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Analysis of LHβ Exon 3 (Gly102Ser) Gene Mutation in Infertile Patients with Endometriosis and Polycystic Ovary Syndrome (PCOS)

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자궁내막증과 다낭성 난포증후군 불임환자에서 LHβ Exon 3 (Gly102Ser) 유전자의 돌연변이 분석

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연구목적: 본 연구는 자궁내막증과 다낭성 난포증후군 불임환자들을 대상으로 LHβ exon 3 (Gly102Ser) 유전자의 돌연변이를 탐색하고자 시도하였다.

연구재료 및 방법: 그 대상으로 26명의 자궁내막증 환자와 52명의 다낭성 난포증후군 환자 그리고, 50명의 출산 경험이 있는 건강한 여성을 대조군으로 사용하였다. 이들을 대상으로 한 돌연변이 탐색은 PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) 방법으로 수행되었다.

결과: 그 결과 자궁내막증과 다낭성 난포증후군 환자 및 출산 경험이 있는 건강한 여성에서 그 변이형이 나타나지 않았다.

결론: 따라서, 자궁내막증과 다낭성 난포증후군 불임환자의 LHβ exon 3 돌연변이형은 중국인 집단에만 존재할 가능성이 높으며, 더 많은 불임환자들을 대상으로 한 연구가 요구된다.

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

Luteinizing hormone (LH) is important in the stimulation of follicular growth and maturation of the oocyte. It has a central role in promoting spermatogenesis and ovulation by stimulating the testes and ovaries, respectively, for steroid synthesis. 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] The mutation in exon 2 of the gene, altering two codons (8 and 15) to the same as those seen in hCG, was

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identified in both healthy and infertile patients,[3,4] however, the evaluation of their infertility was not completely characterized. Recently, another variant with a single missense mutation in exon 3 of the LHβ-subunit gene, replaced Gly102 (1502G) by Ser102 (1502A), has been suggested to be associated with female infertility.[1,5,6] Three populations (Chinese, Malays, Indians) of southeast Asia of the LHβ3 variants recently were studied by Ramanujam *et al.*[5] who found the variant only in the Chinese of Singapore. The LHβ3 variant was not reported in Korean endometriosis, PCOS patients and healthy nonpregnant women.

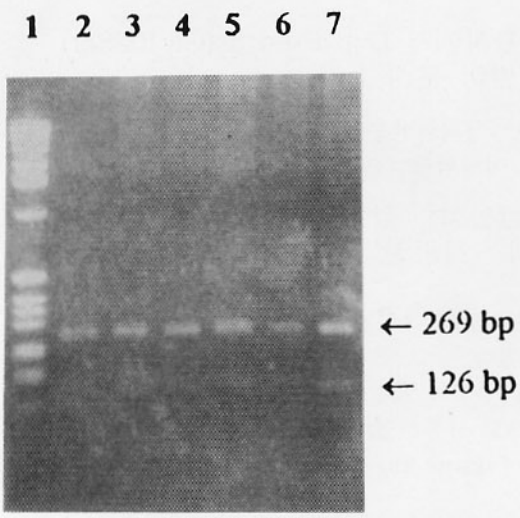

Figure 1. RFLP analysis of the Gly(GGT)102Ser (AGT) mutation in LHβ exon 3 using enzyme Eco 0109I. Lane 1: Marker DNA, Lane 2, 3, 4, 5, 6, 7: Normal homozygous type.

MATERIALS AND METHODS

Samples

Seventy-eight women who were diagnosed with endometriosis (26 individuals) and PCOS (52 individuals) were included in the study at the infertility Medical Center of CHA General Hospital and Pundang CHA General Hospital, defined as endometriosis and PCOS. Fifty healthy fertile and 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 3 (5'-AGTCTGAGACCTGTGGGGTCAGCTT-3') and antisense primer (5'-GGAGGATCCGGGTGTCAGGGCTCCA-3'), respectively. PCR using primers generated a 395 bp fragment. The PCR amplification and RFLP analysis were carried out by the method of Roy *et al.*[7] with slight modification.

RESULTS

The PCR products were analyzed for the mu-

Table 1. Frequency of LHβ exon 3 gene mutation in the Korean, Chinese, Malays and Indians

Population	LHβ3 genotype			Allele frequency	n
	AA	Aa	aa	a	
Chinese	184	7	0	0.018	191
Malays	121	0	0	0	121
Indians	150	0	0	0	150
Korean	128	0	0	0	128

A = Wild-type allele; a = mutant allele.

tation G1502 to A1502 in exon 3 of the LHβ-subunit gene by RFLP using Eco0109I as the restriction enzyme. Agarose gel electrophoresis of the undigested PCR products yield the expected 395 bp fragments in all patients and controls. In a normal LH sequence, Eco0109I digestion generates two separate bands of 269 bp and 126 bp (Figure 1). The absence of any 395 bp fragments demonstrated that no heterozygous or homozygous mutant alleles were present in any patients or control.

DISCUSSION

Recently, Liao *et al.*[1] have identified the exon 3 of LHβ-subunit (LHβ3) variant in 4% of infertile women, and concluded that this mutation in LHβ3 gene might be related to female infertility with endometriosis and PCOS in some women in Singapore (Table 1). Ranamujam *et al.*[6] evaluated that the mutation was also involved in menstrual disorder in Singapore Chinese women. Suganuma *et al.*[8] reported that some patients homozygous for the mutant LH β-subunit had menstrual disorder. Abnormal LH and / or LH receptors have been reported to be linked with endometriosis-associated infertility.[9,10] LH plays an important role in gonadal function. The exon 3 variant may therefore have a number of roles in the pathophysiology of female infertility. Ramanujam *et al.*[6] speculated that the mutant form of LH affected gonadal function and that the microheterogeneity could be related to endometriosis and PCOS with menstrual irregularity. This finding supports that the exon 3 variant may play a part in female infertility.

However, none of 78 patients were homozygous for the missense mutation, suggesting that it is a rare cause of endometriosis and PCOS in Korean women. Moreover, Ramanujam *et al.*[5] also did not find the variant in Malays and Indians (Table 1). Therefore, further studies are required to determine its occurrence in different type of menstrual disorder in relation to menstrual disorder and female infertility. Moreover, the pathophysiological and clinical significance of variant LHβ3 in infertile patients in endometriosis and PCOS with menstrual disorder are still remains to be investigated.

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