International Journal of Biochemistry Research & Review
5(3): 198-206, 2015, Article no.IJBcRR.2015.023
ISSN: 2231-086X

SCIENCEDOMAIN *international*
www.sciencedomain.org

SCIENCEDOMAIN

Association between Follicle Stimulating Hormone Receptor (FSHR) Polymorphism and Polycystic Ovary Syndrome among Egyptian Women

A. F. Abdel-Aziz[1], A. M. A. El-Sokkary[1], A. A. El-Refaeey[2], M. M. A. El-Sokkary[3], H. G. Osman[1] and Rasha A. El-Saeed[1*]

[1]Biochemistry Division, Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt.
[2]Department of Obstetrics and Gynecology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[3]Department of Microbiology, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Authors HGO and AAER designed the study. Authors HGO, AMAES and RAES carried out all laboratories work. Author MMAES shared in doing the PCR technique. Authors AMAES and RAES performed the statistical analyses of the study. Authors AFAA, AAER and RAES managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBcRR/2015/13896
Editor(s):
(1) Dileep G. Nair, Ministry of Higher Education, Sultanate of Oman.
(2) Luisa Di Paola, Chemical and Biochemical Engineering Fundamentals, Faculty of Engineering Università Campus Biomedico, Via Alvaro del Portillo, Roma, Italy.
Reviewers:
(1) Anonymous, University Hospital Miguel Servet, Zaragoza, Spain.
(2) Joselyn Rojas Quintero, Endocrine and Metabolic Diseases Research Center. University of Zulia, Venezuela.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=844&id=3&aid=7084

Original Research Article

Received 8th September 2014
Accepted 24th September 2014
Published 6th December 2014

ABSTRACT

Background: Polycystic ovary syndrome (PCOS) is an endocrine disorder and the criteria are specified by common complex genetic hyperandrogenism, oligomenorrhea or amenorrhea and polycystic ovary morphology. It is a leading cause of female infertility. The prevelance of PCOS among reproductive age women has been estimated to be 4-12%. The association between PCOS and FSH receptor (FSHR) polymorphism attracts wide attention. The aim of the present study was to evaluate whether polymorphism of FSHR at Ala307Thr codon is associated with PCOS and with

**Corresponding author: E-mail: afaziz2012@hotmil.com;*

clinical features of PCOS patients in Egypt.
Results: PCOS patients (n=64) and control subjects (n=65) in the reproductive age were recruited from the outpatient clinic of Obstetrics and Gynecology Department, Mansoura University, Egypt. The Ala307Thr polymorphism in FSHR, and the frequency of respective genotypes was studied and statistical analysis was performed. We found that the heterozygote Ala/Thr genotype was associated with PCOS (64.1%, OR=2.68, CI=0.97, P= 0.033) compared with controls.
Conclusion: The variant of Ala307Thr polymorphism of *FSHR* was associated with PCOS but it may be related to high total testosterone levels. In addition the FSHR polymorphism was not associated with either luteinizing hormone or follicular stimulating hormone. The present study suggests that the variant of the FSHR gene may act as a causative factor of PCOS in Egyptian women.

Keywords: Polycystic ovary syndrome; follicle stimulating hormone; follicle stimulating hormone receptor; single nucleotide polymorphisms.

1. INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a complex endocrine disorder characterized by hyperandrogenism, menstrual irregularity and polycystic ovarian morphology. About 4–12% of women within reproductive age are affected by PCOS. It accounts for about 75% of all cases of anovulatory infertility [1,2]. PCOS is a mutifactorial disease and its pathogenesis is still not fully understood. Although multiple genetic factors including mutations and polymorphisms to several genes have been associated with PCOS risk, the inheritance mode and the molecular genetic mechanisms underlying PCOS risk are not fully understood [3].

Follicle stimulating hormone (FSH) is expressed as a pituitary glycoprotein that plays an essential role during folliculogenesis through stimulation of granulosa cell proliferation and differentiation as well as follicle growth and development [4,5]. The effect of FSH is mediated by the FSH receptor (FSHR) which is expressed in the granulosa cells of the ovary. *FSHR* is a member of the family of G protein-coupled receptors characterized by three domains, extracellular ligand-binding domain, transmembrane domain and intracellular domain [6].

The importance of *FSHR* in the signaling transmission of FSH made *FSHR* gene one of the important candidate genes for PCOS [4]. Mutations in *FSHR* can lead to arrest of follicle development at several phases of growth [7,8]. There are a number of genetic variants in the *FSHR* that have an effect on the phenotype, these effects include variable development of secondary sex characteristics, primary amenorrhea, hypoplastic ovary and serum levels of FSH [9].

The *FSHR* gene is located on chromosome 2p21 and comprises 10 exons and 9 introns [10]. *FSHR* gene contains two important single nucleotide polymorphisms (SNPs) in exon 10, which are in linkage disequilibrium and change two amino acids at positions Ala307Thr and Ser680Asn. Ala307Thr, situated at the extracellular domain of *FSHR*, the site responsible for high affinity hormone binding, has been reported to affect hormone trafficking, signal transduction, and subsequent FSH efficacy [3,11].

Most available studies were focused on position 680, whereas polymorphism 307 was less considered [8,9,12-14]. Studies carried out to establish the association of Ala307Thr polymorphism with the risk of PCOS came out with inconsistent results, moreover, data regarding these associations with PCOS in Egyptian population have not been studied before.

The existence of significant differences in the allele distribution of *FSHR* polymorphism 307 in women with PCOS have been reported in several studies [8,12,13]. On the other hand, this association was not confirmed among other studies [9,4].

The aim of the present study was to investigate the association of Ala307Thr (rs6165) polymorphism of *FSHR* gene with the risk of PCOS among Egyptian women.

2. SUBJECTS AND METHODS

This cross-sectional study was done between August 2011 and April 2013 and included two groups of women. The first group was the PCOS

group which included 64 women with PCOS, with mean age of 25.78±4.53, recruited from the outpatient clinic of Obstetrics and Gynecology Department, Mansoura University Hospitals, Egypt.

The second group was the control group, which included 65 healthy women, matched for age and body mass index (BMI), with mean age of 25.09±4.81, recruited randomly from the fertility care unit, with regular menstrual cycles, no clinical appearance of hirsutism, acne, alopecia, endocrinopathies, or other chronic illness and none of them had PCO picture on ultrasound, also, none of them had a family history of PCOS.

Diagnosis of PCOS was made according to the 2003 Rotterdam ESHRE/ASRM PCOS Consensus Workshop Group's diagnostic criteria. This proposes a diagnosis of PCOS when a patient meets two of the following three criteria: (i) Oligomenorrhea or amenorrhea for at least 6 months; (ii) Clinical and/or biochemical signs of hyperandrogenism; (iii) Polycystic ovaries on ultrasound [15]. Oligomenorrhea was defined as a reduction in the frequency of menses with intervals between 40 days and 6 months and hyperandrogenism was defined as serum testosterone > 0.6 ng/ml. Diagnosis of polycystic ovaries was defined as the presence of 12 or more follicles in each ovary measuring 2-9 mm in diameter and/or increased ovarian volume (10 mm) [9].

The criteria for inclusion were that: patients were in their reproductive age and none of them were on hypoglycemic agents (including metformin) or hormonal therapy (including oral contraceptives) or undergone ovulation induction for at least 3 months before entering the study. Exclusion criteria included other causes of hyperandrogenism, as (androgen-secreting tumors, nonclassical adrenal hyperplasia, and cushing's syndrome), hyperprolactinemia, diabetes, hypertension, thyroid disease, and renal diseases.

All subjects were recruited in the follicular phase of menstrual cycles and BMI of each patient defined as weight (kg)/height (m^2) was calculated [16]. Written informed consent was obtained from each participant and the study protocol was approved by Mansoura ethical committee.

2.1 Collection of Blood Samples

Venous blood samples (5 ml) were taken between 8:00 AM-9:00 AM after 12-h fast. The blood was divided into two parts, 3 ml were placed into plain tubes and allowed to clot for 10-15 minutes, centrifuged and the separated serum was stored at -80°C for further measurement of the biochemical parameters. The other 2 ml of the whole blood were taken in EDTA coated tubes for extraction of DNA and performing genotyping working.

2.2 Biochemical and Genotype Analysis

Serum concentrations of luteinizing hormone (LH) [17], follicular stimulating hormone (FSH) [18] and total testosterone [19] were measured by a microplate chemiluminescence immunoassay (Monobind Inc., Lake Forest, USA).

Genomic DNA was extracted from blood samples using a DNA purification kit (Fermentas, Germany) according to the manufacturer's instructions. The Ala307Thr of FSHR was detected by restriction fragment length polymorphism (RFLP) analysis [3,20]. A 577 bP fragment of *FSHR* was amplified by PCR using the following primers:

F: 5'- CCTGCACAAAGACAGTGATG-3'
R: 5'-TGGCAAAGACAGTGAAAAAG-3'.

Polymerase chain reaction (PCR) was carried out in a 25 µl mixture containing: 2.5 µl 1x PCR buffer, 0.75 µl $MgCl_2$, 1 µl dNTP mixture, 0.7 µl of each primer, 0.5 µl Taq DNA polymerase, 13.85 µl double distilled H_2O, and 5 µl genomic DNA.

The thermal cycler was adjusted for PCR conditions as follows: Initial denaturation cycle at 95°C for 5 minute followed by 30 cycle in the form of 94°C for 30 seconds (denaturation), 55°C for one minute (annealing) and 72°C for one minute (extension) with a final extension cycle of 7 min at 72°C.

The PCR products were digested with enzyme *Eam1105I* (Thermo Fisher Scientific Inc., USA). Digestion of the G allele produced two fragments with lengths 403 and 174 bp, Digestion of the A allele produced three fragments with lengths 403, 143 and 31 bp. The digestion products were resolved after electrophoresis in 4% agarose gel, Fig. 1.

2.3 Statistical Analyses

Statistical analyses were carried out via Statistical Package for Social Science (SPSS)

version 17 program on windows XP. Qualitative data were represented in the form of number and frequency, while quantitative data were represented in the form of mean ± standard deviation (Mean ± SD).

Kolmogrov-smirnov test was used to test normality of quantitative data. χ^2 and Fisher exact tests were used to examine qualitative data. Whereas, ANOVA and Kruskal–Wallis tests were used for normally and non-normally distributed quantitative data. Genetic association was performed by logistic regression. Results were expressed as nominal *P*, odds ratio (OR) and 95% confidence interval (CI). Results were considered significant if *p* value is ≤ 0.05.

3. RESULTS

Table 1 showed that there was no significant difference in the mean age or body mass index between PCOS group and controls (*P* = 0.310, *P* = 0.072, respectively).

LH, FSH, LH/FSH ratio and total testosterone levels were significantly higher in PCOS group compared to controls (*P* = 0.005, *P* = 0.018, *P* = 0.035, *P* < 0.001, respectively), Table 1. The genotype analysis showed that the heterozygote AG genotype was significantly more frequent in PCOS patients than controls [64.1% vs. 40%; OR = 2.68, 95% CI = 0.97-7.5, *P* = 0.033]. Following adjustment for BMI in the comparison between PCOS patients and the control group, this association with PCOS remained statistically significant (*P* = 0.036).

Table 1. Clinical and biochemical characteristics in PCOS and control groups

	PCOS group (n = 64)	Control group (n = 65)	*P*-value
Age (years)			
Mean ± SD	25.78±4.53	25.09±4.81	0.310
BMI (kg/m^2)			
Mean ± SD	29.80±6.12	27.12±5.43	0.072
LH (mIU/ml)			
Mean ± SD	10.11±12.50	4.16±5.56	0.005*
FSH (mIU/m l)			
Mean ± SD	6.46±5.40	4.34 ±2.98	0.018*
LH/FSH ratio			
Mean ± SD	2.95±5.17	1.04±1.33	0.035*
Total testosterone (ng/ml)			
Mean ± SD	0.86±0.27	0.48±0.21	*P* < 0.001*

- (*) significant, P>0.05; (**) highly significant, P>0.001,-BMI: body mass index, LH: luteinizing hormone, FSH: follicle stimulating hormone

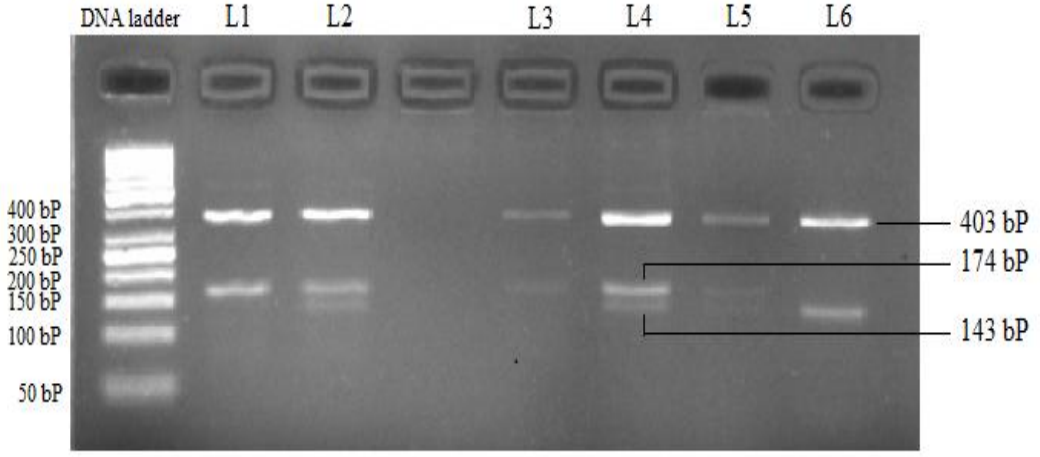

Fig. 1. Electrogram of DNA fragments for Ala307Thr polymorphism after digestion with *Eam1105I*: Homozygote A/A genotype was shown by the bands of 403 bP, 143 bP, and 31 bP (lane 6). Homozygote G/G genotype was shown by the bands of 403 bP and 174 bP (lanes 1 and 3). Heterozygote A/G genotype was shown by the bands of 403 bP, 174 bP, 143 bP, and 31 bP (lanes 2, 4, and 5). Note: The 31 bP bands were run off the gel

No significant differences were observed in the distribution of the homozygote AA or GG genotypes between the two groups (15.6% vs. 26.2% for GG genotype; 20.3% vs. 33.8% for AA genotype), Table 2.

Also, no significant difference was observed in the allele frequencies between PCOS group and controls [64.1% vs. 40%; OR = 0.95, 95% CI = 0.52-1.73, *P* = 0.85] and no significant differences were observed in dominant and recessive frequencies among PCOS and control groups.

According to *FSHR* genotypes, patients with PCOS were divided into three subgroups (carriers of GG, AA, or AG genotypes) (Table 3). PCOS women with homozygote GG, homozygote AA or heterozygote AG genotypes had comparable age and BMI. No significant differences were observed between levels of LH, FSH, or LH/FSH ratio among carriers of different *FSHR* genotypes. While, PCOS patients with heterozygote AG genotypes had significantly high levels of total testosterone compared to women with homozygote AA genotypes (*P* = 0.046).

Table 2. Allele frequencies and genotypes of the *FSHR* polymorphism among women with PCOS and healthy control women

	PCOS group (n = 64)	Controls (n = 65)	P^1- value	P^2- value	OR (95% CI)
Allele frequency					
G	61 (47.7%)	60 (46.2%)			1.00 (Reference)
A	67 (52.3%)	70 (53.8%)	0.85	0.90	0.95 (0.52-1.73)
General genotypes					
GG (Ala/Ala)	10 (15.6%)	17 (26.2%)			1.00 (Reference)
AG (Ala/Thr)	41 (64.1%)	26 (40%)	0.033*	0.036*	2.68 (0.97-7.5)
AA (Thr/Thr)	13 (20.3%)	22 (33.8%)	0.99	0.08	1.005 (0.31-3.23)
Dominant genotype					
GG	10 (15.6%)	17 (26.1%)			1.00 (Reference)
AG+AA	54(84.4%)	48 (73.8%)	0.14	0.14	1.91 (0.74-5.01)
Recessive genotype					
AG+GG	51 (79.7%)	43(66.2%)			1.00 (Reference)
AA	13 (20.3%)	22 (33.8%)	0.084	0.34	0.5 (0.21-1.19)

- Values are given as numbers (percent). OR = odds ratio; CI = confidence interval, P2 value: BMI adjusted P value

Table 3. Clinical and biochemical characteristics of PCOS patients according to FSHR genotypes

	Carriers of GG genotype (n = 10)	Carriers of AG genotype (n = 41)	Carriers of AA genotype (n = 13)	P^1-value	P^2-value
Age (years)					
Mean ± SD	24.33±3.94	26.40±4.54	24.73±5.14	0.196	0.212
BMI (kg/m2)					
Mean ± SD	28.88±5.75	29.85±6.47	27.65±4.90	0.393	0.523
LH (mIU/ml)					
Mean ± SD	10.16±10.80	11.29±14.10	6.38±7.16	0.799	0.224
FSH (mIU/ml)					
Mean ± SD	5.00±0.90	6.19±4.40	8.43±9.04	0.533	0.194
LH/FSH ratio					
Mean ± SD	2.08±2.17	2.89±3.12	3.82±10.16	0.664	0.581
Total testosterone (ng/ml)					
Mean ± SD	0.85±0.16	0.90±0.29	0.72±0.25	0.572	0.046*

-P1-value: AG versus GG; P2-value: AG versus AA, - BMI: body mass index, LH: luteinizing hormone, FSH: follicle stimulating hormone

4. DISCUSSION

The clinical and biochemical characteristics in PCOS and control groups were cited in Table 1. The results depicted in this table revealed that a considerable rise (by 2.5-fold) in levels of LH was accompanied by a significant increase in FSH levels and LH/FSH ratio in polycystic ovary syndrome patients than that of the control group. This is in agreement with previous studies [3,4].

Polycystic ovary syndrome is associated with alterations in the function of the Hypothalamic-Pituitary-Gonadal (HPG) axis that may result from increased frequency of the hypothalamic Gonadotropin Releasing Hormone (GnRH) pulse generator. The normal pulsatile secretion of LH is increased by an increase in LH pulse frequency and amplitude resulting in elevated LH and LH/FSH ratio [21,22].

Moreover, PCOS patients had significantly higher levels of total testosterone compared to controls (Table 1). These results are in agreement with previous studies [9,10,23]. This may be attributed to increased synthesis of testosterone precursors due to a dysregulation of cytochrome P450, (P450c17α), the rate-limiting enzyme in androgen biosynthesis in the theca cells of the ovary in PCOS. Hyperandrogenemia was detected in around 60% to 80% of PCOS cases and results in the common symptoms of androgen excess in PCOS women, as hirsutism, acne and alopecia [23].

Genotypic analysis of A307T polymorphism of *FSHR* gene showed that the heterozygote AG genotype was significantly more frequent in PCOS patients than controls [64.1% vs. 40%; OR = 2.68, 95% CI = 0.97-7.5, *P* = 0.033], Table 2. These results are in accordance with other studies which reported a significant increase in the Ala307Thr frequency among Japanese women with PCOS [8], Chinese women [12] and among women of Italian origin [13]. In contrast, the existence of significant differences in the allele distribution of *FSHR* polymorphism 307 in women with PCOS was not confirmed in women of Korean origin [9] and among Indian women [14].

The heterogeneity of results observed in different studies may be due to a variable definition of PCOS or to some intrinsic characteristics of *FSHR* polymorphism. Further, the *FSHR* genotype distribution varies among different populations, and ethnicity may deeply influence the distribution of allelic variants [24]. Also, a relevant environmental component (diet, exercise, etc.) contributes to PCOS pathogenesis, and PCOS is likely to result from the interaction of genetic with environmental factors [13].

Obesity and abdominal adiposity are typical clinical manifestations of PCOS patients. It has been reported that approximately 38-88% of PCOS women are overweight or obese [25]. In this study, the heterozygote Ala/Thr genotype was significantly more frequent in PCOS patients than controls, even after the adjustment of BMI. This finding suggests that the association of Ala307Thr *FSHR* polymorphism with PCOS susceptibility occurs irrespective of BMI, and confirms the previous study of Wu et al. [10], they observed no correlation between the different obesity standard of PCOS patients and the genotypes of Ala307Thr *FSHR* polymorphism.

In women of a reproductive age, FSH is an important hormone required for the development of follicles, oocyte maturation, and regulation of steroidogenesis in the ovaries. In addition, *FSHR* is involved in the regulation of the FSH levels and it belongs to G-protein coupled receptors, causing phosphorylation of target proteins and adenylate cyclase [9].

Position 307 codifies for an amino-acid located within the extracellular domain in the FSH binding region of the protein, it can affect the hormone-binding ability of the receptor and is crucial for FSH-mediated signal transduction events [11,26]. Amino acid alteration related to the corresponding SNPs might affect the post-translational modifications of the *FSHR* protein, hence the function of the receptor including FSH efficacy [3].

Treatment of infertility of PCOS patients consists of ovulation induction by clomiphene citrate (CC) and/or recombinant FSH (rFSH). Pharmacogenetic studies revealed the varied response of *FSHR* gene polymorphisms to exogenous FSH hormone in patients undergoing *in vitro* fertilization (IVF) [27,28]. Follicular stimulating hormone Ala307Thr polymorphism showed consistent predictive value for estimating the most optimal rFSH dosage in controlled ovarian hyperstimulation. Also, this polymorphism exhibited a potential for the pharmacogenetic assessment of the treatment of PCOS [29].

Interestingly, in a study in Italian PCOS women the *FSHR* Ala307Thr heterozygotes exhibited a higher ovarian responsiveness to exogenous rFSH than subjects homozygous for *FSHR* isoforms, furthermore, the percentage of women considerable as the "best-responders" was significantly higher among Ala307Thr subjects than among homozygotes [15]. The molecular mechanism by which this polymorphism might be altering the ovarian response is still unclear [30].

It has been suggested that the detection of *FSHR* 307 polymorphism could be used to estimate the appropriate FSH starting dose for ovarian stimulation in IVF, also, it could be useful to identify women at higher risk of ovarian hyperstimulation syndrome [13].

In the present work, the association of *FSHR* Ala307Thr polymorphism with clinical and biochemical parameters in PCOS women was examined. PCOS women with homozygotes GG, AA or heterozygote AG genotypes had comparable age and BMI. No significant differences were observed between levels of LH, FSH, or LH/FSH ratio among carriers of different *FSHR* genotypes. However, PCOS women with heterozygote AG genotypes had significantly higher levels of total testosterone compared to women with homozygote AA genotypes (Table 3). It has been suggested that *FSHR* variants are associated with the levels of testosterone and the presence of hyperandrogenism in PCOS subjects [31].

5. CONCLUSION

Our results demonstrated that the Ala307Thr polymorphism of *FSHR* is associated with PCOS in the studied Egyptian population. This polymorphism correlates with the increased levels of total testosterone among PCOS women.

ACKNOWLEDGEMENTS

We would like to thank the editor as well as the anonymous referees very much for their comprehensive comments which helped in improving the paper.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. March WA, Moore VM, Willson KJ, Phillips DI, Norman, et al. The prevalence of polycystic ovary syndrome in a community sample assessed under contrasting diagnostic criteria. Hum Reprod. 2010;25:544-551.
Available:http://www.ncbi.nlm.nih.gov/pubmed/19910321
2. Shabir I, Ganie MA, Zargar MA, Bhat D, Mir MM, Jan A, Shah ZA, Jan V, Rasool R, Naqati A. Prevalence of metabolic syndrome in the family members of women with polycystic ovary syndrome from North India. Indian J Endocrinol Metab. 2014;18:364-369.
Available:http://www.ncbi.nlm.nih.gov/pubmed/24944933
3. Unsal T, Konac E, Yesilkaya E, Yilmaz A, Bideci A, et al. Genetic polymorphisms of *FSHR*, CYP17, CYP1A1, CAPN10, INSR, SERPINE1 genes in adolescent girls with polycystic ovary syndrome. J Assist Reprod Genet. 2009;26:205-216.
Available:http://www.ncbi.nlm.nih.gov/pubmed/19387820
4. Fu L, Zhang Z, Zhang A, Xu J, Huang X, 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;30:717-721
Available:http://www.ncbi.nlm.nih.gov/pubmed/23536150
5. Shi Y, Zhao H, Cao Y, Yang D, Li Z, et al. Genome-wide association study identifies eight new risk loci for polycystic ovary syndrome. Nat Genet. 2012;44:1020-1025.
Available:http://www.ncbi.nlm.nih.gov/pubmed/22885925
6. George JW, Dille EA, Heckert LL. Current concepts of follicle stimulating hormone receptor gene regulation. Biol Reprod. 2011;84:7-17.
Available:http://www.ncbi.nlm.nih.gov/pubmed/20739665
7. Kuechler A, Hauffa BP, Koninger A, Kleinau G, Albrecht B, Horsthemke B, Gromoll J. An unbalanced translocation unmasks a recessive mutation in the follicle-stimulating hormone receptor (FSHR) gene and causes FSH resistance. Eur J Hum Genet. 2010;18:656-661.
Available:http://www.ncbi.nlm.nih.gov/pubmed/20087398

8. Casarini L, Pignatti E, Simoni M. Effects of polymorphisms in gonadotropin and gonadotropin receptor genes on reproductive function. Rev Endocr Metab Disord. 2011;12:303-321. Available:http://www.ncbi.nlm.nih.gov/pubmed/21912887
9. Gu BH, Park JM, Baek KH. Genetic variations of follicle stimulating hormone receptor are associated with polycystic ovary syndrome. Int J Mol Med. 2010;26:107-112. Available:http://www.ncbi.nlm.nih.gov/pubmed/20514429
10. Wu XQ, Xu SM, Liu JF, Bi XY, Wu YX, et al. Association between *FSHR* polymorphisms and polycystic ovary syndrome among Chinese women in north China. J Assist Reprod Genet. 2014;31:371-377. Available:http://www.ncbi.nlm.nih.gov/pubmed/24390680
11. Yan Y, Gong Z, Zhang L, Li Y, Li X, et al. Association of follicle-stimulating hormone receptor polymorphisms with ovarian response in Chinese women: A prospective clinical study. PLoS One. 2013;8:e78138. Available:http://www.ncbi.nlm.nih.gov/pubmed/24167601
12. Du J, Zhang W, Guo L, Zhang Z, Shi H, et al. Two *FSHR* variants, haplotypes and meta-analysis in Chinese women with premature ovarian failure and polycystic ovary syndrome. Mol Genet Metab. 2010;100:292-295. Available:http://www.ncbi.nlm.nih.gov/pubmed/20399696
13. Dolfin E, Guani B, Lussiana C, Mari C, Restagno G, et al. FSH-receptor Ala307Thr polymorphism is associated to polycystic ovary syndrome and to a higher responsiveness to exogenous FSH in Italian women. J Assist Reprod Genet. 2011;28:925-930. Available:http://www.ncbi.nlm.nih.gov/pubmed/21792664
14. Kambalachenu H, Paul S, Nellepalli S, Venkatachalam P. Study on follicle stimulating hormone receptor gene polymorphisms in South Indian women with polycystic ovarian syndrome. Am Med J. 2013;4:160-167. Available:http://thescipub.com/abstract/10.3844/amjsp.2013.160.167
15. 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;19:41-47. Available:http://www.ncbi.nlm.nih.gov/pubmed/14688154
16. Nishida C, Ko GT, Kumanyika S. Body fat distribution and non communicable diseases in populations: Overview of the 2008 WHO Expert Consultation on Waist Circumference and Waist-Hip Ratio. Eur J Clin Nutr. 2010;64:2-5. Available:http://www.ncbi.nlm.nih.gov/pubmed/19935820
17. Kosasa TS, Levesque LA, Taymor ML, Goldstein DP. Measurement of early chorionic activity with a radioimmunoassay specific for human chorionic gonadotropin following spontaneous and induced ovulation. Fertil Steril. 1974;25:211-216. Available:http://www.ncbi.nlm.nih.gov/pubmed/4814076
18. Odell WD, Parlow AF, Cargille CM, Ross GT. Radioimmunoassay for human follicle-stimulating hormone: Physiological studies. J Clin Invest. 1968;47:2551-2562. Available:http://www.ncbi.nlm.nih.gov/pubmed/5725274
19. Lashansky G, Saenger P, Fishman K, Gautier T, Mayes D, Berg G, Di Martino-Nardi J, Reiter E. Normative data for adrenal steroidogenesis in a healthy pediatric population: Age- and sex-related changes after adrenocorticotropin stimulation. J Clin Endocrinol Metab. 1991;73:674-686. Available:http://www.ncbi.nlm.nih.gov/pubmed/1651957
20. Sundblad V, Chiauzzi VA, Escobar ME, Dain L, Charreau EH. Screening of FSH receptor gene in Argentine women with premature ovarian failure (POF). Mol Cell Endocrinol. 2004;222:53-59. Available:http://www.ncbi.nlm.nih.gov/pubmed/15249125
21. Pfeifer SM, Kives S. Polycystic Ovary Syndrome in the Adolescent. Obstetrics and Gynecology Clinics of North America. 2009;36:129-152. Available:http://www.sciencedirect.com/science/article/pii/S0889854508001034
22. Elizabeth M, Leslie NS, Critch EA. Managing polycystic ovary syndrome: A cognitive behavioral strategy. Nursing for Women's Health. 2009;13:292-300. Available:http://www.ncbi.nlm.nih.gov/pubmed/19686552

23. Allahbadia GN, Merchant R. Polycystic ovary syndrome and impact on health. Middle East Fertility Society Journal. 2011;16:19-37.
Available:http://www.sciencedirect.com/science/article/pii/S111056901000141X
24. Kuijper EA, Blankenstein MA, Luttikhof LJ, Roek SJ, Overbeek A, et al. Frequency distribution of polymorphisms in the FSH receptor gene in infertility patients of different ethnicity. Reprod Biomed Online. 2010;20:588-593.
Available:http://www.ncbi.nlm.nih.gov/pubmed/21575851
25. Song do K, Lee H, Oh JY, Hong YS and Sung YA. FTO Gene Variants Are Associated with PCOS Susceptibility and Hyperandrogenemia in Young Korean Women. Diabetes Metab J. 2014;38:302-310.
Available:http://www.ncbi.nlm.nih.gov/pubmed/25215277
26. Chen DJ, Ding R, Cao JY, Zhai JX, Zhang JX, Ye DQ. Two follicle-stimulating hormone receptor polymorphisms and polycystic ovary syndrome risk: A meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2014;182:27-32.
Available:http://www.ncbi.nlm.nih.gov/pubmed/25218548
27. Mohiyiddeen L, Nardo LG. Single-nucleotide polymorphisms in the FSH receptor gene and ovarian performance: Future role in IVF. Hum Fertil. 2010;13:72-78.
Available:http://www.ncbi.nlm.nih.gov/pubmed/20184506
28. Simoni M, Casarini L. Mechanisms in endocrinology: Genetics of FSH action: A 2014-and-beyond view. Eur J Endocrinol. 2014;170:91-107.
Available:http://www.ncbi.nlm.nih.gov/pubmed/24288354
29. Laan M, Grigorova M, Huhtaniemi IT. Pharmacogenetics of follicle-stimulating hormone action. Curr Opin Endocrinol Diabetes Obes. 2012;19:220-227.
Available:http://www.ncbi.nlm.nih.gov/pubmed/22499219
30. Desai SS, Roy BS, Mahale SD. Mutations and polymorphisms in FSH receptor: functional implications in human reproduction. Reproduction. 2013;146:235-248.
Available:http://www.ncbi.nlm.nih.gov/pubmed/24051057
31. Valkenburg O, Uitterlinden AG, Piersma D, Hofman A, Themmen AP, et al. Genetic polymorphisms of GnRH and gonadotrophic hormone receptors affect the phenotype of polycystic ovary syndrome. Hum Reprod. 2009;24:2014-2022.
Available:http://www.ncbi.nlm.nih.gov/pubmed/19403562

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=844&id=3&aid=7084