FSH Receptor Gene Polymorphism in Indonesian Women with Polycystic Ovarian Syndrome (PCOS)

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Abstract. Polycystic ovary syndrome (PCOS) is a reproductive problem that often occurs among women in reproductive age, but until now, the etiopathogenesis of PCOS is still unclear. One of the alleles for candidate genes associated with the PCOS incident is the FSH receptor (FSHR) gene. This research was aimed at analyzing the genotypes within Asn680Ser FSHR with PCOS pathogenesis. The total research subject was 142 subjects. DNA isolation was used for performing the RFLP analysis toward Asn680Ser FSHR polymorphism. There was no mean value difference of the genotype distribution within Asn680Ser FSHR between the PCOS and non-PCOS groups ($p>0.05$). There was no difference of the basal FSHR levels based on the Asn680Ser FSHR genotyping variation between the PCOS and the non-PCOS groups ($p >0.05$). The Asn680Ser FSHR genotype does not determine the individual susceptibility to suffering from PCOS. There was no difference in basal FSH levels between PCOS and non-PCOS groups based on the Asn680Ser FSHR genotype variation. However, there are differences in the frequency distribution pattern of the FSHR genotype at position 680 in the PCOS group compared to the previous research findings in several countries. **Keywords**: FSHR, PCOS, Polymorphism

1. Introduction

Polycystic ovary syndrome (PCOS) is a reproductive problem that frequently happens among 5-10% of women in reproductive age to be fertile, however, the main cause has not been found yet up until now [1][2]. PCOS can be considered as a heterogeneous disorder with elevated androgen levels and various levels of reproductive and metabolic problems determined by the interaction of various genetic factors and environment [3]. The general illustrations for PCOS patients are including oligomenorrhoea or amenorrhea, syndrome of ovarian dysfunction characterized by the existence of anovulation, and polycystic ovarian morphology, hyperandrogenism such as hirsutism, acne, alopecia, and often followed by obesity [4]. Genome-wide association studies (GWAS) has identified some variances of genetic significantly related to PCOS. Up until now, a large number of genetic studies have identified more than 200 genes functionally related to PCOS. One of the genes related to PCOS is Follicle stimulating hormone (FSH) receptor gene [5].

FSH plays a role in inducing the proliferation of granulosa cells, the recruitment of secondary follicle, the selection of dominant follicle, and regulating the activity of aromatase enzyme in granulosa cells for converting androgen into estrogen (aromatization process). In running the function, FSH interacts with the receptor namely FSHR. FSH and LH will interact with the targeted cell and they will affect the function through a cell surface receptors in the form of integral proteins in the cellular membrane. In women, the affinity of FSH binding toward the receptor in the surface of follicle granulosa cells will determine the capacity of a follicular cell to proliferate and synthesize the estrogen [6].

The mutation in FSHR gene can cause a problem in the activity of the FSH receptor. One of the most investigated things is rs6166 FSHR SNP (Asn680Ser). From the previous studies, it was reported that polymorphism of the FSHR gene is Ser680 mutant allele associated with the lower response of receptor and it is significantly connected with the high level of basal FSH; besides, it changes the response towards the ovarian stimulation by using exogenous FSH for in-vitro fertilization [7] [8]. In most of PCOS patients, there is an increase in excessive secretion of LH that can result in the increase of androgen synthesis in ovarian. It has been found out an increased LH/FSH ratio (>3) [9]. The study in PCOS patients in *RSCM Jakarta* shows that 79% of the PCOS patients have an increased LH to FSH ratio. Both PCOS patients with and without insulin resistance experience an increased LH to FSH ratio of 81.5% and 90.9% respectively [10]

The first study conducted in Singapore at Thr307Ala and Ser680Asn, the variants of FSHR gene mutation related to the susceptibility to PCOS in 124 PCOS patients found that there was no mutation in the PCOS patient group. The study proceeded to analyze the polymorphism at the same point with 236 individuals classified in a healthy group as the control and the finding showed that the distribution was not different from the variants of allele and protein isoform in PCOS patients who had been investigated previously. The research finding showed that mutation in the coding area of the FSHR gene was not the factor of PCOS in women in Singapore [11]. In the study of polymorphism of FSHR Ser680Asn and Ala307Thr in Korea that involved 235 PCOS patients and 128 healthy individuals as the control subject, and all samples classified into reproductive age found that the polymorphism was significantly related to PCOS. The FSHR polymorphism is also associated with the concentration of endogenous serum FSH and Prolactin. However, based on the data, there was no hormone from the endocrine system related to other PCOS and no clinical pregnancy levels in PCOS patients recorded in that study [12].

In the report written by Du, et al., (2010), only a study in Chinese women revealed the significance between polymorphism of FSHR and PCOS. This study involved 60 PCOS patients and 92 healthy individuals as control. It stated that in one side, FSHR gene SNPs determines the susceptibility to PCOS and in another side; it also affects the sensitivity of the receptor of exogenous FSH during the therapy of induced ovulation [13]. Based on previous research reported that the Asn680Ser polymorphism of the FSH receptor gene is known to reduce FSH receptor activity with different results on race and ethnicity, this study aims to analyze the genotypes within Asn680Ser FSHR with PCOS pathogenesis in Indonesian Women.

2. Research Method

2.1. Subjects

The research subject was women at 18-40 years old who suffered from PCOS and non-PCOS women collected from the patients who underwent the IVF assisted fertilization program with a total of 142 people (Approval letter No. 106/H2.F1/ETIK/2013). The isolated DNA was collected from 2 subject groups namely women with PCOS who underwent the IVF assisted fertilization program diagnosed based on the Rotterdam 2003 criteria and had a willingness to be the research subject. The women with PCOS who underwent IVF assisted fertilization program and the infertility cause was diagnosed such as the male factor issues, fallopian tube, idiopathic infertility (unexplained), and had a willingness to be the research subject.

2.2. Fragment Amplification of Targeted DNA

The Asn680Ser rs 6166 polymorphism of the FSHR gene was identified by using PCR-RFLP technique based on the site of BsrI restriction enzyme (Promega) in the area of exon 10. The amplification of DNA fragment was performed by using PCR method with BioMix Red kit (BiolineTM) (IDT). Pairs of primer for the amplification of FSHR gene in exon 10 at position 680 (Asn680Ser), was left primer /forward (F): 5'GAG CAA GTG TGG CTG CTA TG 3', and right primer/reverse (R): 5'TGT AGA AGC ACT GTC AGC TC 3'. The amplification was conducted for 30 cycles, with initial denaturation at 95°C for 6 minutes, continued with the denaturation cycle at 95°C for 1 minute, annealing at 49°C for 1 minute, and elongation at 72°C for 1.5 minutes. At the end of the cycle, it was continued with elongation at 72°C for 5 minutes [14][15][16]. By using a pair of prime above, it would result in a PCR product with a size of 258bp. An amplicon was separated using electrophoresis technique in 2% agarose gel at a voltage of 80 volts for 1 hour.

2.3. Restriction Fragment Length Polymorphism (RFLP)

The restriction fragment was performed by using BsrI restriction enzyme (New England Biolabs). The digestion mixture was made using a composition consisting of a 10µl DNA fragment from the amplification result (DNA concentration ≈ 10µg) and 2µl buffer 3(1x), and 10 units of BsrI restriction enzyme, then ddH20 was added until the volume reached 30µL. The mixture was incubated at 65°C for 3 hours. The result of RFLP was detected using electrophoresis in 3% agarose gel in 1X TAE Buffer solution at a voltage of 80 volts for 1 hour. The visualization of DNA bands was observed using illuminator UV (UV long life™ Filter Spectroline® and it was captured using a digital camera. The result of RFLP toward FSHR gene of Asn680Ser using BsrI restriction enzyme was in the form of single DNA band that was not cut by BsrI restriction enzyme of 258pb in size with homozygous Asn/Asn genotype (wild type); it was formed two DNA bands of 136pb and 122pb respectively with homozygous Ser/Ser genotype (mutant type) and three DNA bands of 258pb, 136pb, and 122pb respectively with Asn/Ser genotype (heterozygote).

2.4. Sequencing Analysis

The sequencing of DNA was conducted using the Sanger method to determine the polymorphic site and the change of nucleotide from some samples by using the result of amplicon that had polymorphic sites collected from the result of PCR-RFLP. The sequencing of DNA was conducted in the 1st Base, Singapore. The analysis of sequencing result was done using *Finch TV program* Version 1.4.0 and then the process of BLAST in the NCBI site to conduct alignments between the DNA sequences of Asn680Ser RefSeq NG_008146.1 FSHR gene and the DNA sequences from the sample taken from a sequencing result.

2.5. Statistical Analysis

The statistical analysis conducted to determine the relevancy between the Asn680Ser polymorphism of the FSHR gene and the susceptibility of an individual towards PCOS was the Pearson Chi-square test (X^2 test) with a p-value of ≤ 0.05. Statistical analysis namely one way ANOVA test ($p \leq 0.05$) was done to prove the difference of the variation in the genotype of FSHR in exon 10 at position 680 and the basal FSH levels in both PCOS and non PCOS women [17].

3. Results and Discussion

3.1. The Characteristic of research subject

The total research subject was 142 people consisting of 66 PCOS women (46.6%) and 76 non-PCOS women (53.5%) (Table 1).

Table 1. The Characteristic of the Research Subjects in PCOS and Non-PCOS groups.

Paramater	Group		*p-value*
	PCOS	Non PCOS	
Age (years)	32.21 ± 0.499 (n=66)	32.80 ± 0.516 (n=76)	0.416[b]
BMI (Kg/m^2)	24.425 ± 0.585 (n=53)	22.84 ± 0.494 (n=59)	0.034[b]
FSH (mIU/mL)	5.715 ± 0.299 (n=48)	5.186 ± 1.033 (n=33)	0.233[b]
LH (mIU/mL)	5.626 ± 1.444 (n=56)	6.234 ± 0.976 (n=65)	0.002[b]
Prl (ng/mL)	12.27 (3.26-148.40) (n=50)	15.98 (6.32-179.80) (n=25)	0.018[a]
P (ng/mL)	2.133 ± 1.084 (n=22)	0.740 ± 0.163 (n=44)	0.083[b]
E2 (pg/mL)	32.40 (5.0-198.6) (n=41)	41.49 (2.07-300) (n=33)	0.132[a]
AMH (ng/mL)	5.705 ± 1.092 (n=49)	2.988 ± 0.793 (n=43)	0.000[b]

The data is presented in mean value ± SEM (*Standard Error Mean*)
[a] Non-parametric Mann Whitney test, significant at a p-value of <0.05
[b] Independent t-test, significant at a p-value of <0.05; n = total subject

3.2. Genotyping Analysis

The PCR technique was conducted to amplify the targeted DNA for FSHR in exon 10 at position 680 containing polymorphic sites and recognized by BsrI restriction enzyme that has a restriction site (5'AGTGGN3'). The polymorphism of FSHR gene at position 680 (Asn680Ser) was detected by finding the amino acid code namely Serine (A**G**T) (mutant type), which replaced the amino acid code namely Asparagine (A**A**T) (wild type); there was a change from nucleotide base namely Adenine into Guanine (A→G). The DNA amplicon was cut by BsrI enzyme to know the susceptibility of DNA toward restriction enzyme. If the result of the PCR was cut, the sample experienced mutation at that point. The result of DNA amplicon cutting would be electrophoresed on 3% agarose gel afterward (Figure 1.)

It was obtained three RFLP illustrations namely homozygote Ser/Ser (mutant type) with a restriction site for BsrI so that the PCR amplicon was divided into two bands of 136pb and 122pb respectively, Asn/Asn homozygote (wild type) that was not cut so that it has one band of 258pb, heterozygote Asn/Ser that has RFLP illustration with three DNA bands of 258pb, 136pb, and 122pb respectively. To clarify the polymorphism of the FSHR gene at position 680, the sequencing was done towards 10 samples that were randomly chosen.

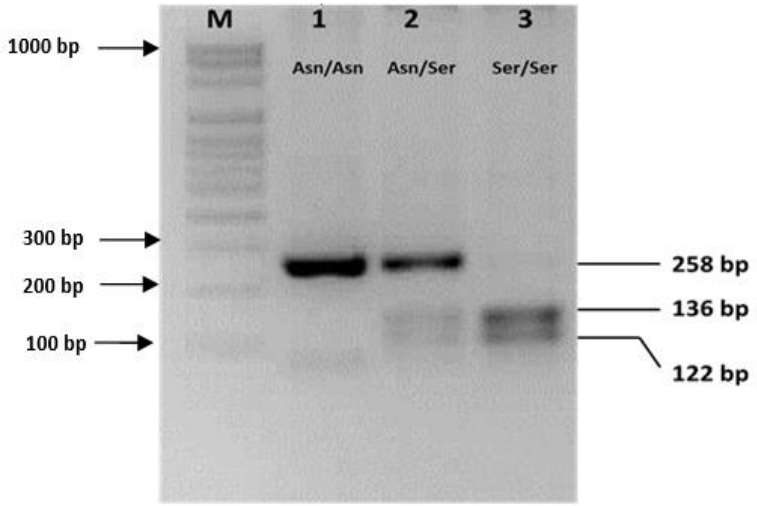

Figure 1. The result of the PCR-RFLP for Asn680Ser FSHR gene was cut using BsrI Enzyme M= DNA 100bp marker, well 1 = sample 1; homozygous Asn/Asn genotype, well 2 = sample 2; heterozygous Asn/Ser genotype, well 3 =sample 3; homozygous Ser/Ser genotype.

3.3. Sequencing Analysis

The result of sequencing showed that there was a change from nucleotide base namely Adenine into Guanine (A→G) at position 196710 that caused the change in amino acid code namely Asparagine (A**A**T) (wild type) into Serine (A**G**T) (mutant type) (see Figure 2.)

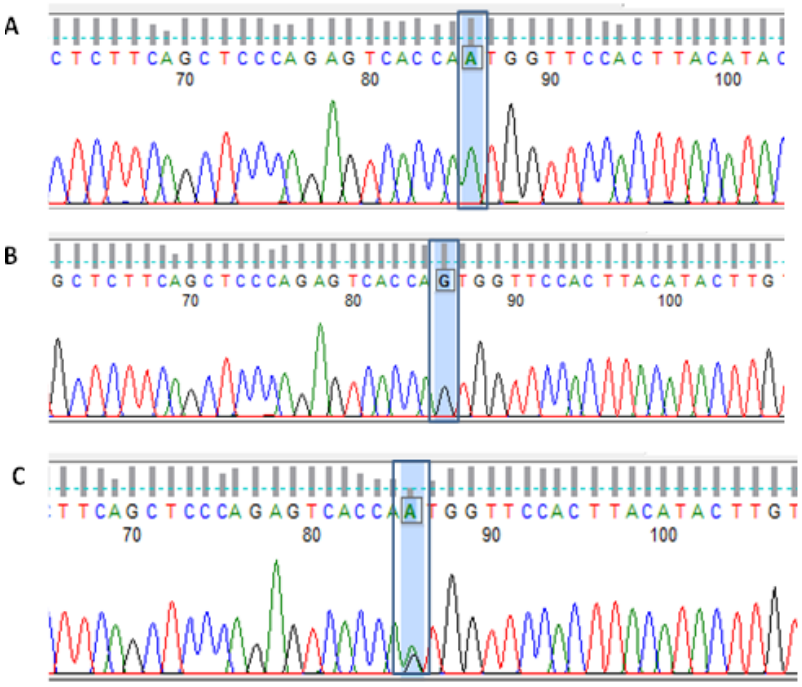

Figure 2. The result of sequencing uses the primer of FSHR at position 680. The result of sequencing shows the change in base namely the wild type adenine (A), into Guanine (G). A. The illustration of a sequencing result with a wild type sample (Asn/Asn). B. The sequencing result with a mutant type sample (Ser/Ser) C. The sequencing result with a sample that has a heterozygote type (Asn/Ser).

3.4. The Frequency Distribution of Genotype and Allotype within Asn680Ser FSHR gene in exon 10

In PCOS group, around 36 people (54%) had Asn/Asn genotype, 24 people (37%) had Asn/Ser genotype, and 6 people (9%) had Ser/Ser genotype. In non-PCOS group, around 40 people (52.63%) had Asn/Asn genotype, 25 people (32.90%) had Asn/Ser genotype, and 11 people (14.47%) had Ser/Ser genotype.

In the PCOS group, the frequency of Asn allele was 0.73 (73%) and Ser allele was 0.27 (27%). In the non-PCOS group, the frequency of Asn allele was 0.69 (69%) and Ser allele was 0.31 (31%). It is the comparison between the distribution of genotype and allotype within Asn680Ser FSHR gene in both PCOS and non-PCOS groups. The distribution of Asn680Ser FSHR genotype in PCOS group was in accordance with Hardy–Weinberg law ($p>0.05$), while in the non-PCOS group, it was not in accordance with Hardy–Weinberg law ($p<0.05$). The Asn allele that is the normal type (wild type) has a more dominant proportion towards Ser allele as a mutant allele.

Table 2. The frequency distribution of genotype and allotype within Asn680Ser FSHR in exon 10 in both PCOS group and non-PCOS group.

Group	Genotype			Alleles	
	Asn/Asn	Asn/Ser	Ser/Ser	Asn	Ser
PCOS (66)	54% (36)	37% (24)	9% (6)	0.73	0.27
Non-PCOS (76)	52.63% (40)	32.90 (25)	14.47 (11)	0.69	0.31

The result of the Person-Chi-Square test showed that the distribution of Asn680Ser FSHR genotype in PCOS group was not extremely different from the non-PCOS group ($n= 142$; $df=2$; p-value >0.05) and the distribution of FSHR allotype (Asn680Ser) in PCOS group was not extremely different from the non-PCOS group ($n=284$; $df=1$; p-value>0.05). This research finding showed that the allotypes within Asn680Ser FSHR gene in exon 10 did not affect the susceptibility for the occurrence of polycystic ovarian syndrome.

The genotype frequency within Asn680Ser FSHR in this research showed that the individual with homozygous Asn/Asn genotype (wild type) had a higher frequency than an individual with heterozygote Asn/Ser and an individual with homozygote Ser/Ser (mutant type) in both PCOS group and non-PCOS group. The result of the Person-Chi-Square test showed that the genotype distribution within Asn680Ser FSHR in PCOS group was not extremely different from the non-PCOS group ($p>0.05$) and the allotype distribution within Asn680Ser FSHR in PCOS group was not different from the non-PCOS group ($p >0.05$).

The distribution of Asn680Ser FSHR genotype in PCOS group in this research was different if it was compared to the similar research finding conducted by the previous researchers. In this research, the Asn/Asn genotype was more dominant than other genotype variants such as Asn/Ser and Ser/Ser. The previous studies related to the polymorphism of FSHR gene have been reported with various results in different races. Sudo, et al., [18] investigated the influence of the FSHR gene polymorphism variant at codon 307 (Alanine → Threonine) and at codon 680 (Asparagine → Serine) toward the ovarian function in 522 women in Japan. The frequency of Asn680Ser FSHR genotype in PCOS group was 16.7% Asn/Asn, 66.7% Asn/Ser, and 16.7% Ser/Ser. In the PCOS group, the percentage of Thr307-Asn680/Ala307-Ser680 variant was significantly bigger than a woman with a normal ovulation cycle. Conway, et al., [19] investigated the prevalence of FSHR isoforms in 93 PCOS women in England. The frequency of genotype was 24.7% Asn/Asn, 51.6% Asn/Ser, and 23.7% Ser/Ser. There was no significant difference in genotype distribution [20]. The research about Asn680Ser FSHR polymorphism in 518 PCOS women in a Caucasian population obtained a genotype frequency as follows: 24.8% Asn//Asn, 50.1% Asn/Ser, and 25.1% Ser/Ser [21]. Bon, et al reported the frequency of Asn680Ser

FSHR genotype in 235 PCOS women in Korea namely 58.72% Asn/Asn, 38.72% Asn/Ser, and 2.56% Ser/Ser [19]. Wahyono, et al investigated the FSHR polymorphism genotype in 91 women at reproductive age and normogonadotropic. The finding showed that among the Indonesian women population, the frequency of FSHR genotype at position 680 was 48.4% Asn/Asn, 41.7% Asn/Ser, and 9.9 % Ser/Ser [15].

Figure 3 shows the comparison of the frequency of Asn680Ser FSHR genotype in PCOS women in various race populations. It is very clear the there is a difference in the frequency distribution pattern of FSHR genotype at position 680. Caucasian population (Europe), represented by England, Italy, and Turkey, has a higher genotype frequency in the form of heterozygote Asn/Ser, while for Mongoloid population (Asian) represented by Japan, China, Korea, Thailand, and Indonesia, the higher genotype frequency is homozygote Asn/Asn (wild type), except for Japan. The frequency of Asn680Ser FSHR genotype in Japanese people population is almost similar to the Caucasian race namely heterozygote Asn/Ser that is higher. The distribution pattern of the European population is 1:2:1 for homozygote:heterozygote:homozygote ratio. Apparently, there is an association between the distribution of Asn680Ser FSHR genotype and the geographical variations and races. To prove those things, further research with more number of samples is needed. Besides, the research should be multicenter.

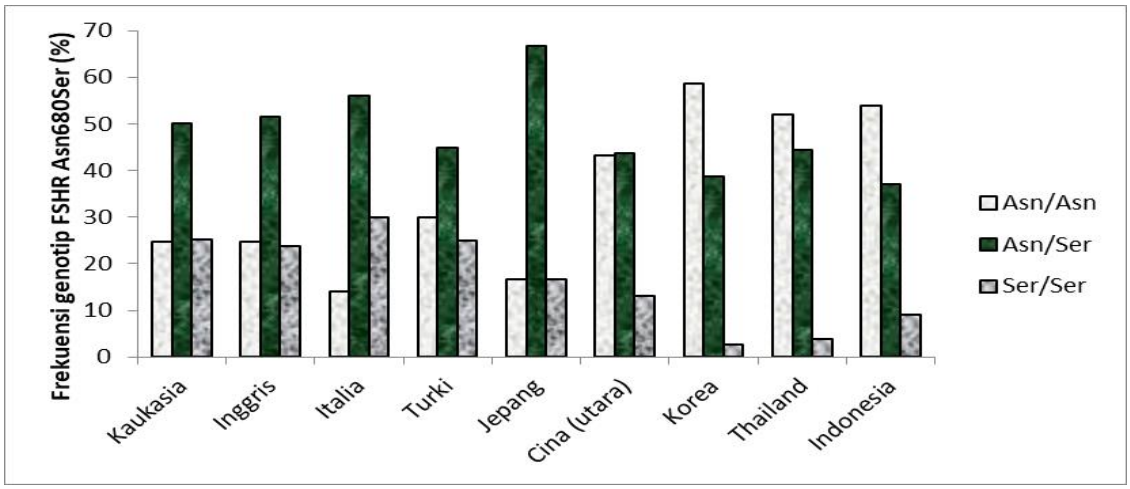

Figure 3. The comparison of the frequency of Asn680Serine FSHR genotype in PCOS women in a Caucasian population, England, Italy, Turkey, Japan, China (north), Korea, Thailand, and Indonesia.

The analysis of FSHR gene polymorphism at codon 680 in PCOS women has been conducted by the previous researchers in 50 PCOS patients compared to the normal women. The result of genotype frequency in PCOS group was 32% Asn/Asn, 44% Asn/Ser, and 24% Ser/Ser while in normal group was 34% Asn/Asn, 50% Asn/Ser, and 16% Ser/Ser [22]. However, the result is different from the illustration of Asn680Ser FSHR genotype frequency found in this research. In the previous studies, it was obtained a higher frequency of heterozygous Asn/Ser genotype (44%) than two genotype variations namely Asn/Asn (32%) and Ser/Ser (24%). In this research, it is shown that the higher frequency of homozygous genotype namely Asn/Asn (54%) than the other genotype variations namely Asn/Ser (37%) and Ser/Ser (9%).

Based on the explanation above, the distribution of FSHR genotype at position 680 between the PCOS group and non-PCOS group did show a significant difference. This explains that there is no relationship between the polymorphism of Asn680Ser FSHR gene and the pathogenesis of PCOS in PCOS women in Indonesia. It is in line with the research finding in Thailand about the polymorphism of FSHR in women who suffer from chronic anovulation [23]. The study conducted by Valkenburg, et al [21] in Caucasian women stated that the FSHR variant was not associated with the risk of being affected by PCOS; yet the FSHR variant was strongly associated with the severity level of PCOS

characteristic illustration such as gonadotropin levels and hyperandrogenism. The result of a meta-analysis study conducted by Dao, et al [24] reported that there was no relationship between two FSHR polymorphisms namely Thr307Ala and Asn680Ser and the susceptibility toward PCOS. The FSHR polymorphism was not associated with the increased risk of PCOS.

The result of a study on Asn680Ser FSHR polymorphism associated with the pathogenesis of PCOS in PCOS women in Korea successfully proved that Asn680Ser FSHR was identified as the potential factor for the change of PCOS pathophysiology. In that study, it was shown that the Ser680Asn FSHR polymorphism caused infertility and folliculogenesis that deviates in PCOS patients [19]. The pathogenesis of PCOS can be caused by the other involved genes by the regulation of folliculogenesis, the regulation of synthesis, and androgen action, the regulation of synthesis, and insulin action. It is associated with the hyperandrogenemia, the insulin resistance or the increased level of LH associated with the anovulation situation as the cause of infertility in PCOS women. Another factor that plays a role in determining the severity of PCOS is a gene involved in lipid metabolism and chronic inflammation [25][26][21].

The different ovarian response and the success of ovarian stimulation towards the FSH stimulation depend on the FSHR genotype. The FSHR genotype variation at codon 680 from each individual that affects the condition has been proven by some previous researchers [27]. Other researchers found that the splicing alternative that could affect the ligand-binding part in the extracellular domain from the FSHR receptor caused the reduced cAMP activation in the FSHR variants compared to the normal one. The research was successful in identifying that the FSHR alternative splicing variant (deletion in exon 2) was associated with the reduced follicle growth. It is supposed that the different individual response toward the ovarian stimulation in the in-vitro fertilization program [26]. One of the problems of PCOS patients is the different response towards the ovarian stimulation and the low oocyte quality that can affect the pregnancy success rate, which until now, it cannot be explained well. Further knowledge about the etiology that can help in determining the parameter that determines the response of an individual towards the ovarian stimulation in an assisted reproductive program in treating infertility in PCOS [12][27][28].

3.5. The relationship between Asn680Ser FSHR genotype and basal FSH levels in both PCOS and non-PCOS groups

A statistical analysis namely ANOVA test to prove the difference between the FSHR genotype variation at position 680 in exon 10 toward the basal FSH levels in each PCOS group and non-PCOS group. The result of the one-way ANOVA test in PCOS group is shown in Table 3.

Table 3. The relationship between the Asn680Ser FSHR genotype variation and the basal FSH levels in PCOS group.

Genotype	n	The Average of basal FSH levels	p
Asn/Asn	27	5.774 ± 1.471	
Asn/Ser	17	5.308 ± 1.477	0.512
Ser/Ser	4	6.125 ± 2.458	

Based on the Asn680Ser FSHR genotype variation namely Asn/Asn, Asn/Ser, and Ser/Ser, there was no significant difference in the average level of basal FSH levels in PCOS group ($p > 0.05$). The result of the one-way ANOVA test in the non-PCOS group is shown in Table 4.

Table 4. The relationship between the Asn680Ser FSHR genotype variation and the basal FSH levels in the non-PCOS group.

Genotype	n	The average of basal FSH levels	p
Asn/Asn	20	6.159 ± 1.801	
Asn/Ser	11	6.701 ± 2.298	0.709
Ser/Ser	2	5.800 ± 0.424	

Based on the Asn680Ser FSHR genotype variation namely Asn/Asn, Asn/Ser, and Ser/Ser, there was no significant difference in the average of basal FSH levels in the non-PCOS group ($p > 0.05$).

The average of basal FSH levels was 5.715±0.299 mIU/mL in the PCOS group and 5.186±1.033mIU/mL in the non-PCOS group. It is seen that the average of basal hormone in PCOS group tends to be higher than the non-PCOS group, however, the difference is not statistically significant ($p>0.05$). The basal FSH levels in both PCOS group and non-PCOS group are still within normal limits of around 5-9IU/L [30]. The average basal FSH levels based on the genotype variation in PCOS group show a difference, but based on the statistical analysis, the difference is not significant ($p >0.05$).

Table 4 shows a tendency that Ser/Ser genotype group has a higher average basal FSH level of 6.125 ± 2.458 mU/mL than the Asn/Asn genotype group and Asn/Ser have namely 5.774 ± 1.471mlU/mL and 5.308 ± 1.477mlU/mL respectively. In the non-PCOS group, the highest average of basal FSH levels was in Asn/Ser genotype that was 6.701±2.298 mlU/mL, while the Asn/Asn genotype was 6.159± 1.801mlU/mL and Ser/Ser genotype was 5.800±0.424 mlU/mL. It is shown the there is a difference but it is not statistically significant ($p >0.05$).

The previous research findings stated that the individual as the carrier of Ser680 allele had a higher basal FSH level than the individual the carrier of Asn680 allele had [30][18][31]. This research could not prove that there was a significant difference in basal FSH levels in both allele groups. Nevertheless, it tended to have a difference in basal FSH levels based on FSHR genotype variation in both PCOS group and non-PCOS group.

The inconsistency of result obtained from some studies that investigated the relationship between polymorphism and PCOS has been reported by some researchers. It might be because of the methodological reason such as Population stratification (genetic heterogeneity, the history of the population), the definition of illness/conflicting characteristics, problems in selecting the subjects or an incidental finding. One of the main problems with the study about candidate gene was the sample size that was frequently insufficient. The inconsistent result might occur because of the characteristic for polymorphism such as incomplete penetration, genetic heterogeneity, and interaction between genes or interaction between gene and environment. Other weaknesses of using candidate gene/marker to analyze the association are including the fact that only a small part of the genome that is being studied, and this is performed independently from each interaction that may be involved [12]. Another thing that should be considered is the responsibility factor toward the association of PCOS with some disturbances, which is important for clinical diagnosis in PCOS patients.

4. Conclusion

Based on this research finding, it can be concluded that: (1) there is a frequency distribution pattern of FSHR genotype at position 680 in PCOS group that is different from the previous research findings, (2) Asn680Ser FSHR genotype does not determine the susceptibility of an individual to suffer from PCOS, and (3) there is no difference of basal FSH levels in women with PCOS compared to non PCOS patients based on the Asn680Ser FSHR genotype.

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