FSH receptor gene p. Thr307Ala and p. Asn680Ser polymorphisms are associated with the risk of polycystic ovary syndrome

Jin Ju Kim^{1,2} · Young Min Choi^{2,3} · Min A. Hong³ · Soo Jin Chae⁴ · Kyuri Hwang⁵ · Sang Ho Yoon⁶ · Seung Yup Ku^{2,3} · Chang Suk Suh^{2,3} · Seok Hyun Kim^{2,3}

Received: 30 November 2016 / Accepted: 15 May 2017 / Published online: 25 May 2017
© Springer Science+Business Media New York 2017

Abstract

Purpose The purpose of this study was to investigate whether the follicle-stimulating hormone receptor (FSHR) gene p. Thr307Ala (c.919A>G, rs6165) and p. Asn680Ser (c.2039A>G, rs6166) polymorphisms are associated with susceptibility to polycystic ovary syndrome (PCOS).

Methods Genotyping was performed in 377 women with PCOS and 388 age-matched controls. Difference in the genotype distribution was assessed using a Fisher's exact or chi-square test, and continuous variables were compared using a Student's *t* test. To evaluate the association between the presence of PCOS status and SNP, logistic regression analyses were performed.

Electronic supplementary material The online version of this article (doi:10.1007/s10815-017-0953-z) contains supplementary material, which is available to authorized users.

✉ Young Min Choi
ymchoi@snu.ac.kr

Results Linkage disequilibrium between the two polymorphisms was approximately complete ($r^2 = 99\%$). The genotype distributions of the PCOS group significantly differed from those of the control group (Thr/Thr, Thr/Ala, and Ala/Ala frequencies were 38.5, 46.7, and 14.9% for the PCOS group and 46.6, 45.4, and 8.0% for the controls, respectively, $P = .005$; Asn/Asn, Asn/Ser, and Ser/Ser frequencies were 39.5, 47.2, and 13.3% for the PCOS group and 46.4, 45.4, and 8.2% for the controls, respectively, $P = .035$). Using the wild-type genotypes as the references, the odds ratios that a woman has PCOS were 2.23 (95% confidence intervals 1.38–3.68) for the Ala/Ala genotype, 1.87 (95% confidence intervals 1.14–3.06) for the Ser/Ser genotype, and 1.96 (95% confidence intervals 1.19–3.24) for the homozygous variant combination (Ser/Ser-Ala/Ala). However, there were no significant differences in serum hormonal, ovarian, and metabolic markers according to each genotype.

Conclusions Findings of this study suggest a significant association between *FSHR* gene p. Thr307Ala or p. Asn680Ser coding sequence change and PCOS. The variant homozygote genotype results in a higher risk of PCOS.

Keywords FSH receptor gene · Polycystic ovary syndrome · Polymorphism

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age with a prevalence that ranges from 11.9 to 19.9% by the Rotterdam criteria [1, 2]. Chronic anovulation and hyperandrogenism are cardinal features of this syndrome, and obesity and insulin resistance are also common. Thus, diabetes, metabolic syndrome, and other cardiovascular disease risk factors are known to be increased in women with PCOS, and a risk of developing type 2 diabetes (DM) is

- ¹ Department of Obstetrics and Gynecology, Healthcare System Gangnam Center, Seoul National University Hospital, Seoul, South Korea
- ² Department of Obstetrics and Gynecology, The Institute of Reproductive Medicine and Population, Medical Research Center, Seoul National University College of Medicine, 28 Yungun-dong, Chongno-ku, Seoul 110-744, South Korea
- ³ Department of Obstetrics and Gynecology, Seoul National University College of Medicine, Seoul, South Korea
- ⁴ Department of Obstetrics and Gynecology, Maria Fertility Hospital, Seoul, South Korea
- ⁵ Department of Obstetrics and Gynecology, Seoul Municipal Boramae Hospital, Seoul, South Korea
- ⁶ Department of Obstetrics and Gynecology, Graduate School of Medicine, Dongguk University, Seoul, Republic of Korea

approximately seven times greater in these women than unaffected women [3]. Despite considerable detrimental impact on women's reproductive and metabolic health, PCOS etiology remains poorly understood.

Follicle-stimulating hormone (FSH) plays an important role in follicle development, oocyte maturation, and steroidogenesis. FSH exerts its effects via a specific receptor (FSHR) which is located on the granulosa cells of the ovary. The *FSHR* gene contains 10 exons and 9 introns, and is located at chromosome 2p21. Variants of the *FSHR* are known to be very rare [4, 5]; however, two polymorphisms, p. Thr307Ala (c.919A>G, rs6165) and p. Asn680Ser (c.2039A>G, rs6166), have been identified in exon 10 [6]. Earlier studies showed that the p. Asn680Ser variant allele was associated with higher basal FSH levels although within the normal range, with longer menstrual cycles, and with a higher ovarian threshold to ovulation induction, suggesting a difference in receptor sensitivity [7–11].

Although the etiology of PCOS has not been elucidated, numerous studies have suggested that genetic factors play important roles in its etiology and pathogenesis. Based on the knowledge that PCOS is characterized by failure of follicular growth, several studies have extensively investigated the association between *FSHR* p. Thr307Ala or p. Asn680Ser coding sequence changes and PCOS with inconsistent and controversial results [8, 11–24]. In this study, we compared the genotype distribution of *FSHR* gene p. Thr307Ala and p. Asn680Ser polymorphisms between PCOS patients and controls, and investigated whether serum hormonal, ovarian, and metabolic markers were affected according to these polymorphisms.

Materials and methods

Subjects

Three hundred seventy-seven premenopausal women were enrolled as PCOS diagnosis. PCOS diagnosis was based on the 2003 Rotterdam consensus meeting guidelines [25]. Oligomenorrhea was defined as fewer than eight periods per year or cycles longer than 35 days, and amenorrhea was defined as the absence of menstruation for more than 3 months without pregnancy. Clinical hyperandrogenism was defined by a modified Ferriman and Gallwey score (mF-G score) of 6 or greater in our population [26]. Biochemical hyperandrogenism was defined by an elevation of serum androgen levels beyond the 95% confidence limits measured in 89 ovulatory, non-hirsute controls in our population who did not have a PCO based on ultrasonography (total testosterone [T] >0.68 ng/ml, free T > 1.72 pg/ml, free androgen index [FAI] >5.36) [27]. All PCOS patients were screened to exclude hyperprolactinemia and thyroid dysfunction. Serum 17-hydroxyprogesterone (OHP) was also measured in all PCOS patients. If a patient's serum 17-OHP level was greater than 2 ng/ml, a repeat test

was performed during the early morning follicular phase. Patients who showed continuous elevation of 17-OHP were excluded from the study group.

A total of 388 premenopausal women were matched with patients based on age (± 1 year). These women visited the healthcare center of our hospital as part of a group checkup for work and lacked specific health problems. All controls had regular (21–35-day) menstrual cycles and mF-G scores below 6. They all received a transvaginal or transrectal pelvic ultrasound examination to evaluate ovarian morphology, and were excluded if a polycystic ovarian morphology was identified. Neither the cases nor the controls had taken hormonal medications, including oral contraceptives, for at least 3 months. The review board for human research of the Seoul National University Hospital approved this project (IRB No. 1005-050-319), and written informed consent was obtained from all individual participants included in the study.

Clinical and biochemical measurements

Clinical variables such as body weight, height, waist circumference, and blood pressure (BP) were assessed, and body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2).

Basal gonadotropin hormone levels were measured in all PCOS subjects, including serum luteinizing hormone (LH), FSH, and estradiol (E_2) levels. All PCOS women were evaluated for serum total T, free T, 17-OHP, dehydroepiandrosterone sulfate (DHEAS), and sex hormone-binding globulin (SHBG) using radiomimunoassay (Siemens, Los Angeles, CA, USA). Plasma insulin levels were measured using a commercial kit (BioSource Europe S.A., Belgium). FAI was calculated as $(\text{total T} / \text{SHBG}) \times 100$, and the values for T were converted from nanograms per milliliter to nanomoles per liter using the following index provided by the manufacturer: 1 ng/ml = 3.467 nmol/l. Fasting and 2-h glucose and insulin levels were evaluated by a 75-g glucose tolerance test to assess glucose intolerance and insulin resistance in PCOS patients. The homeostatic model for insulin resistance (HOMA-IR) was calculated as $\text{glucose (mg/dl)} \times \text{insulin } (\mu\text{U/ml}) / 405$, and $\text{HOMA}_{\beta\text{cell}} (\%)$ was calculated as follows: $(20 \times \text{fasting insulin}) / (\text{fasting glucose} - 3.5)$. Serum cholesterol, triglyceride (TG), high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol levels were measured using a 200FR system (Toshiba, Tokyo, Japan). In most of the controls, serum total T, free T, SHBG, fasting glucose and insulin, total cholesterol, TG, HDL cholesterol, and LDL cholesterol levels were also measured. The intra- and inter-assay coefficients of variation were 4.0–11.0 and 5.9–11.0% for total testosterone, 4.0–17 and 8.0–18.3% for free T, 5.0–7.1 and 5.0–11.0% for 17-OHP, 3.8–5.1 and 6.3–11.0% for DHEAS, 2.8–5.3 and 7.9–8.5% for SHBG, and 1.6–2.2 and 6.1–6.5% for plasma insulin, respectively.

Genomic DNA analysis

Genomic DNA was extracted from peripheral blood samples with the Wizard DNA Purification Kit (Promega, Madison, WI). Allelic discrimination was done using the MGB-NFQ primer/probe TaqMan assay on the ABI Prism 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Primer pairs and probes for *FSHR* p.Thr307Ala and *FSHR* p.Asn680Ser were Assays-on-Demand, C__2676873_30 (Applied Biosystems), and Assays-on-Demand, C__2676874_10 (Applied Biosystems), respectively. The PCR mixture consisted of 10 µl of TaqMan Universal PCR Master Mix 2× (Applied Biosystems) and 25 ng DNA. The PCR cycling conditions consisted of one 2-min cycle at 50 °C, and one 10-min cycle at 95 °C, followed by 40 cycles at 95 °C for 15 s and 60 °C for 1 min. We used distilled water as a negative PCR control in each amplification.

Statistical analysis

Power calculations were performed using the QUANTO v.1.2.4 (log-additive model) software (<http://hydra.usc.edu/gxe/>). A 12% population prevalence of PCOS was assumed, and minor allele frequency was obtained from other Korean studies [1, 18]. Based on the sample size (377 patients with PCOS and 388 controls), the power to detect an allelic odds ratio of 1.5 at an α value of 0.05 was 83.2%. Possible deviations from the normality of the data distribution were tested by visual inspection of quantile-normal plots or the Shapiro-Wilk test of normality. If variables did not follow Gaussian distributions, normal distributions were achieved by natural logarithmic or square root transformation. In these cases, data were shown as geometric means with 95% confidence intervals (95% CI). Hormonal and other metabolic parameters were compared using either Student's *t* test or analysis of variance (ANOVA). The differences in genotype distribution and allele frequency were tested using a Fisher's exact or chi-square test. Genotype distribution was also examined for significant departures from the Hardy-Weinberg equilibrium by a goodness-of-fit chi-square test. To evaluate the association between the presence of PCOS status and SNP, logistic regression analyses were performed using the wild-type homozygote as the reference category. All data analyses were performed using the Statistical Package for the Social Sciences software (version 22.0, IBM SPSS, NY, USA), and a haplotype analysis was performed using Haploview software (version 4.2; Broad Institute). Significance was accepted for two-sided *P* values <.05.

Results

The clinical and endocrine characteristics of the PCOS patients and controls are shown in Table 1. By definition, there were significant differences in hirsutism score and serum

androgen levels between women with PCOS and controls. Women with PCOS were more obese than controls, and as expected, there were also significant differences in metabolic parameters between the two groups except HDL cholesterol and fasting glucose levels.

The genotype distribution in the control group was in the Hardy-Weinberg equilibrium, and a linkage disequilibrium (LD) between p. Asn680Ser and p. Thr307Ala polymorphisms was approximately complete ($r^2 = 99\%$). The genotype distributions of the PCOS group significantly differed from those of the control group (Asn/Asn, Asn/Ser, and Ser/Ser frequencies were 39.5, 47.2, and 13.3% for the PCOS group and 46.4, 45.4, and 8.2% for the control group, respectively, $P = .035$; Thr/Thr, Thr/Ala, and Ala/Ala frequencies were 38.5, 46.7, and 14.9% for the PCOS group and 46.6, 45.4, and 8.0% for the control group, respectively, $P = .005$) (Table 2). Using the wild-type genotype as the reference, the odds that a woman has PCOS was 1.87 (95% CIs 1.14–3.06) times higher if she has a Ser/Ser genotype and 2.23 (95% CIs 1.38–3.68) times higher if she has an Ala/Ala genotype (Table 2). We additionally evaluated distributions of p. Asn680Ser and p. Thr307Ala genotype combinations, and found significant differences between the women with PCOS and the controls (Supplementary Table 1). The Asn/Asn-Thr/Thr, Asn/Ser-Thr/Ala, and Ser/Ser-Ala/Ala genotype combinations made up almost the whole subject pool (366/377 in women with PCOS and 387/388 in controls). Distributions of these three major genotype combinations significantly differed between the PCOS patients and controls (Asn/Asn-Thr/Thr, Asn/Ser-Thr/Ala, and Ser/Ser-Ala/Ala frequencies were 38.5, 45.6, and 13.0% for the PCOS group and 46.4, 45.4, and 8.0% for the controls, respectively, $P = .026$) (Table 2). Using the wild-type genotype combination (Asn/Asn-Thr/Thr) as the reference, the odds that a woman has PCOS was 1.96 (95% CIs 1.19–3.24) times higher if she has a homozygous variant combination (Ser/Ser-Ala/Ala).

For further analysis, subjects were divided according to genotype, and we assessed whether serum hormonal, ovarian, and metabolic markers were affected by the p. Asn680Ser or p. Thr307Ala genotypes (Table 3). However, we could not find any significant differences in these parameters that were correlated with genotype in women with PCOS (nor in controls, data not shown).

Discussion

The aim of the present study was to evaluate the association between *FSHR* gene p. Thr307Ala or p. Asn680Ser coding sequence polymorphisms and PCOS in Korean women. According to our data, a nearly complete LD between p. Thr307Ala and p. Asn680Ser polymorphism exists, and the *FSHR* p. Asn680Ser or p. Thr307Ala genotype distribution of

Table 1 Clinical and biochemical features of PCOS patients and age-matched controls

	PCOS (<i>n</i> = 377)	Controls (<i>n</i> = 388)	<i>P</i> value
Age (years) ^a	28.5 ± 4.9	28.5 ± 4.9	.955
Body mass index (kg/m ²) ^a	22.2 ± 4.0	20.1 ± 2.3	<.001
Waist circumference (cm) ^a	74.1 ± 10.6	74.4 ± 6.8	.615
Hirsutism score ^b	5 (0–22)	0 (0–5)	<.001
Total testosterone (ng/ml) ^c	0.32 (0.30, 0.34)	0.25 (0.24, 0.26) (<i>n</i> = 210)	.002
Free testosterone (pg/ml) ^c	1.0 (0.9, 1.1)	0.6 (0.5, 0.7) (<i>n</i> = 210)	.028
Sex hormone-binding globulin (nmol/l) ^c	39.0 (34.6, 43.4)	64.3 (60.5, 68.1) (<i>n</i> = 197)	<.001
Free androgen index ^c	3.2 (2.9, 3.5)	1.5 (1.3, 1.7) (<i>n</i> = 197)	<.001
Luteinizing hormone (mIU/ml) ^c	7.3 (6.7, 8.0)	Not available	
Follicle-stimulating hormone (mIU/ml) ^c	4.6 (4.3, 4.9)	Not available	
Estradiol (pg/ml) ^c	46.1 (42.1, 50.5)	Not available	
Fasting plasma glucose (mg/dl) ^a	88.3 ± 13.1	87.3 ± 8.2	.240
Fasting insulin (μU/ml) ^c	8.5 (6.6, 10.4)	6.2 (4.5, 7.9)	<.001
Homeostatic model assessment for insulin resistance ^c	1.86 (1.52, 2.20)	1.32 (1.24, 1.40)	<.001
Homeostatic model assessment _{βcell} (%) ^c	131.8 (130.0, 133.6)	97.7 (95.6, 99.8)	<.001
Systolic blood pressure (mmHg) ^a	108.4 ± 15.7	104.5 ± 14.4	.001
Diastolic blood pressure (mmHg) ^a	69.6 ± 10.8	65.7 ± 10.8	<.001
Total cholesterol (mg/dl) ^a	180.6 ± 31.1	173.3 ± 26.9	.002
Triglyceride (mg/dl) ^c	75.9 (74.2, 77.6)	67.6 (66.2, 69.0)	.001
High-density lipoprotein cholesterol (mg/dl) ^a	63.1 ± 14.9	63.9 ± 13.9	.513
Low-density lipoprotein cholesterol (mg/dl) ^a	101.3 ± 26.7	94.8 ± 21.8	.006
Hemoglobin A1c (%) ^a	5.53 ± 0.253	5.43 ± 0.26	<.001

P values are indicated for the differences in groups as analyzed by Student's *t* test

^a Data are shown as means ± SD

^b Median (range)

^c Geometric mean and 95% CI

Table 2 Genotype distributions of the p. Asn680Ser and p. Thr307Ala polymorphisms in the follicle-stimulating hormone receptor gene in PCOS patients and controls

Genotype	PCOS (<i>n</i> = 377)	Control (<i>n</i> = 388)	<i>P</i> value ^a	Odds ratio (95% CIs)	<i>P</i> value ^b
p. Asn680Ser					
Asn/Asn (%)	149 (39.5%)	180 (46.4%)	.035		
Asn/Ser (%)	178 (47.2%)	176 (45.4%)		1.21 (0.89–1.63)	.219
Ser/Ser (%)	50 (13.3%)	32 (8.2%)		1.87 (1.14–3.06)	.013
p. Thr307Ala					
Thr/Thr (%)	145 (38.5%)	181 (46.6%)	.005		
Thr/Ala (%)	176 (46.7%)	176 (45.4%)		1.25 (0.92–1.69)	.150
Ala/Ala (%)	56 (14.9%)	31 (8.0%)		2.23 (1.38–3.68)	.001
Genotype combination					
Asn/Asn-Thr/Thr (%)	145 (38.5%)	180 (46.4%)	.026		
Asn/Ser-Thr/Ala (%)	172 (45.6%)	176 (45.4%)		1.21 (0.90–1.64)	.212
Ser/Ser-Ala/Ala (%)	49 (13.0%)	31 (8.0%)		1.96 (1.19–3.24)	.008

CI confidence interval, PCOS polycystic ovary syndrome

^a Evaluated by chi-square test in comparison with the control group

^b Evaluated by logistic regression models using the wild-type Asn/Asn genotype, Thr/Thr genotype, or Asn/Asn-Thr/Thr genotype combination as the reference

Table 3 Hormonal and metabolic variables according to the p. Asn680Ser and p. Thr307Ala polymorphisms in the follicle-stimulating hormone receptor gene in women with polycystic ovary syndrome

	Asn/Asn (n = 149)	Asn/Ser (n = 178)	Ser/Ser (n = 50)	<i>P</i> ^a	Thr/Thr (n = 145)	Thr/Ala (n = 176)	Ala/Ala (n = 56)	<i>P</i> ^a
Age (years) ^a	26.1 ± 1.3	26.9 ± 0.9	27.2 ± 1.4	.398	26.5 ± 1.4	26.3 ± 0.9	26.4 ± 1.4	.203
Body mass index (kg/m ²) ^a	22.2 ± 4.2	22.0 ± 3.9	22.4 ± 3.8	.913	22.2 ± 4.1	22.0 ± 3.9	22.5 ± 3.7	.721
Total testosterone (ng/ml) ^b	0.32 (0.28, 0.36)	0.36 (0.33, 0.39)	0.33 (0.29, 0.37)	.141	0.32 (0.28, 0.36)	0.36 (0.33, 0.39)	0.34 (0.29, 0.39)	.129
Free testosterone (pg/ml) ^b	1.0 (0.8, 1.2)	1.1 (1.0, 1.2)	1.1 (0.9, 1.3)	.068	1.0 (0.8, 1.2)	1.1 (1.0, 1.2)	1.1 (0.9, 1.3)	.067
Sex hormone binding globulin (nmol/l) ^b	34.6 (29.6, 40.0)	34.1 (30.7, 37.7)	37.1 (31.2, 43.6)	.336	34.5 (29.7, 39.3)	34.1 (30.7, 37.7)	37.2 (31.3, 43.7)	.595
Free androgen index ^b	3.37 (2.72, 4.18)	3.83 (3.32, 4.41)	3.12 (2.45, 3.96)	.313	3.38 (2.74, 4.02)	3.83 (3.32, 4.41)	3.14 (2.47, 3.81)	.734
Total cholesterol (mg/dl) ^a	181.9 ± 33.8	176.9 ± 29.2	189.7 ± 26.7	.079	181.3 ± 33.9	177.7 ± 29.6	188.2 ± 28.7	.176
Triglycerides (mg/ml) ^b	75.4 (65.5, 87.8)	70.9 (64.0, 79.2)	82.4 (72.0, 89.3)	.569	75.5 (65.5, 85.5)	71.4 (64.0, 78.8)	82.5 (72.1, 92.9)	.527
High density lipoprotein cholesterol (mg/ml) ^a	62.3 ± 14.7	63.4 ± 15.6	63.4 ± 14.5	.790	62.1 ± 14.2	63.5 ± 15.6	63.4 ± 15.2	.695
Low density lipoprotein cholesterol (mg/ml) ^a	101.6 ± 28.0	98.7 ± 24.6	108.7 ± 28.5	.188	102.8 ± 29.5	100.6 ± 25.5	105.7 ± 26.9	.628
Fasting plasma glucose (mg/dl) ^a	90.0 ± 17.8	87.0 ± 8.8	87.8 ± 8.5	.153	90.1 ± 17.8	87.9 ± 8.9	87.9 ± 8.7	.156
Fasting insulin (μU/ml) ^b	9.2 (7.9, 10.7)	10.0 (9.1, 11.0)	9.5 (8.4, 10.9)	.768	9.2 (7.9, 10.7)	10.0 (9.1, 11.0)	9.6 (8.4, 10.8)	.502
Homeostatic model assessment for insulin resistance ^b	2.04 (1.74, 2.39)	2.20 (1.98, 2.44)	1.99 (1.73, 2.30)	.758	2.05 (1.74, 2.36)	2.20 (1.98, 2.44)	2.19 (1.73, 2.65)	.995
Homeostatic model assessment _{βcell} (%) ^b	144.7 (142.8, 146.6)	147.7 (145.7, 149.7)	159.1 (157.3, 160.9)	.458	144.7 (142.8, 146.6)	158.1 (145.6, 170.6)	159.8 (157.3, 162.3)	.195
2-h post-load plasma glucose (mg/dl) ^a	108.2 ± 40.7	103.5 ± 28.0	107.7 ± 25.8	.611	108.2 ± 41.2	103.7 ± 27.9	106.6 ± 25.5	.636
2-h post-load plasma insulin (μU/ml) ^b	41.7 (39.2, 44.2)	36.3 (34.1, 38.5)	46.8 (44.3, 49.3)	.313	41.7 (39.2, 44.2)	36.3 (34.1, 38.5)	46.8 (44.3, 49.3)	.329
Hemoglobin A1c ^a	5.53 ± 0.26	5.55 ± 0.24	5.53 ± 0.24	.861	5.54 ± 0.25	5.55 ± 0.24	5.54 ± 0.31	.859
Lt. ovarian volume ^b	6.26 (4.37, 8.15)	5.75 (3.92, 7.58)	4.81 (2.99, 6.63)	.085	6.45 (4.51, 8.40)	5.84 (3.97, 7.71)	4.98 (3.14, 6.82)	.090
Lt. ovarian follicle number ^a	14.7 ± 5.4	14.9 ± 6.2	14.6 ± 5.4	.947	14.9 ± 5.6	15.1 ± 6.2	14.7 ± 5.4	.896
Rt. ovarian volume ^b	7.67 (5.92, 9.42)	7.57 (5.78, 9.36)	6.24 (4.27, 8.21)	.913	7.69 (5.93, 9.45)	7.54 (5.84, 9.24)	6.40 (4.63, 8.17)	.398
Rt. ovarian follicle number ^a	16.7 ± 6.1	17.0 ± 7.0	15.5 ± 6.7	.402	16.8 ± 6.3	16.9 ± 6.7	15.7 ± 6.7	.431
Luteinizing hormone ^b	6.88 (4.83, 8.93)	8.03 (6.07, 9.99)	9.40 (7.21, 11.59)	.080	6.88 (4.84, 8.92)	7.99 (6.05, 9.93)	9.41 (7.21, 11.61)	.081
Follicle stimulating hormone ^a	4.93 ± 2.58	4.94 ± 2.25	5.54 ± 2.55	.391	4.81 ± 2.37	5.07 ± 2.37	5.35 ± 2.56	.512
Estradiol ^b	43.0 (40.6, 45.4)	44.7 (42.5, 46.6)	43.4 (41.3, 45.5)	.932	43.1 (40.6, 45.6)	44.9 (42.4, 47.4)	44.4 (41.3, 47.5)	.608

P values are indicated for the differences in groups as analyzed by ANOVA

^a Data are shown as means ± standard deviations

^b Data are shown as geometric means and 95% confidence intervals

the PCOS group was significantly different from that of the control group. Using the wild-type genotypes as the references, the odds ratios that a woman has PCOS were 1.87 (95% CIs 1.14–3.06) for the Ser/Ser genotype, 2.23 (95% CIs 1.38–3.68) for the Ala/Ala genotype, and 1.96 (95% CIs 1.19–3.24) for the homozygous variant combination (Ser/Ser-Ala/Ala). However, in women with PCOS, there were no significant differences in the serum levels of hormones, biochemical variables, or ovarian markers associated with any genotype.

FSH plays a key role in human reproduction. Based on the knowledge that PCOS is characterized by failure of follicular growth, an association between p. Thr307Ala or p. Asn680Ser polymorphism and PCOS has been extensively studied across ethnicity with conflicting results [8, 11–24]. Most studies were performed within small numbers of subjects, but two large studies could not find an association between *FSHR* polymorphism and PCOS. The largest one was the study by Valkenburg et al., which compared 518 Caucasian PCOS patients and 2996 controls, and the second largest one was the

study by Fu et al., which compared 384 Chinese women with PCOS and 768 controls [16, 20]. Our study contained the third largest number of subjects (377 patients and 386 controls) ever reported of women with PCOS in which p. Asn680Ser polymorphism was analyzed, and our current study might offer data to the literature about the significance of *FSHR* polymorphism in PCOS. One interesting point was that the frequency of the Ser680Ser variant genotype (13.3% in women with PCOS and 8.2% in controls) was lower than that observed in Caucasians (22–25%). Another study performed in Italian women with PCOS also found higher frequency (30–40%) of the Ser680Ser variant genotype, suggesting the importance of ethnicity background [14].

The FSH receptor is a G-protein-coupled receptor, and has transmembrane, intracellular, and extracellular domains. The intracellular domains are encoded by exon 10, in which the p. Asn680Ser variant is located [28]. Although in vitro studies could not find different activity associated with variants of the FSH receptor [13, 29], substantial studies reported that *FSHR* gene polymorphism was associated with phenotype in women with PCOS. In addition, the *FSHR* p. Asn680Ser variant allele was associated with higher levels of FSH or clomiphene citrate resistance [11, 13, 16, 20–22, 24]. We also assessed the possibility that *FSHR* gene polymorphism affects ovarian markers represented by serum FSH concentrations, antral follicle count, or ovarian size, but could not find any association between genotype and phenotype. FSH levels in women with PCOS are generally within reference values, thus there is a possibility that small differences in serum FSH levels within the normal range may need larger numbers of subjects to demonstrate an association. Alternatively, the p. Asn680Ser variant might be involved only in local regulation of *FSHR* in women with PCOS. Either way, the mechanism by which *FSHR* gene polymorphism modulates the pathophysiology of PCOS needs to be determined.

Genome-wide association (GWA) studies on Chinese and European ancestry women found *FSHR* as PCOS susceptibility locus. Especially, the variant (rs2268361) in the *FSHR* gene intron region at 2p16.3 was associated with PCOS, although functional significance was not clear [30–33]. Recently, two European GWA studies reported that chromosome 11p14.1 SNP (rs11031006), which lies near the FSH β -subunit coding region, was strongly associated with PCOS diagnosis [34, 35]. Whether *FSHR* gene or *FSH* gene polymorphisms have complementarity in the PCOS pathogenesis is uncertain; these findings suggest that gonadotropin action modulation may have some roles in the PCOS pathogenesis.

In conclusion, the current study suggests a significant association between *FSHR* gene p. Thr307Ala or p. Asn680Ser coding sequence changes and PCOS. Since disorder in follicle development is a key feature of PCOS, *FSHR* polymorphism studies which might affect receptor function need to be performed continuously.

Compliance with ethical standards

Ethical approval All procedures performed in studies involving human participants were in accordance with ethical standards of the institutional and/or national research committees and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of interest The authors declare that they have no conflict of interest.

Funding This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (20100009075).

References

1. March WA, Moore VM, Willson KJ, Phillips DI, Norman RJ, Davies MJ. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. *Hum Reprod*. 2010;25:544–51.
2. Yildiz BO, Bozdag G, Yapici Z, Esinler I, Yarli H. Prevalence, phenotype and cardiometabolic risk of polycystic ovary syndrome under different diagnostic criteria. *Hum Reprod*. 2012;27:3067–73.
3. Legro RS, Kusanman AR, Dodson WC, Dunaif A. Prevalence and predictors of risk for type 2 diabetes mellitus and impaired glucose tolerance in polycystic ovary syndrome: a prospective, controlled study in 254 affected women. *J Clin Endocrinol Metab*. 1999;84:165–9.
4. Layman LC, Amde S, Cohen DP, Jin M, Xie J. The Finnish follicle-stimulating hormone receptor gene mutation is rare in North American women with 46, XX ovarian failure. *Fertil Steril*. 1998;69:300–2.
5. Gromoll J, Simoni M. Genetic complexity of FSH receptor function. *Trends Endocrinol Metab*. 2005;16:368–73.
6. Simoni M, Gromoll J, Nieschlag E. The follicle-stimulating hormone receptor: biochemistry, molecular biology, physiology, and pathophysiology. *Endocr Rev*. 1997;18:739–73.
7. Perez MM, Gromoll J, Behre HM, Gassner C, Nieschlag E, Simoni M. Ovarian response to follicle-stimulating hormone (FSH) stimulation depends on the FSH receptor genotype. *J Clin Endocrinol Metab*. 2000;85:3365–9.
8. Laven JS, Mulders AG, Suryandari DA, Gromoll J, Nieschlag E, Fauser BC, et al. Follicle-stimulating hormone receptor polymorphisms in women with normogonadotropic anovulatory infertility. *Fertil Steril*. 2003;80:986–92.
9. Greb RR, Grieshaber K, Gromoll J, Sonntag B, Nieschlag E, Kiesel L, et al. A common single nucleotide polymorphism in exon 10 of the human follicle stimulating hormone receptor is a major determinant of length and hormonal dynamics of the menstrual cycle. *J Clin Endocrinol Metab*. 2005;90:4866–72.
10. de Koning CH, Benjamins T, Harms P, Homburg R, van Montfrans JM, Gromoll J, et al. The distribution of FSH receptor isoforms is related to basal FSH levels in subfertile women with normal menstrual cycles. *Hum Reprod*. 2006;21:443–6.
11. Overbeek A, Kuijper EA, Hendriks ML, Blankenstein MA, Ketel IJ, Twisk JW, et al. Clomiphene citrate resistance in relation to follicle-stimulating hormone receptor Ser680Ser-polymorphism in polycystic ovary syndrome. *Hum Reprod*. 2009;24:2007–13.
12. Tong Y, Liao WX, Roy AC, Ng SC. Absence of mutations in the coding regions of follicle-stimulating hormone receptor gene in Singapore Chinese women with premature ovarian failure and polycystic ovary syndrome. *Horm Metab Res*. 2001;33:221–6.

13. Sudo S, Kudo M, Wada S, Sato O, Hsueh AJ, Fujimoto S. Genetic and functional analyses of polymorphisms in the human FSH receptor gene. *Mol Hum Reprod*. 2002;8:893–9.
14. Orio F Jr, Ferrarini E, Cascella T, Dimida A, Palomba S, Gianetti E, et al. Genetic analysis of the follicle stimulating hormone receptor gene in women with polycystic ovary syndrome. *J Endocrinol Investig*. 2006;29:975–82.
15. Unsal T, Konac E, Yesilkaya E, Yilmaz A, Bideci A, Ilke Onen H, et al. Genetic polymorphisms of FSHR, CYP17, CYP11A1, CAPN10, INSR, SERPINE1 genes in adolescent girls with polycystic ovary syndrome. *J Assist Reprod Genet*. 2009;26:205–16.
16. Valkenburg O, Uitterlinden AG, Piersma D, Hofman A, Themmen AP, de Jong FH, et al. Genetic polymorphisms of GnRH and gonadotrophic hormone receptors affect the phenotype of polycystic ovary syndrome. *Hum Reprod*. 2009;24:2014–22.
17. Du J, Zhang W, Guo L, Zhang Z, Shi H, Wang J, et al. Two FSHR variants, haplotypes and meta-analysis in Chinese women with premature ovarian failure and polycystic ovary syndrome. *Mol Genet Metab*. 2010;100:292–5.
18. Gu BH, Park JM, Baek KH. Genetic variations of follicle stimulating hormone receptor are associated with polycystic ovary syndrome. *Int J Mol Med*. 2010;26:107–12.
19. Mohiyiddeen L, Salim S, Mulugeta B, McBurney H, Newman WG, Pemberton P, et al. PCOS and peripheral AMH levels in relation to FSH receptor gene single nucleotide polymorphisms. *Gynecol Endocrinol*. 2012;28:375–7.
20. Fu L, Zhang Z, Zhang A, Xu J, Huang X, Zheng Q, et al. Association study between FSHR Ala307Thr and Ser680Asn variants and polycystic ovary syndrome (PCOS) in Northern Chinese Han women. *J Assist Reprod Genet*. 2013;30:717–21.
21. Singhasena W, Pantasri T, Piromlertamom W, Samchinchom S, Vutyavanich T. Follicle-stimulating hormone receptor gene polymorphism in chronic anovulatory women, with or without polycystic ovary syndrome: a cross-sectional study. *Reprod Biol Endocrinol*. 2014;12:86.
22. Wu XQ, Xu SM, Liu JF, Bi XY, Wu YX, Liu J. Association between FSHR polymorphisms and polycystic ovary syndrome among Chinese women in north China. *J Assist Reprod Genet*. 2014;31:371–7.
23. Almawi WY, Hubail B, Arekat DZ, Al-Farsi SM, Al-Kindi SK, Arekat MR, et al. Luteinizing hormone/choriogonadotropin receptor and follicle stimulating hormone receptor gene variants in polycystic ovary syndrome. *J Assist Reprod Genet*. 2015;32:607–14.
24. Valkenburg O, van Santbrink EJ, König TE, Themmen AP, Uitterlinden AG, Fauser BC, et al. Follicle-stimulating hormone receptor polymorphism affects the outcome of ovulation induction in normogonadotropic (World Health Organization class 2) anovulatory subfertility. *Fertil Steril*. 2015;103:1081–8.
25. The Rotterdam ESHRE/ASRM-sponsored PCOS consensus workshop group. Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). *Hum Reprod*. 2004;19:41–7.
26. Kim JJ, Chae SJ, Choi YM, Hwang SS, Hwang KR, Kim SM, et al. Assessment of hirsutism among Korean women: results of a randomly selected sample of women seeking pre-employment physical check-up. *Hum Reprod*. 2011;26:214–20.
27. Chae SJ, Kim JJ, Choi YM, Hwang KR, Jee BC, Ku SY, et al. Clinical and biochemical characteristics of polycystic ovary syndrome in Korean women. *Hum Reprod*. 2008;23:1924–31.
28. Simoni M, Nieschlag E, Gromoll J. Isoforms and single nucleotide polymorphisms of the FSH receptor gene: implications for human reproduction. *Hum Reprod Update*. 2002;8:413–21.
29. Simoni M, Gromoll J, Hoëppner W, Kamischke A, Krafft T, Staëhle D, et al. Mutational analysis of the follicle-stimulating hormone (FSH) receptor in normal and infertile men: identification and characterization of two discrete FSH receptor isoforms. *J Clin Endocrinol Metab*. 1999;84:751–5.
30. Chen ZJ, Zhao H, He L, Shi Y, Qin Y, Shi Y, et al. Genome-wide association study identifies susceptibility loci for polycystic ovary syndrome on chromosome 2p16.3, 2p21 and 9q33.3. *Nat Genet*. 2011;43:55–9.
31. Shi Y, Zhao H, Shi Y, Cao Y, Yang D, Li Z, et al. Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. *Nat Genet*. 2012;44:1020–5.
32. Mutharasan P, Galdones E, Peñalver Bernabé B, Garcia OA, Jafari N, Shea LD, et al. Evidence for chromosome 2p16.3 polycystic ovary syndrome susceptibility locus in affected women of European ancestry. *J Clin Endocrinol Metab*. 2013;98:E185–90.
33. Saxena R, Georgopoulos NA, Braaten TJ, Bjorntjes AC, Koika V, Panidis D, et al. Han Chinese polycystic ovary syndrome risk variants in women of European ancestry: relationship to FSH levels and glucose tolerance. *Hum Reprod*. 2015;30:1454–9.
34. Day FR, Hinds DA, Tung JY, Stolk L, Styrkarsdottir U, Saxena R, et al. Causal mechanisms and balancing selection inferred from genetic associations with polycystic ovary syndrome. *Nat Commun*. 2015;6:8464.
35. Hayes MG, Urbanek M, Ehrmann DA, Armstrong LL, Lee JY, Sisk R, et al. Genome-wide association of polycystic ovary syndrome implicates alterations in gonadotropin secretion in European ancestry populations. *Nat Commun*. 2015;6:7502.