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# Polymorphism gen Follicle Stimulating Hormone Receptor Ala307 Thr (rs 6165) And Ser 680 Asn (rs 6166) Related To Polycystic Ovary Syndrome With Insulin Resistance

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## Abstract

Follicle stimulating hormone (FSH) has an important role in female reproduction, follicular development and ovarian steroidogenesis, through binding to FSH with its receptors in ovarian granulosa cells. The study design used was a study with an observational approach with a cross sectional approach, which is a group of cases originating from subjects with Polycystic Ovary Syndrome (SOPK). Wahidin Sudirohusodo and hospital education network and private hospitals. there was no significant relationship between the occurrence of FSHR Ala307Thr gene polymorphism with the incidence of insulin resistance Odd Ratio, 95% Confidence Interval 0.69 (0.23-2.12) with a value of p = 0.518. The same was also found in the Ser680Asn FSHR gene polymorphism with the incidence of insulin resistance Odd Ratio, 95% Confidence Interval 0.70 (0.27-1.84) with a p value = 0.470, as well as the polymorphism of the G935A LHR gene with the incidence of insulin resistance Odd Ratio , 95% Confidence Interval 2.24 (0.88-5.73).

***Keywords:*** **Ala 307Thr, Ser 680 Asn, Restensi insulin, SOPK**

**Introduction**

Polycystic ovary syndrome (PCOS) is an endocrinopathic disorder that most often occurs in women of reproductive age with an incidence of 4 to 20%. Follicle stimulating hormone (FSH) has an important role in female reproduction, follicular development and ovarian steroidogenesis, through binding to FSH with its receptors in ovarian granulosa cells[1].

Other genetic factors that play a role in SOPK are LHB and LHCGR polymorphisms. Some studies want to prove this but have opposite results with each other. Bassiouny and friends first demonstrated the link between LHCGR G935A SNP and PCOS in women in Egypt. El-Shal AS and colleagues (2015) succeeded in proving the linkage of gene polymorphisms (LHB G1502A and LHCGR (G935A and ins18LQ) and SOPK events Research that evaluates the relationship of these polymorphisms to the occurrence of PCOS is still very limited, but it is very important to continue evaluating the occurrence of SOPK[2,3,4].

Research conducted on SOPK research samples showed a wide variety of polymorphisms of A307T, 56% N680S, 30% S680S and 14% N680N. reported the presence of FSHR Ala307Thr polymorphisms and Ser680 Asn in PCOS women in [5.6,7].From a meta-analysis of several studies, Seoul totaling 235 PCOS patients and 128 control patients, they found that Ser 680Asn from FSHR was significantly associated with PCOS, it turns out that also studies in South Korea from 377 PCOS samples and 388 controls found a significant association of Ala307Thr gene polymorphism and Ser680Asn with SOPK. Research in Turkey shows a significant relationship, whereas in caucasian women there is no meaningful relationship[8,9].

Insulin resistance and hyperinsulinemia are key factors in the pathogenesis of ovulation disorders and hyperandrogenism in PCOS. Increased adrenal activity is thought to cause insulin receptor phosphorylation which causes insulin resistance. Hyperinsulinemia also decreases the production of sex hormone binding globulin (SHBG) in the liver so that free testosterone levels will increase[10,11,12]. Hormonal abnormalities are associated with carbohydrate imbalance, hyperinsulinemia and insulin resistance, with consequences developing into type 2 diabetes and impacting approximately 50% in PCOS patients both obese and thin 13,14. A meta-analysis of 28 studies found lower insulin sensitivity in women with PCOS[15,16.]

Considering SOPK can occur with insulin resistance and without insulin resistance as well as several studies on the polymorphism of the Ala 307Thr gene (rs 6165) and the Ser680Asn gene (rs 6166), so it is necessary to know whether this insulin resistance is related to polymorphisms in these genes. Insulin resistance and hyperinsulinemia are key factors in the pathogenesis of ovulation disorders and hyperandrogenism in PCOS[17,18,5]. In the ovary, high insulin levels will stimulate the enzyme 17α-hydroxylase which will increase the process of converting progesterone to androstendione. Hyperperulinulinemia will increase the activity of the pituitary-pituitary-adrenal axis. Increased adrenal activity is thought to cause insulin receptor phosphorylation which causes insulin resistance. Hyperinsulinemia also decreases the production of sex hormone binding globulin (SHBG) in the liver so that free testosterone levels will increase[19,20]

**MAT**

**RIALS AND METHODS**

The study design used was an observational approach with a cross sectional approach, which is a group of cases originating from subjects with Polycystic Ovary Syndrome (PCOS).

The location of the study was conducted at the RSUP dr. Wahidin Sudirohusodo and hospital education network and private hospitals. Sampling was carried out in Prodia clinical laboratory, for examination of fasting blood glucose and fasting insulin in the calculation of HOMA-IR and NECHRI Laboratory of Hasanuddin University Makassar for examination of FSH receptor polymorphisms and LH receptors.

The study was conducted in January 2019 - February 2020 study sample that is all female sex patients aged 25 to 40 years, the selection method until is by consecutive sampling. the number of samples was 23 research samples for each group, namely 23 groups with HOMA-IR ≥2 and 23 groups with HOMA-IR < 2.

**PCR Real Time Checking**

Genomic DNA was extracted from peripheral blood samples with the DNA Wizard Purification Kit (Promega, Madison, WI). Allelic discrimination is carried out using the MGB-NFQ primer / TaqMan probe assay on the ABI Prisma 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). Plug pairs and probes for FSHR p. Thr307Ala and FSHR p. Asn680Ser is assays-on-demand, C-

2676873_30 (Applied Biosystems), and assays-on-demand, C-2676874_10 (Applied Biosystems), respectively. The PCR mixture consists of 10 μL from TaqMan Universal PCR Master Mix 2 × (Applied Biosystems) and 25 ng DNA. The PCR cycle consists of one 2-min cycle at 50 ° C, and 1 10-min cycle at 95 ° C, followed by 40 cycles at 95 ° C for 15 s and 60 ° C for 1 minute.

In this study primary data were obtained using questionare, on the sheets provided, then record the results of physical and laboratory examinations on the sheets provided. Polymorphism of FSHR 307, 680 and LhR 935 and HOMA IR were examined. After the primary data is collected and recorded, the data obtained is organized and processed using the SPSS computer program.

**RESULT**

**Tabel 1.** Characteristic of responden

| | **N** | **Result** |
|---|---|---|
| Age in a year (mean, SD) | 83 | 27.9 ± 3.9 |
| Age (N, %) | | |
| ≥ 30 Year | 23 | 27.7 |
| < 30 Year | 60 | 72.3 |
| Weight in kg (mean, SD)<br>Height in cm (mean, SD) | 83 | 65.5 ± 8.5 |
| High mass indeks cm (mean, SD) | 83 | 157.2 ± 5.7 |
| Body Mass indeks (mean, SD) | 83 | 26.5 ± 3.0 |
| Body Mass indeks (N, %) | | |
| Obesity | 46 | 55.4 |
| Non obesitity | 37 | 44.6 |
| Polimorfisme gen (N, n%) | | |
| Ala307Thr | 83 | 15 (18.1) |
| Ser680Asn | 83 | 23 (27.7) |
| G935A | 83 | 56 (67.5) |
| Dosis (median, interquartile range) | | |
| Fasting Sugar (mg/dl) | 83 | 90 (84-98) |
| Insulin (μU/ml) | 83 | 10.2 (5.3-16.8) |
| HOMA-IR | 83 | 2.4 (1.1-4.1) |

Table 1, shows that the mean age and standard deviation (SD) is 27.9 ± 3.9 which found 23 people (27.7%) in the age group ≥30 years and in the age group <30 years as many as 60 (72.3%) people, then the mean and sd for body weight and height respectively mean ± sd 65.5 ± 8.5 and mean ± sd 157.2 ± 5.7.

From a total of 83 samples, there were 46 (55.4%) samples that resulted in Body Mass Index (BMI) in the obesity category while non-obese were 37 (44.6%) Sample. The mean and standard deviation for BMI of the whole sample is mean ± sd 26.5 ± 3.0. the results of Body Mass Index (BMI) in the category of obesity while non-obese as many as 37 (44.6%) samples. The mean and standard deviation for BMI of the whole sample is mean ± sd 26.5 ± 3.0

Based on the examination of fasting blood sugar, fasting insulin and IR HOMA obtained median and interquartile range (IQR) respectively 90 (84-98) mg / dl, 10.2 (5.3-16.8) μU / ml and 2.4 (1.1- 4.1)

**Table 2**
**Content HOMA IR with Polimorfisme gen Ala307Thr,**
**And Ser608Asn FSHR**

| | **N** | **HOMA-IR (median, IQR)** | ***p*-value*** |
|---|---|---|---|
| FSHR Ala307Thr | | | |
| Negative | 68 | 2.57 (1.11-3.96) | 0.519 |
| Positive | 15 | 1.98 (1.16-4.30) | |
| FSHR Ser680Asn | | | |
| Negative | 60 | 2.57 (1.12-4.05) | 0.472 |
| Positiv | 23 | 1.98 (1.13-4.27) | |
| Positive | 56 | 2.76 (1.17-4.38) | |

IQR: interquartile range, *Uji Mann-Whitney

There was no significant difference in IR HOMA levels between polymorphisms and without polymorphisms in all FSHR genes namely Ala307Thr (p = 0.519) and Ser608Asn (p = 0.472)

**Table 3**.
Relationship of FSHR Ala307Thr polymorphism, And Ser680Asn
to insulin resistance

| | **n** | **n (%)** | **OR [95% CI]** | ***p*-value** |
|---|---|---|---|---|
| FSH Ala307Thr | | | | |
| Negative | 68 | 38 (55.9) | reference | 0.518 |
| Positive | 15 | 7 (46.7) | 0.69 [0.23-2.12] | |
| FSH Ser680Asn | | | | |
| Negative | 60 | 34 (56.7) | reference | 0.470 |
| Positive | 23 | 11 (47.8) | 0.70 [0.27-1.84] | |

[a]association based on univariate logistic model. The number of positives (n) of the total population examined (N). OR: Odds ratio, CI: Confidence intervals.

Table 3 shows that there is no significant relationship between the occurrence of FSHR gene Ala307Thr gene polymorphism with the incidence of insulin resistance Odd Ratio, 95% Confidence Interval 0.69 (0.23-2.12) with a value of p = 0.518. The same was also found in the Ser680Asn FSHR gene polymorphism with the incidence of insulin resistance Odd Ratio, 95% Confidence Interval 0.70 (0.27-1.84) with a p value = 0.470, as well as the polymorphism of the G935A LHR gene with the incidence of insulin resistance Odd Ratio , 95% Confidence Interval 2.24 (0.88-5.73).

**Table 4**.
Multivariate analysis between polimorfisme FSHR Ala307Thr, FSHR Ser680Asn to resistensi insulin[a]

| | **adjusted OR [95% CI]** | ***P*-value** |
|---|---|---|
| Polimorfisme FSHR Ala307Thr | 0.69 [0.18-2.70] | 0.590 |
| Polimorfisme FSHR Ser680Asn | 0.57 [0.17-1.92] | 0.367 |

[a]Multivariate model. CI: Confidence intervals.

In multivariable analysis of these two polymorphisms it was found that the FSHR polymorphism both Ala307Thr with an odds ratio of 0.69 and Ser680Asn with an odds ratio of 0.57 had nothing to do with the incidence.

## DISCUSSION

FSH receptor position codon 307 is clearly known that plays an important role for ligand receptor interaction, whereas position 680 which is located intra-cellular is involved in transduction of the FSH signal [21].This was proven by Valkenburg and colleagues, the polymorphism of the Ser 680 allele had low estradiol levels when that is stimulated with FSH when undergoing assisted reproductive technology programs.

This shows that the polymorphism that occurs in the FSH receptor can cause a decrease. In the sensitivity of the receptor to thein the sensitivity of the receptor to the FSH hormone with insulin resistance there is a A polymorphism in genes that encode insulin-like growth factor (IGF-2) which is thought to stimulate androgen secretion[22,23].

The results of his study in Italy in reporting from 40 samples contained 26 (65%) Ala307Thr FSH receptor gene polymorphisms, Wu Xue-qing and friends in 2014 in North China reported from 215 samples there were Ala307Thr gene polymorphisms and Ser680Asn FSH receptor respectively. as many as 95 (44.2%)

and 94 (43.7%), Sujatha T and colleagues in 2016 in India reported from 204 samples with PCOS that occurred Polymorphism of Ser680Asn 99 (48.52)%.

LHR gene polymorphism can cause an increase in receptor bioactivity or overexpression of the LH hormone, this is according to research by Joanes and colleagues reported[18,23].Significant abnormality in the LH hormone can have an increased androgen production in PCOS and cause anovulation. (El-Shal A et al., 2015)

Some things that can be a cause of SOPK at a younger age include because of an increasingly unhealthy lifestyle, an unbalanced diet, passive physical activity, so that more and more at risk of becoming obese and metabolic syndrome [3,6,18],. (This study found related to this, from the sample group with age <30 years there were 41 obese samples namely BMI ≥ 25 kg / m2, whereas BMI <25 kg / m2 were 19 samples from 60 total samples from that group. And from the obese sample group there were 26 samples with IR HOMA ≥ 2 and 15 samples with IR HOMA <2.

Insulin resistance and hyperinsulinemia are key factors in the pathogenesis of ovulation disorders and hyperandrogenism in PCOS. In the ovary, high insulin levels will stimulate the enzyme 17α-hydroxylase which will increase the process of converting progesterone to androstendione. In the adrenal gland, hyperinsulinemia will increase the activity of the pituitary-pituitary-adrenal axis. Increased activity of the pituitary-pituitary-adrenal axis increases the activity of the enzyme 17α-hydroxylase which converts the compound 17OH Pregnolone to Dehydroepiandrosteronesulphate (DHEAS). Increased adrenal activity is thought to cause insulin receptor phosphorylation which causes insulin resistance. Hyperinsulinemia also decreases the production of sex hormone binding globulin (SHBG) in the liver so that free testosterone levels will increase[14,18]

## CONCLUSION

There is no correlation between Ala307Thr polymorphism and Asn680Ser gene follicle stimulating hormone receptor to the incidence of SPOK with insulin resistance.

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**Conflict Of Interest-** None of the authors has competing interests

**Ethical Clearance-** This research was approved by the Research Ethics Commission of the Faculty of Medicine, Hasanuddin University Makassar, (No. 1140/UN4.6.4.5.31//PP36/2019), and all

research subjects gave written informed consent.

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