大韓不妊學會雜誌: 第27卷 第2號 2000
Kor. J. Fertil. Steril., Vol. 27, No. 2, 2000, 6

Molecular Variants of the LHβ-subunit in Infertile Patients with Polycystic Ovary Syndrome (PCOS) in Korean Women

Nam Keun Kim[1,2], Hyung Min Chung[2], Eu Gene Lee[1], Yoon Sung Nam[3], Dong Hee Choi[2], Tae Jong Sohn[1], Sook Hwan Lee[1,2], Jung Jae Ko[1,2], Kwang Yul Cha[1,2]

[1]Department of Biochemistry and Institute of Medical Research, College of Medicine, Pochon CHA University, Pochon, 487-800; [2]Infertility Medical Center, CHA General Hospital, Seoul, 135-081; [3]Department of Obstetrics & Gynecology and Infertility Medical Center, Pundang CHA General Hospital, Sungnam 463-712, Korea

한국인의 다낭성 난포증후군 불임환자에서 황체형성 호르몬 유전자의 분자변이 연구

[1]포천중문 의과대학교 생화학교실 및 기초의학연구소 중앙연구실;
[2]차병원 여성의학 연구소; [3]분당 차병원 산부인과 및 불임센터

김남근[1,2] · 정형민[2] · 이유진[1] · 남윤성[3] · 최동희[2] · 손태종[1]
이숙환[1,2] · 고정재[1,2] · 차광열[1,2]

연구목적: 한국여성의 다낭성 난포증후군 환자에서 황체형성 호르몬 exon 2 유전자의 변이를 탐색하여 이들 변이와 질환과의 관련성 여부를 밝히고자 하였다.

연구재료 및 방법: 21명의 다낭성 난포증후군 환자를 대상으로 황체형성 호르몬 exon 2 (Trp8Arg; TGG to CGG and Ile15Thr; ATC to ACC)의 변이를 탐색하였다. 혈액에서 Genomic DNA를 추출하여 PCR로 증폭한 후 RFLP 방법으로 변이형을 구분하였다.[4]

결 과: 황체형성 호르몬 exon 2의 변이형이 다낭성 난포증후군 환자에서 28.6%로 이미 조사된 바 있는 대조군의 16.7% 보다 약간 높게 나타났으나 통계적으로 유의한 차이는 없었다 ($p>0.05$).

결 론: 황체형성 호르몬 exon 2의 변이가 한국인의 다낭성 난포증후군 발병과 관련이 있는지를 밝히기 위해 더 많은 개체에 대한 연구가 요구된다.

Key Words: Polycystic ovary syndrome (PCOS), Luteinizing hormone (LH), Missense mutation

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.[6,8,15] A large scale study of the frequency of these LHβ variants recently was performed by Nilsson *et al.*,[9] who found the highest prevalence in the Lapps of Finland (42%) and the lowest prevalence in U.S. residents of hispanic origin (7.1%). It was found to have higher in vitro bioactivity and a shorter half-life than the wild type LH.[3]

PCOS has the feature of excessive LH, hy-

*교신저자: 김남근, 경기도 포천군 포천읍 동교리 198-1, 포천중문 의과대학교 생화학교실 및 기초의학연구소
Tel: 0357) 543-1814, Fax: 0357) 543-2818, E-mail: nkkim@netsgo.com

perandrogenism, obesity (overweight) and disturbance of folliculogenesis. PCOS is a heterogeneous disorder containing no simple definition. This disease is often characterized by hyperandrogenism, menstrual disorders, and obesity in women with enlarged polycystic ovaries.[14] The two mutations in exon 2 of the gene, altering two codons (8 and 15, Trp8Arg; TGG to CGG and Ile15Thr; ATC to ACC) are the same as those seen in hCG; these were identified in both healthy and infertile patients.[10] Mutations in the human LHβ-subunit gene recently have been reported and linked with infertility.[2,8,15] However, the evaluation of their infertility was not completely characterized, especially in PCOS.[1,5]

In this study, we investigated whether there was any correlation between these variants of LHβ exon 2 (LHβ2) subunits and PCOS in Korean women.

MATERIALS AND METHODS

Sample

Twenty-one women who were diagnosed with PCO were included in the study at the infertility Medical Center of CHA General Hospital. Samples of venous blood were collected from consenting individuals and their 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'-TCTTTGTGGGTGGTGTACCACGC-3') and antisense primer (5'-GGAGGATCCGGGT-GTCAGGGCTCCA-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.*[13] with slight modifications.

Data Analysis

The data were analyzed using the chi-square (χ^2) test. $p<0.05$ was considered statistically significant.

RESULTS

Twenty-one women with PCO and fifty-four nonpregnant controls were investigated in the two loci of LHβ2 (Table 1). The phenotypic frequencies of LHβ2 (Trp8Arg) in PCO patients

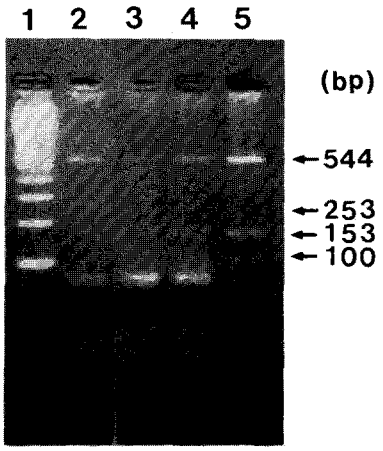

Figure 1. RFLP analysis of the Trp(TGG)8Arg (CGG) mutation in LHβ exon 2 using enzyme NcoI. Lane 1: Marker DNA, Lane 2: Heterozygote type, Lane 3, 4, 5: Homozygote type.

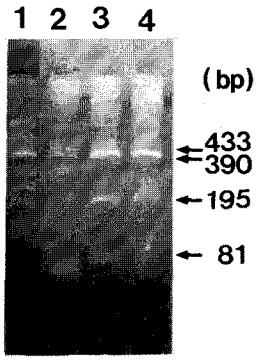

Figure 2. RFLP analysis of the Ile(ATC)15Thr (ACC) mutation in LHβ exon 2 using enzyme FokI. Lane 1: Marker DNA, Lane 2: Heterozygote type, Lane 3, 4: Homozygote type.

Table 1. Comparison of allele frequencies of the LHβ variants in the PCO and nonpregnant Korean women

Study group	LHβ Phenotype			Allele Frequency	N
Trp8Arg	1-1	2-1	2-2	2	
PCO	18	3	0	0.071	21
Nonpregnant women (Kim *et al*)[4]	49	5	0	0.046	54
Ile15Thr	TT	TC	CC	C	
PCO	17	4	0	0.095	21
Nonpregnant women (Kim *et al*)[4]	50	4	0	0.037	54

1,T=Wild type allele: 2,C=mutant allele.

Table 2. LHβ exon 2 variants in 21 infertile patients with PCO and 54 nonpregnant women in Korea

Study group	No. of patients	Frequency of indicated condition (%)	
		Variant LH group	Normal LH group
PCO	21	6 (28.6)	15 (71.4)
Nonpregnant women (Kim *et al*)[4]	54	9 (16.7)	45 (83.3)

*; $p<0.05$.

Table 3. Frequency of LHβ-subunit gene mutations in the Korean and Japanese infertile women

Study group	Frequency of LHβ-subunit variant (%)		
	Korean	Japanese	UK
PCO	28.6 (*present study*)	7.7 (Furui *et al*)[2]	20.9 (Rajkhowa *et al*)[12]
Nonpregnant women	16.7 (Kim *et al*)[4]	12.0 (Nilsson *et al*)[9]	14.6 (Rajkhowa *et al*)[12]

were LHβ2 1-1 type (85.7%) and LHβ2 2-1 type (14.3%) (Figure 1). The allele frequencies of $LH\beta2^1$ and $LH\beta2^2$ were 0.93 and 0.07, respectively. The phenotypic frequencies of LHβ2 (Ile 15Thr) in PCO patients were LHβ2 TT type (81.0%) and LHβ2 TC type (19.0%) (Figure 2). The allele frequencies of $LH\beta2^T$ and $LH\beta2^C$ in PCO patients were 0.90 and 0.10, respectively.

The frequency of LHβ2 variant was higher in the PCO patients (28.6%) than in the controls (16.7%) ($p>0.05$). PCO patients with the variant were more frequent than nonpregnant women with the variant (Table 2).

DISCUSSION

Elter *et al.*[1] found that the women with variant LH had lower serum levels than the healthy women. Kurioka *et al.*[5] also initially failed to confirm a case of PCOS because of an underestimation of LH concentrations due to a variant form of this hormone resulted in a misleadingly low LH/FSH ratio. These abnormal characteristics of variant LH may lead to abnormalities in the pituitary-gonadal endocrine pathways.[7,15] A reportedly 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.[3,11,12,14] Suganuma *et al.*[14]

reported that some patients homozygous for the mutant LHβ-subunit had menstrual disorders. In a Finnish population, homozygotes reportedly were fertile, but homozygous Japanese subjects have been reported to be infertile.[2,3,9]

The role of LH in the pathogenesis of PCOS has been debated. Variant LH, as a result of its increased bioacivity, may cause hyperandrogenemia and PCOS. Furui *et al.*[2] and Suganuma,[14] who also defined the LH variant, reported that two of five cases homozygous for the variant LH were diagnosed as PCOS. Rajkhowa *et al.*[12] and Elter *et al.*,[1] however, observed a similar frequency of the LH variant in women with PCOS and healthy women.

Takahashi *et al.*[15] reported a significantly higher prevalence of luteinizing hormone β-subunit variant (53.3%) in Japanese patients with premature ovarian failure (POF). They suggested that variant LH secretion may induce ovulatory disorders, luteal insufficiency, amenorrhea caused by weight loss, or hyperprolactinemia with consequent infertility and PCOS. However, we showed that there was a significantly higher prevalence of LHβ variant in Korean PCO patients (28.6%) than that of Japanese patients (7.7%) (Table 3).

The pathophysiological and clinical significance of variant LH in infertile patients with PCOS is still unclear.

REFERENCES

1. Elter K, Erel T, Cine N, Ozbek U, Hacihanefioglu B, Ertungealp E. Role of the mutations $Trp^8 \Rightarrow$ Arg and Ile15 $\Rightarrow$ Thr of the human luteinizing hormone β-subunit in women with polycystic ovary syndrome. Fertil Steril 1999; 71(3): 425-30.
2. Furui K, Suganuma N, Tsukahara S-I, Asada Y, Kikkawa F, Tanaka M, *et al.* Identification of two point mutations in the gene coding luteinization hormone (LH) β-subunit, associated with immunologically anomalous LH variants. J Clin Endocrinol Metab 1994; 78(1): 107-13.
3. Haavisto AM, Pattersson K, Bergendahl M, Virkkamaki A, Huhtaniemi I. Occurrence and biological properties of a common genetic variant of luteinizing hormone. J Clin Endocrinol Metab 1995; 80: 1257-63.
4. Kim NK, Lee EG, Nam YS, Lee SH, Chung KW, Ko JJ, *et al.* The analysis of LHβ gene mutation in infertile patients with endometriosis and amenorrhea. Kor J Fertil Steril 2000; 27(1): 107-10.
5. Kurioka H, Takahashi K, Irikoma M, Okada M, Ozaki T, Ueda T, *et al.* Diagnostic difficulty in polycystic ovary syndrome due to an LH-beta-subunit variant. Eur J Endocrinol 1999; 140(3): 235-8.
6. Layman L. Mutations in human gonadotropin genes and their physiologic significance in puberty and reproduction. Fertil Steril 1999; 71(2): 201-18.
7. Lee SJ, Kim JY, Park KH, Choi KH. The endocrine changes and alteration of the ovarian response to clomiphen citrate after laparascopic laser vaporization in patients with polycystic ovary syndrome. Kor J Fertil Steril 1999; 26(3): 483-90.
8. Liao W-X, Roy AC, Chan C, Arulkumaran S, Ratnam SS. A new molecular variant of luteinizing hormone associated with female infertility. Fertil Steril 1998; 69(1): 102-6.
9. Nilsson S, Matzuk MM, Pettersson K, Huhtaniemi IT, Millar RP, Coerver KA. Worldwide frequency of a common genetic variant of luteinizing hormone: an international collaborative research. Fertil Steril 1997; 67(6): 998-1004.
10. Okuda K, Yamada T, Imoto H, Komatsubara H, Sugimoto O. Antigenic alterations of an anomalous human luteinizing hormone caused by two chorionic gonadotropin-type amino acid substitutions. Biochem Biophys Res Commun 1994; 200: 584-90.
11. Raivio T, Huhtaniemi I, Anttila R, Siimes MA, Hagenas L, Nilsson C, *et al.* The role of luteinizing hormone β gene polymorphism in the onset and progression of puberty in healthy boys. J Clin Endocrinol Metab 1996; 81: 3278-82.
12. Rajkhowa M, Talbot JA, Johns PW, Patterson K, Haavisto M, Huhtaniemi I, *et al.* Prevalence

of a immunological LHβ-subunit variant in a UK population of healthy women and women with polycystic ovary syndrome. Clin Endocrinol (Oxf) 1996; 43: 297-303.

13. Roy AC, Liao W-X, Chen Y, Arulkumaran S, Ratnam SS. Identification of seven novel mutation in LHβ-subunit gene by SSCP. Mol Cell Biochem 1996; 165: 151-3.
14. Suganuma N, Asada Y, Furui K, Kikkawa F, Furuhashi M, Tomada Y. Screening of the mutations in luteinizing hormone β-subunit in patients with menstrual disorders. Fertil Steril 1995; 63(5): 989-95.
15. Takahashi K, Ozaki T, Okada M, Kurioka H, Kanasaki H, Miyazaki K. Increased prevalence of luteinizing hormone β-subunit variant in patients with premature ovarian failure. Fertil Steril 1999; 71(1): 96-101.