大韓不妊學會雜誌: 第27卷 第1號 2000
Kor. J. Fertil. Steril., Vol. 27, No. 1, 2000, 3

The Analysis of the LHβ Gene Mutation in Infertile Patients with Endometriosis and Amenorrhea Women

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자궁내막증과 무월경 불임환자에서 LHβ 유전자의 돌연변이 분석

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연구목적: 본 연구는 자궁내막증과 무월경 불임환자들을 대상으로 LHβ exon 2 유전자의 돌연변이를 탐색하고자 시도하였다.

연구재료 및 방법: 그 대상으로 22명의 자궁내막증 환자와 12명의 무월경 환자 그리고, 54명의 건강한 비임신 여성을 대조군으로 사용하였다. 이들을 대상으로 한 돌연변이 탐색은 PCR-RFLP (polymerase chain reaction-restriction fragment polymorphism) 방법으로 수행되었다.

결 과: 그 결과 자궁내막증과 무월경증 환자에서 그 변이의 비율이 각각 18.2%, 16.7% 그리고, 대조군에서 역시 16.7%의 빈도를 나타냈다.

결 론: 따라서, 자궁내막증과 무월경증 환자는 LHβ exon 2 돌연변이와는 서로 관련이 없거나 매우 적음을 알 수 있었다.

Key Words: Endometriosis, Luteinizing hormone, Missense mutation, Amenorrhea, Infertility

Luteinizing hormone (LH) is important in the development of follicle growth, stimulation of steroidogenesis, and maturation of the oocyte. Abnormal LH secretion induces anovulation, leuteal insufficiency, and premature oocyte maturation, leading to menstrual disorders, polycystic ovary syndrome (PCOS), recurrent miscarriage, and infertility.[1,2] Mutations in the human LHβ-subunit gene recently have been reported and linked with infertility. The two mutation in exon 2 of the gene, altering two codons (8 and 15) to the same as those seen in hCG, were identified in both healthy and infertile patients,[3,4] however, the evaluation of their infertility was not completely characterized. A large scale study of the frequency of these LHβ variants recently was performed by Nilsson *et al.*[5] who found the highest prevalence in the Lapps of Finland

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Table 1. Clinical profile and LHβ exon 2 variants in 34 Korean infertile patients with endometriosis and amenorrhea, and 54 healthy nonpregnant women

Study group	No. of patients	Frequency of indicated condition (%)	
		Variant LH group	Normal LH group
Endometriosis	22	4 (18.2)	18 (81.8)
Amenorrhea	12	2 (16.7)	10 (83.3)
Healthy nonpregnant women	54	9 (16.7)	45 (83.3)

Table 2. Frequency of LHβ-subunit gene mutations in the Korean and Japanese women with endometriosis

Study group	Frequency of LHβ-subunit variant (%)	
	Korean	Japanese
Endometriosis	18.2 (*present study*)	10.3 (Furui *et al.*, 1998)
Healthy fertile women	No data	8.5 (Furui *et al.*, 1998)
Healthy nonpregnant women	16.7 (*present study*)	12.0 (Nilsson *et al.*, 1997)

(41.9%) and the lowest prevalence in U.S. residents of hispanic origin (7.1%).[6] However, the LH variants were not reported in Korean endometriosis, amenorrhea patients and healthy nonpregnant women.

We found that two loci of LHβ exon 2 (LHβ2) variant was detected in endometriosis, amenorrhea healthy nonpregnant women. Endometriosis patients with the variant was slightly more frequent than healthy fertile nonpregnant women with the variant.

MATERIALS AND METHODS

Samples

Thirty-four women who were diagnosed with endometriosis (22 individuals) and amenorrhea (12 individuals) were included in the study at the infertility Medical Center of CHA General Hospital and Pundang CHA General Hospital, defined as endometriosis and amenorrhea. Fifty-four healthy nonpregnant unrelated Korean women were used for the control group, respectively. Samples of venous blood were collected from consenting individual DNA extracted by standard methods.

PCR Amplification, Restriction Fragment Length Polymorphism (RFLP) from Genomic DNA for Mutation Detection

PCR reaction contained 50 μl 10x reaction buffer (500 mM KCl, 100 mM Tris-Cl, pH 8.3), 2.5 mM $MgCl_2$, 0.8 mM dNTP, 2.0 U Taq polymerase and 50 pM of sense primer of LHβ exon 2 (5'-TCTTTGTGGGTG GTGTACCACGC-3') and antisense primer (5'-GGAGGATCCGGG TGTCAGGGCTCCA-3'), respectively. The primers used in these experiments span exon 2, intron 2 and exon 3 of the LHβ gene. PCR using primers generated a 794 bp fragment. The PCR amplification and RFLP analysis were carried out by Roy *et al.*[7] with slight modification.

RESULTS

Twenty-two women with idiopathic 46,XX endometriosis, 12 amenorrhea and 54 healthy fertile and nonpregnant women were investigated (Table 1). The two loci of LHβ exon 2 (Trp8 Arg; TGG to CGG and Ile15Thr; ATC to ACC) were studied in the three different groups. The variant frequencies of LHβ2 (Trp8Arg and Ile15

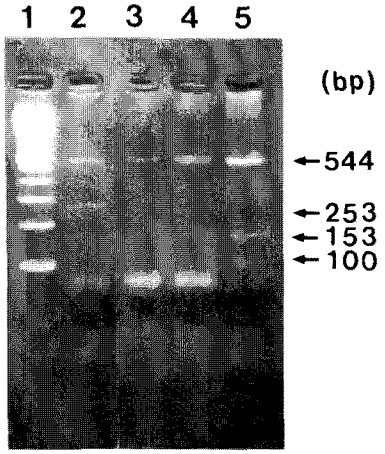

Figure 1. RFLP analysis of the Trp(TGG)8Arg (CGG) mutation in LHβ exon 2 using enzyme NcoI. Lane 1: Marker DNA, Lane 2: Heterozygote type, Lane 3, 4, 5: Homozygote type.

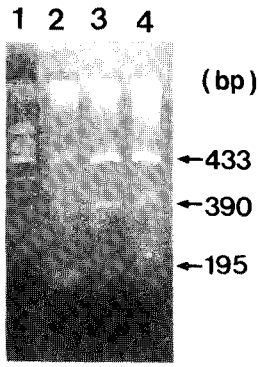

Figure 2. RFLP analysis of the Ile(ATC)15Thr (ACC) mutation in LHβ exon 2 using enzyme FokI. Lane 1: Marker DNA, Lane 2: Heterozygote type, Lane 3, 4: Homozygote type.

Thr) in endometriosis patients were 18.2% and 16.7%, respectively. In healthy nonpregnant women, the variant frequency of LHβ2 was 16.7% (Table 1 and Figures 1, 2).

The LHβ2 variants were slightly more frequent in endometriosis patients (18.2%) than in healthy nonpregnant women (16.7%). However, amenorrhea patients (16.7%) with the variant were the same frequency as healthy nonpregnant women with the variant (Table 2).

DISCUSSION

In the present study, the frequencies of the LHβ variant were not significantly higher in endometriosis (18.2%) and amenorrhea (16.7%) than healthy nonpregnant Korean women (16.7%) (Table 1). However, Takahashi *et al.*[2] reported that significantly increased prevalence of luteinizing hormone β-subunit variant (53.3%) in Japanese patients with premature ovarian failure (POF). In a Finnish population, homozygote mutants were reported fertile, but homozygous Japanese subjects have been reported to be infertile.[3,5,8] However, homozygote mutant type was not found in Korean infertile patients with endometriosis and amenorrhea women (Figures 1, 2). A survey of the frequency of the LHβ variant in various populations has found it to be a ubiquitous polymorphism occuring in 7.1 ~41.9% of individuals in healthy populations with considerable geographic and racial variation.[5,9]

A shorter half-life of variant LH in the circulation, lower bioactivity in vivo, and increased bioactivity in vitro may be related to these interesting associations.[8,10,11,12] These abnormal characteristics of variant LH may lead to abnormalities in the pituitary-gonadal endocrine pathways.[2,13] Suganuma *et al.*[10] reported that some patients homozygous for the mutant LHβ-subunit had menstrual disorders. Takahashi *et al.*[2] suggested that variant LH secretion may induce ovulatory disorders (55.6%), luteal insufficiency (44.4%), amenorrhea caused by weight loss (37.5%), or hyperprolactinemia (44.4%), with consequent infertility and endometriosis (10.3%). However, the pathophysiological and clinical significance of variant LH in infertile patients with endometriosis and amenorrhea women is still unclear.

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