgCl_2$ (25 mmol/L), 2.5 μL 10× PCR buffer, 0.3 μL dNTP (25 mmol/L), and 1 Unıte Taq polymerase (Fermentas, Shenzhen, China). The PCR primers, PCR programs, product size, and restriction enzymes used are given in Table 2. The amplified products were run on a 3% agarose gel and examined under ultraviolet light.

2.3 | Statistical analysis

Statistical analyses were carried out using Open Epi Info Software Version 3.2.2 (CDC, Atlanta, GA). Our results in leiomyoma patients and control groups were compared by chi-square or Fisher's exact test. The odds ratios (ORs) and 95% confidence intervals (CIs) were calculated when chi-square or Fisher's exact test was significant. Chi-square test was used to test for quality of fitness of genotypic distributions and Hardy-Weinberg equilibrium using Arlequin Software ver. 2000 (University of Geneva, Switzerland).

TABLE 3 Genotype and allele frequencies of ERα-351 *Xba*I A/G and ERα-397 *Pvu*II T/C gene polymorphisms in leiomyoma patient and control groups

	Leiomyomas, n = 103	Controls, n = 110	*P*
ERα-351*XbaI* A/G			
A/A	25 [24%]	21 [19%]	0.5447
A/G	49 [48%]	60 [55%]	
G/G	29 [28%]	29 [26%]	
Allele frequencies			
A	99 [48%]	102 [46%]	0.364
G	107 [52%]	118 [54%]	
ERα-397 PvuII T/C			
T/T	35 [34%]	44 [40%]	0.6226
T/C	55 [53%]	55 [50%]	
C/C	13 [13%]	11 [10%]	
Allele frequencies			
T	125 [61%]	143 [65%]	0.179
C	81 [39%]	77 [35%]	

CI, confidence interval; OR, odds ratio.

3 | RESULTS

A significant difference was not found among leiomyoma patient and healthy control groups for ERα-351 *XbaI* A/G and ERα-397 *PvuII* T/C gene polymorphisms and allele distributions ($P > 0.05$). The 1374-bp PCR products were digested with XbaI restriction enzyme for ERα-351 XbaI gene polymorphism (Figure 1) and PvuII restriction enzyme for ERα-397 PvuII gene polymorphism (Figure 2). ERα-351 *XbaI** AA/AG/GG frequencies were 24/48/28% and 19/55/26% in patient and control groups, respectively. In leiomyoma patient and healthy control groups, T allele was the most common for ERα-397 *PvuII* T/C gene polymorphism. ERα-397 *PvuII** TT/TC/CC frequencies were 34/53/13% and 40/50/10% in leiomyoma patient and control groups, respectively (Table 3).

The proportions of PGR PROGINS polymorphism, and the presence of 159-bp-long T1 allele and 465-bp-long T2 allele (306 bp) were compared in patient and healthy control groups (Figure 3). Frequencies of progesterone receptor genotypes T1/T1-T1/T2-T2/T2 were 76/23/1% and 80/15/5% in leiomyoma patient and control groups, respectively. Allele frequencies of T1 and T2 were 87/13% and 87/13% in leiomyoma patient and control groups, respectively. According to our data, PGR PROGINS polymorphism was not significant for genotype or allele frequencies in study groups (Table 4).

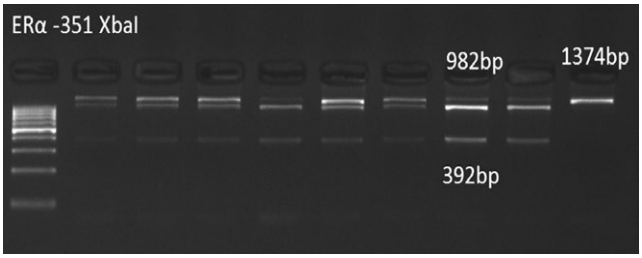

FIGURE 1 The 1374-bp PCR products were digested with XbaI restriction enzyme. Line 1 50 bp marker, Line 10 A/A, Line 2,3,4,6,7 A/G, Line 5,8,9 A/G

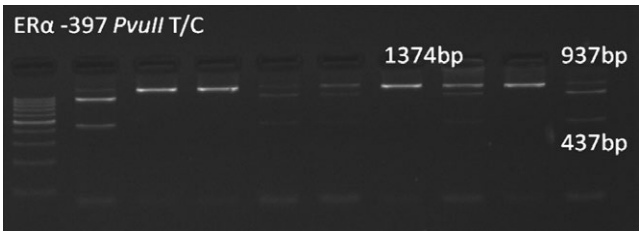

FIGURE 2 The 1374 bp PCR products were digested with PvuII restriction enzyme. Line 1 50 bp marker, Line 3,4,7,9 T/T, Line 5,6,8 T/C, Line 2, 10 C/

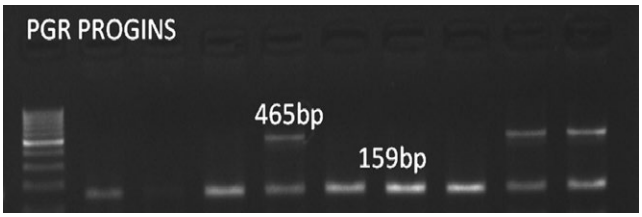

FIGURE 3 Line 1 50 bp marker, Line 2,4,6,7,8 159 bp (D), Line 5,9,10 465 bp and 159 bp (I/D)

4 | DISCUSSION

In the present study, genotype frequencies of estrogen receptor polymorphisms, ERα-351 *XbaI* A/G and ERα-397 *PvuII* T/C, and of PGR PROGINS polymorphisms, were investigated. A significant difference was not observed among leiomyoma patient and healthy control groups for ERα polymorphisms and allele distributions. Moreover, frequencies of 159-bp T1 allele (wild type) and 465-bp T2 allele (PROGINS ALU insertion) were investigated in leiomyoma patient and control groups. The genotype frequencies of PGR PROGINS polymorphism were not significantly different in the study groups.

There are some studies investigating the relationship between leiomyoma development and estrogen receptor gene polymorphisms, and the results are contradictory. Such a study found no association between leiomyoma development and estrogen receptor α-397 *PvuII* T/C gene polymorphism in Caucasians.[18] On the other hand, another study on different races showed that leiomyoma development risk increased in Black or White women carrying these ERα polymorphisms while no increase was found in Hispanic women.[19]

Massart et al[20] found different results for the association between leiomyoma development and ERα-351 *XbaI* A/G and ERα-397 *PvuII* T/C gene polymorphisms in two different studies conducted on Italian women. In the first study, showed an association in 119 patients between leiomyoma development and gene polymorphism. In the second study where the patient number was increased to 413,[21] no association was found. As can be seen in these studies, an association between receptor gene polymorphism and leiomyoma development could appear in different ethnic groups. In addition, different results could be obtained in the same society when the number of patients changed.

TABLE 4 Genotype and allele frequencies of PGR PROGINS polymorphism in leiomyoma patient and control groups

	Leiomyomas, n = 103	Controls, n = 110	*P*
PGR PROGINS			
T1/T1	78 [76%]	88 [80%]	0.0584
T1/T2	24 [23%]	16 [15%]	
T2/T2	1 [1%]	6 [5%]	
Allele frequencies			
T1	180 [87%]	192 [87%]	0.488
T2	26 [13%]	28 [13%]	

CI, confidence interval; OR, odds ratio.

ERα gene PvuII polymorphism has been assessed in different ethnic groups with conflicting results in endometriosis. Govindan et al[22] indicated that significant association of C allele with endometriosis in the Indian population. A study by Hsieh et al[10] found an association between ERα-351 *XbaI**G- and ERα-397 *PvuII**C genotypes and endometriosis or leiomyoma frequency. On the other hand, Shaik et al[23] carried out a study on ERα-397 *PvuII* T/C polymorphism and found that frequency of ERα-397 *PvuII* C/C genotype was higher in premenopausal leiomyoma patients and contributed to tumorigenesis. Based on elevated ERα and MTCYB expression levels in premenopausal leiomyoma patients and association of the condition with ERα-397 C/C genotype, investigators concluded that mitochondria-mediated estrogen could be a promoting factor for leiomyoma development.[23]

Similarly, Veronica et al[24] found an association between ERβ and PGR gene polymorphism and leiomyoma development. They proposed that ERβ −13 950 T/C and PGR +331 G/A polymorphisms could be used as markers for leiomyoma development. Besides, there are various other studies reporting associations of ER gene polymorphisms with many estrogen-related diseases.[25,26]

Estrogen is usually the major stimulator of leiomyoma. However, biochemical, histological, and clinical evidence indicates that estrogen and progesterone have similar abilities to promote tumorigenesis.[27] Some investigators stressed that progesterone and PGR play significant roles in mitotic efficiency, growth factors and growth factor receptors.[28] In their studies on relations among progesterone receptor polymorphism and uterine leiomyoma, endometriosis and breast cancer, Govindan et al[29] suggested that PROGINS was a risk factor for breast cancer, but they did no t find any predisposing risk factor for uterine leiomyoma and endometriosis. Gomes et al[30] investigated the association between uterine leiomyoma and progesterone receptor gene polymorphisms in different races and found a significant association in non-White women, while there was no association in White women. Similarly, our results did not indicate any tendency of progesterone receptor polymorphisms to leiomyoma development.

In conclusion, it has been suggested that there are many genetic factors involved in leiomyoma development. Among them are estrogen and progesterone gene polymorphisms. However, our results showed that neither estrogen nor progesterone gene polymorphisms contribute to leiomyoma development. Different results were obtained for different races. Limited number of patients and ethnicity could have affected the results in the present study. Studies involving a larger patient population, including patients of different ethnic origins, are needed to better reveal the association between estrogen and PGR PROGINS polymorphisms and leiomyoma development.

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AUTHOR CONTRIBUTIONS

M.T., Ö.A., and F.D. contributed to the concept of the study; Ö.A., S.S.S., N.B., A.Z.Ö., B.C., and F.D. designed the study; M.T., C.B., and H.Y.D. contributed to materials; M.T., S.S.S., N.B., and H.D.Y. contributed to data collection and/or processing; Ö.A., S.S.S., N.B., M.T., and B.C. analyzed and/or interpreted data; M.T., A.Z.Ö., S.S.S., N.B., and Ö.A. contributed to the literature review and writing of the manuscript; M.T., Ö.A., A.Z.Ö., S.S.S., N.B., B.C., H.Y.D., İ.B.D., and F.D. critically reviewed the manuscript.

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