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Influence of the *CYP1B1* gene polymorphisms and uterine leiomyoma risk in Iranian women

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Abstract

Uterine leiomyoma (UL) is the most common gynecological benign tumor with unknown etiology and pathogenesis. This case- control study was performed to investigate the association between *CYP1B1*gene polymorphisms with uterine leiomyoma in Iranian women. Polymorphisms *CYP1B1* Arg48Gly, Ala119Ser, Leu432Val and Asp449Asp were genotyped by using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) method in 300 premenopausal women with diagnosed UL (cases) and 200 healthy normal (controls). A significant difference was found for the C allele frequencies of the *Leu432Val* C>G polymorphism between the two groups (OR = 0.71, P = 0.04, 95 % CI = 0.48 – 1.03). However, no significant difference was found for the *CYP1B1* Arg48Gly, Ala119Ser and Asp449Asp polymorphisms between the two groups (p>0.05). Our findings indicated that *CYP1B1* Leu432Val CC genotype may be involved in susceptibility to UL in Iranian women.

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Introduction

Uterine leiomyoma (UL) is the most common benign smooth muscle cell tumor of the female genital tract (Buttram Jr and Reiter, 1981). This tumor affects 30% of women in the reproductive age (Nierth-Simpson *et al.*, 2009). Despite of its high prevalence, the exact mechanisms of initiation, survival and growth of uterine leiomyomas remain unclear. Uterine leiomyomas can cause a variety of symptoms including menometrorrhagia, pelvic pain, spontaneous abortion and infertility and are the most frequent cause of hysterectomy (Haney, 2000).

Studies indicate that the growth and the development of UL is estrogen dependent (Lethaby and Vollenhoven, 2002, Maruo *et al.*, 2004, Walker and Stewart, 2005). Estrogens are eliminated from the body by metabolic changes to estrogenically inactive metabolites. Several enzymes are involved in the synthesis and degradation of estrogen (Ashton *et al.*, 2010). *CYP1A1* and *CYP1B1* are the main *CYP450* enzymes involved in estrogen catabolism (Ye *et al.*, 2008).

Hydroxylation of estrogens is performed by cytochrome P450 enzymes, such as *cytochrome P450 1B1 (CYP1B1)* (Martucci and Fishman, 1993). This enzyme converts estrogens to 4-hydroxy estrogens, which can induce DNA damage (Spink *et al.*, 1994). The *CYP1B1* gene is located in chromosome 2p21–p22 and encoding a 543-amino acid protein (Tang *et al.*, 1996). At present, approximately 42 common *CYP1B1* allele polymorphisms have been reported (Sowers *et al.*, 2006).

Studies have shown that polymorphisms in this gene alter the activity of the encoded protein (Bailey *et al.*, 1998, Shimada Tsutomu *et al.*, 1999). Determination of the single nucleotide polymorphism *(SNPs)* is a key way to study the etiology of multifactorial disorders such as uterine leiomyoma. The purpose of this study was to evaluate the association of four *CYP1B1* polymorphisms with uterine leiomyoma in Iranian women.

Material and methods

Subjects

In this case control study, we recruited 300 uterine leiomyoma Patients (mean age, 38.47years; range, 20-50 years) and 200 normal controls(mean age, 42.63years; range, 18- 49 years) who were selected from the Department of Gynecology at Shahrekord Hajar Hospital from 2012 to 2013. Uterine leiomyoma diagnosed by transvaginal sonographic examination and confirmed by the histological test after surgical intervention. Volunteers were matched for age, gender and ethnicity. Subjects with diseases or conditions, including genital tract neoplasms, abnormal uterine bleeding, adenomyosis, pregnancy, estrogen receptors (ESR) dependent cancers, alcohol intake, permanent or current use of oral contraceptives and smoking habit were excluded from the study. Informed consent was obtained from all participants and the Ethics Committees of the Shahrekord University of Medical Sciences was approved all the study protocol.

Genotyping

Genomic DNA was extracted from EDTA blood by using a standard phenol/chloroform extraction method. The genotypes of *CYP1B1 gene* Arg48Gly, Ala119Ser, Leu432Val, Asp449Asp were determined by using PCR–RFLP method, the PCR primers were designed based on the Gen-Bank reference sequence and described previously(Gallegos-Arreola *et al.*, 2008, Zhou *et al.*, 2004). The PCR reaction contained 0/3µl of both forward and reverse primers (10 PM), Mgcl2 (50mM) 2µl, TaqDNA l buffer (10X) 2.5µl, Mix dNTP (10mM) 0.5µl, 0.1µl Taq DNA Polymerase (5U/µl) and 1.2µl of DNA (about 100ng) in a total volume of 25 µl. The PCR protocol was run in a TECHNE TC_5000 Thermocycler. Table 1 demonstrates the primer sequences, PCR conditions, restriction enzymes, digestion products and their length. The PCR products were digested with the specific endonuclease enzymes according to protocols recommended by the manufacturer. PCR and digestion products were analyzed directly by vertical non-denaturing 8% polyacrylamide gel electrophoresis and visualized by silver staining. To

confirm the genotyping results, a subset of PCR products were examined by DNA sequencing, and the results were 100% concordant.

Statistical methods

Data analyses were performed using the statistical software package SSPS 17 (SPSS, Chicago, IL, USA). Genotypes and allelic frequencies for individual polymorphisms were compared between cases and controls using the χ2 test. The associations between alleles and genotype and disease risks were calculated by odds ratios (OR) with a 95% confidence interval (CI). P<0.05 was considered statistically significant.

Results

The allele and genotype frequencies of the Leu432Val (C/G), Asp449Asp (T/C), Arg48Gly (C/G) and Ala119Ser (C/T) in the healthy and UL groups are shown in Table 2 and 3. As shown in Table 2 and 3, the CC genotype and the C allele frequency of the Leu432Val (C/G) were higher in UL patients compared to healthy controls (GG vs. CC, OR 2.62, p= 0.004, 95 % CI 1.34–5.1; G vs. C, OR 0.71, p= 0.04, 95 % CI 0.48-1.03). Thus, the CC genotype seems to be a risk factor for UL. However, There was no significant difference in the genotype and allele frequencies of the *CYP1B1* gene Arg48Gly, Ala119Ser and Asp449Asp polymorphisms between the two groups (p>0.05).

Table 1. The primer sequences, PCR conditions and Restriction enzymes for *CYP1B1*gene polymorphisms.

Polymorphism	Primer sequences	Denature	Annealing	Extension	Cycle	Restriction enzyme	Product size (bp)
rs10012 Arg48Gly (C/G)	5′-GGCAACGGAGGCGGCAGCAC-3′ 5′GGAAAACGTCGCCGTAGCGCCG-3′	95 °C,30 sec	67 °C, 30sec	72 °C ,52sec	36	BSHTI	135
rs1056827 Ala119Ser (C/T)	5′-TCGGCCTTCGCCGACCGGCC-3′ 5′-TGCGCGCCGCTGCACCTTCCAG-3′	95 °C, 1min	60 °C, 1min	72 °C, 50sec	35	PdiI	108
rs1056836 Leu432Val (C/G)	5′-CACCACTGCCAACACCTCTGTC-3′ 5′-AGTTCTCCGGGTTAGGCCACTTAA-3′	9 °C,1min	61 °C, 1min	72 °C, 50sec	30	BSPTI	113
rs1056837 Asp449Asp (T/C)	5′-CCAGCTCGATTCTTGGACAAGGA-3′ 5′-CTGGTGAGCCAGGATGGAGATG-3′	95 °C, 1min	60 °C, 1min	72 °C, 50sec	35	FokI	147

Discussion

Uterine leiomyoma is a complicated disease that is caused by multiple genes, hormones, growth factors, cytokines, gene–environment involvement (Hsieh *et al.*, 2004). Evidence indicates that estrogens and its metabolic product have effects on the occurrence and development of uterine leiomyoma (Ye *et al.*, 2008). *CYP1B1* is a phase I enzyme that converts estrogens to 4-hydroxy estrogens which was linked to estrogen-induced carcinogenesis in laboratory animals and human (Hayes *et al.*, 1996, Newbold and Liehr, 2000). In this study, we observed that frequencies of Leu432Val CC genotype and C allele were significantly increased in patients with uterine leiomyoma compared to healthy controls. This result was in accordance with Hsieh *et al* who reported a significant association of the Leu432Val polymorphism with an increased risk of breast cancer (Hsieh *et al.*, 2003). Yager and Liehr reported predominant 4-hydroxylase activities in human uterine myometrium and benign uterine leiomyomas(Yager and Leihr, 1996). Napoli *et al.* indicated that American women carrying the C allele (CC, CG) had a higher urinary estrogen metabolites in compared to the GG genotype (Napoli *et al.*, 2009). On the other hand, several functional studies indicated that the *CYP1B1* Leu432Val variants gene have different catalytic activities (Aklillu *et al.*, 2002, Li *et al.*, 2000, Shimada T *et al.*, 2001, Tang *et al.*, 2000). Also the association of *CYP1B1* polymorphism with several estrogen dependent diseases such as breast cancer (Gaudet *et al.*, 2006), endometriosis (Cho *et al.*, 2007) and endometrial cancer (Sasaki *et al.*, 2003) have been reported. According to results of these studies, polymorphism in *CYP1B1* Leu432Val gene might be a risk factor of uterine leiomyoma in Iranian women due to a change in the enzymatic activity. In our study, no differences were found in

either genotype or allele frequencies of the *CYP1B1* Arg48Gly, Ala119Ser and Asp449Asp polymorphism between patient and control groups. In study that carried out by Ye Y *et al* in China, no association was found between *CYP1B1* Arg48Gly, Ala119Ser and Asp449Asp gene polymorphism and UL (Ye *et al*., 2008). In conclusion, *CYP1B1* Leu432Val polymorphism may be a risk factor on UL in Iranian women. Further studies with a larger sample size in other populations are required to confirm this possible association. We suggest that interaction other gene polymorphisms and environmental factors must be surveyed.

Table 2. The allele frequencies of *CYP1B1* between uterine leiomyoma patients and control subjects.

SNP	Allele	Cases (n = 300) (%)	Controls (n = 200) (%)	OR (95 % CI)	P
rs10012	C	193(0.64)	127(0.64)	1.00	0.46
Arg48Gly (C/G)	G	107(0.36)	73(0.36)	0.96(0.66-1.6)	
rs1056827	C	189(0.63)	133(0.66)	1.00	0.24
Ala119Ser (C/T)	T	111(0.37)	67(0.34)	1.17(0.80-1.70)	
rs1056836	C	120(0.40)	64(0.32)	1.00	0.04
Leu432Val (C/G)	G	180(0.60)	136(0.68)	0.71(0.48-1.03)	
rs1056837	C	216(0.72)	142(0.71)	1.00	0.44
Asp449Asp (T/C)	T	84(0.28)	58(0.29)	0.95 (0.64-1.41)	

Table 3. The genotype frequencies of *CYP1B1* between uterine leiomyoma patients and control subjects.

SNP	Genotype	Cases (n = 300) (%)	Controls (n = 200) (%)	OR (95 % CI)	P
rs10012	CC	118(39.3)	87(43.5)	1.00	-
Arg48Gly (C/G)	CG	148(49.3)	80(40)	0.73(0.49-1.08)	0.12
	GG	34(11.3)	33(16.5)	1.32(0.76-2.29)	0.33
rs1056827	CC	111(37)	91(45.5)	1.00	-
Ala119Ser (C/T)	CT	156(52)	83(41.5)	0.65(0.44-0.95)	0.03
	TT	33(11)	26(13)	0.96(0.54-1.72)	0.90
rs1056836	CC	43(14.4)	14(7)	1.00	-
Leu432Val (C/G	CG	156(52)	100(50)	1.97 (1.02–3.78)	0.04
	GG	101(33.6)	86(43)	2.62(1.34-5.1)	0.004
rs1056837	CC	146(48.6)	94(47)	1.00	-
Asp449Asp (T/C)	CT	140(46.7)	96(48)	1.06(0.74-1.54)	0.74
	TT	14(4.7)	10(5)	1.11(0.47-2.6)	0.81

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Conflict of interest

The authors declare no conflict of interest.

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