Reports of Biochemistry & Molecular Biology
Vol.5, No.1, Oct 2016

Original article

www.RBMB.net

Association of FAS A-670G Polymorphism and Risk of Uterine Leiomyoma in a Southeast Iranian Population

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Abstract

Background: Uterine leiomyoma (UL) is a benign tumor of uterine smooth muscle that affects women in reproductive ages. FAS has an important role in initial stages of apoptosis. Previous studies have shown an association between the FAS gene and tumorigenesis. In the present study, we evaluated the relationship between FAS A-670G (rs 1800682) and UL risk.
Methods: The FAS gene polymorphism of 155 women with UL and 157 healthy controls was analyzed by the polymerase chain reaction restriction fragment length polymorphism method.
Results: The AA, AG, and GG genotype frequencies of the FAS A-670G polymorphism were respectively 37.4, 42.6, and 20% in women with UL, and 46, 42.6, and 11.5% in healthy controls. The risk of UL in women was 1.5-fold greater in GG-genotype women than in AA-genotype women. The G allele frequencies were 41% in women with UL and 33% in healthy controls and statistically different *(P = 0.03)*.
Conclusions: The FAS polymorphism was associated with the risk of UL in a sample of Iranian women.

Keywords: FAS, PCR-RFLP, Polymorphism, Uterine leiomyoma

Introduction

Uterine leiomyoma (UL), also known uterine myoma, is a common pelvic neoplasm in women (1). Uterine leiomyoma arises from smooth muscle cells and is composed of extracellular matrix components that include collagen, fibronectin, and proteoglycan (2). Some prevalent symptoms include heavy bleeding during periods, prolonged menstrual periods, pain in the lower belly or pelvis, and reproductive dysfunction (3).

Although there are many treatment options for women with UL, hysterectomy is the most common, accounting for about 300,000 procedures performed annually in the USA alone (4-6). The estimated occurrence of UL is 20-40%; however, the pathogenesis of UL is unknown. Proposed risk factors for UL include age, race, pregnancy, and parity. Evidence suggests that heredity plays a significant role in UL pathogenesis (7). The genetic predisposition is supported by the fact that hysterectomy and hospitalization ratios due to UL are greater in monozygotic than in dizygotic female twins (8).

Benign tumors, including UL, have been reported due to overexpression of the inhibiting apoptosis genes; therefore, they have roles in UL development, while inducer apoptotic genes do not (9). Apoptosis, also known as programmed cell death, is an important mechanism of cellular hemostasis (10); any

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Received: Apr 10, 2016; Accepted: May 20, 2016

disturbance in this process can lead to disease that can be caused by decreased cell death and the creation and growth of tumor cells (11).

In various cells and tissues the FAS/Fas ligand (FAS/FasL) (CD95) pathway initiates apoptosis. FAS, also known as TNFRSF6/CD95/APO-1, is a member of the transmembrane receptor family and plays a significant role in apoptotic signaling (12). De-regulation of this pathway, e.g. via mutations, may inhibit the immune system from removing freshly-formed tumor cells, and thus lead to tumor formation (13). Although FAS is found on the surface of resting cells, it is often expressed on the surface of activated T-cells (14). FAS triggers apoptosis by binding FasL, resulting in FAS-expressing cell death. FAS expression is reduced in many kinds of human tumors (15, 16).

Some studies showed that FAS is not exclusively restricted to lymphoid or malignant cells (17). Although its role is unknown, FAS is expressed in epithelial tissues including prostate, lung, and uterus (18). Despite FAS expression, many cells are resistant to FAS-mediated apoptosis. Coexistence of FAS-sensitive and -resistant cells has been demonstrated in the same lineage (19).

Several polymorphisms have been recognized in the FAS gene promotor region; these include a guanine to adenine substitution at nucleotide position -1377 in the silencer region and an adenine to guanine substitution at nucleotide position -670 in the enhancer region (20).

To our knowledge, an association between the FAS A-670G (rs1800682) polymorphism and the risk of UL has not been reported; therefore, in the present study, we aimed to analyze the effect of the FAS polymorphism on UL risk in an Iranian population sample.

Materials and Methods

This study was performed at the Zahedan Cellular and Molecular Research Center from August through October, 2015. The study included 150 UL females as the patient group and 157 healthy premenopausal women as the control group. We received ethical approval from the University Ethics Board, and approval was obtained from all participants.

Iranian women with UL, referring to Ali-ebn-Abitaleb Hospital women's clinic, whose disease was diagnosed by medical examinations and ultrasounds, but lacked pathological examinations, were selected for the patient group. Premenopausal women with no UL pathology were selected for the control group. None of the participants had histories of blood injection. Women with systemic diseases and/or histories of malignancies were excluded from the study.

Genotype analysis

Two ml of blood were collected from all subjects into tubes containing EDTA and stored at -20 ˚C until analysis. Genomic DNA was extracted from peripheral blood leukocytes using the salting out method.

A-670G polymorphism of FAS gene

The fragment containing FAS A-670 SNP was amplified using the following forward and reverse primers:
5′-CTACCTAAGAGCTATCTACCGTTC-3′
and 5′GGCTGTCCATGTTGTGGCTGC-3′, respectively (21).

The fragment was amplified by PCR using an initial denaturation step for 6 min at 94 °C, and then by 30 amplification cycles of denaturation at 94 °C for 30 s, annealing at 61 °C for 30 s, extension at 72 °C for 60 s, and a final extension step at 72 °C for 6 min.

Seven µl of the PCR products were digested with *MvaI* (Fermentas, Vilnius, Lithuania) for 16 h at 37 °C. The 233-bp wild-type fragment was undigested while the mutant allele was digested and produced 189 and 44-bp fragments. The fragments were analyzed by electrophoresis on 2% agarose gels containing ethidium bromide.

Statistical analysis

Statistical analysis was performed using SPSS software, version 20 (SPSS, Chicago, IL, USA). Quantitative data were presented as means ± standard deviations. We used the Chi-square test for comparison of the frequency of genotypes and alleles between the patient and control groups. Allele frequencies were calculated by the gene-counting method.

P-values <0.05 were considered statistically significant.

Results

Demographic and clinical characteristics of women with UL and controls are shown in Table. 1. The UL patient and control groups were not significantly different with respect to age, BMI, marital status, age of menarche, or duration of menses and menstrual cycles. The frequencies of bleeding and pain were higher in the women with UL than in controls.

The genotype and allele frequencies of the FAS A-670 polymorphism for women with UL and controls are shown in Table 2.

Table 1. Clinical and demographic characteristics of the women with UL and controls

Criteria	Women with UL (n=155)	Controls (n=157)	P-value
Age (years)	38.5 ± 9.7	38.4 ± 7.9	NS
Marriage status; n (%)	146 (94)	151 (96)	NS
BMI (Kg/m2)	25.8 ± 5.3	25.3 ± 4.6	NS
Age of menarche (years)	13.5 ± 1.6	13.2 ± 1.5	NS
Duration of menses (days)	6.1 ± 1.6	5.9 ± 1.6	NS
Menstrual cycle (days)	28.2 ± 3.6	28.5 ± 2.9	NS
Bleeding; n (%)	94 (61)	6 (3.8)	< 0.0001
Pain; n (%)	43 (28)	10 (6.4)	< 0.0001

Table 2. Genotype and allele frequencies of FAS A-670G polymorphisms in women with UL and controls

FAS A-670G	Women with UL (N=155)	Control (N=157)	P-value	Non-Adjusted OR* (95% CI)	P-value	Adjusted OR (95% CI)**
Genotypes						
AA (%)	58 (37.4)	72 (46)				
AG (%)	66 (42.6)	66 (42.6)	0.4	1.2 (0.8-2)	0.4	1.2 (0.8-2)
GG (%)	31 (20)	18 (11.5)	0.03	1.5 (1-2.1)	0.03	1.5 (1-2.1)
Allele						
A (%)	182 (59)	211 (67)				
G (%)	128 (41)	103 (33)	0.03	1.4 (1-2)		

*Odds Ratio

**Adjusted for age

Genotype frequencies of the FAS A-670G polymorphism were in Hardy–Weinberg equilibrium. Frequencies of the AA, AG, and GG genotypes were 37.4, 42.6, and 20% in women with UL, and 46, 42.6, and 11.5% in healthy controls, respectively. The frequency of GG genotype was significantly higher in women with UL and the risk of UL was 1.5-fold greater in individuals with the GG genotype compared to AA genotype (1.5, [95% CI, 1–2.1]; *P = 0.03*).

The frequency of G allele was 41% in women with UL and 33% in controls which was statistically different (*P = 0.03*).

Discussion

In the present study, we evaluated a single nucleotide polymorphism in FAS, an apoptosis-related gene, in women with UL and found higher frequency of FAS -670GG genotype and FAS -670G allele in UL women compared to controls (*P = 0.03*).

Uterine leiomyoma is a multi-factorial disease associated with an interaction between various genes, cytokines, and growth and environmental factors, and likely develops in response to certain types of injury to the myometrium, such as hypoxia (8).

FAS has a key role in initial apoptosis; it binds the FAS receptor, which leads to caspase 8 activation, a major apoptosis pathway in many cell types, and is associated with tumor formation (22).

Although there was no published report about the association between FAS polymorphism and UL susceptibility, the role of the FAS polymorphisms has been investigated in the etiology of several female disorders including endometriosis and cervical, ovarian, and gynecological cancers.

Soon-Cen Huang et al. evaluated expression of FAS and FasL in UL. They reported that FAS gene expression was significantly less in women with UL than in controls (18).

In 2008, Kordi et al. studied 400 women with cervical cancer and 400 healthy controls from the North Indian population. They reported significant association between AG and combined AG + GG genotypes with the risk of cervical cancer (23).

In 2006, Ueda et al., in a case-control study, investigated the association of FAS A-670G polymorphism and gynecological cancer in Japan. They reported that there was no significant difference in the allele frequency between controls and endometrial patients; however, the FAS-670 GG genotype was associated with the risk of cervical cancer. They found that the risk of cervical cancer was greater with the G allele than with the A allele (24).

Li Y et al. (2003) investigated the FAS gene functional polymorphism and epithelial ovarian cancer risk. They found no correlation in the FAS genotype distribution frequency between control subjects and epithelial ovarian cancer patients (25).

Similarly, Fernandez et al. (2005) found no significant association between the FAS gene promotor A-670G polymorphism (rs1800682) and endometriosis (26).

In conclusion, our findings showed that the FAS polymorphism was associated with the risk of UL in an Iranian population in southeast Iran. Further studies with different populations are required to evaluate the association between the FAS polymorphism and UL.

Acknowledgement

The authors acknowledge the Research Deputy of Zahedan University of Medical Sciences for financial support. The authors declare no conflict of interests.

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