

# Analysis of CYP17 gene polymorphism on increasing androstenedione levels in polycystic ovary syndrome women with obesity in Surabaya



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## ABSTRACT

**Introduction:** CYP17 gene polymorphism causes susceptibility, can interfere with the enzyme biosynthesis mechanism in steroidogenesis, and becomes a possible genetic factor for hyper-androgen in PCOS. The study aimed to analyze the relationship between CYP17 gene polymorphism and increased androstenedione levels in PCOS women with obesity.

**Methods:** This study is an observational case-control analytic. The sample is 23 obese PCOS women and 23 obese non-PCOS women, using a purposive sampling technique. CYP17 gene polymorphism examination with PCR and RFLP. Examination of androstenedione hormone by ELISA. The examination results were tested using a logistic regression statistical test with  $\alpha = 0.05$ , the hypothesis being accepted if  $p\text{-value} < 0.005$ .

**Results:** The study subjects were 20-30 years old, 50-60 kg body weight, with BMI more than 25%. The distribution of CYP17 gene genotypes in the case group was TC, 47.8%, while in the control group, it was TT, 100%, Chi-Square test on CYP17 gene polymorphism in PCOS and control groups showed significant differences ( $P = 0.000$ ). Wilcoxon-Mann Whitney test of androstenedione levels in cases compared to controls  $P = 0.000$ , eta CYP17 correlation test with androstenedione levels  $P = 0.061$ .

**Conclusion:** There is a difference in CYP 17 gene polymorphism in obese PCOS women compared to obese non-PCOS women. Androstenedione levels are higher in PCOS women than in obese non-PCOS women and there is no association of CYP17 with Androstenedione levels in PCOS women with obesity.

**Keywords:** CYP17 gene polymorphism, androstenedione, PCOS.

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## INTRODUCTION

PCOS is a multifactorial endocrine condition characterized by anovulatory menstrual periods, infertility, abnormal gonadotropin levels, obesity, and polycystic ultrasonography.<sup>1</sup> PCOS is a heterogeneous syndrome caused by a mix of hereditary and environmental variables.<sup>2</sup>

The presence of particular polymorphisms in genes contributing to susceptibility to the onset of PCOS has been widely recognized in PCOS research. CYP11A1, CYP17, CYP19, and StAR 3 are genes that influence the synthesis, function, and regulation of androgens.<sup>3</sup>

According to the European Society for Human Reproduction and Embryology/ American Society for Reproductive Medicine, the prevalence of PCOS in the

world is believed to be around 6-10%, whereas the prevalence of PCOS in reproductive age is 15-20%.<sup>4</sup> There is no specific incidence rate in Indonesia. However, it is thought to be around 5-10% and mainly occurs in women of reproductive age aged 15 to 40 years with the largest frequency occurring in the age range of 26-30 years, which is 45.7%.<sup>4,5</sup> According to a Punjab research report, 14.8% of overweight and 13.8% of obese women have contributed to the rise in PCOS cases.<sup>6</sup>

According to the findings of a 2013 global study funded by the Bill & Melinda Gates Foundation and published in *The Lancet* in May 2014, Indonesia ranked 10th in the population with obesity. According to RISKEDAS 2013, more than 10% of the Indonesian population is obese, beginning in childhood and adolescence,

and increasing in adulthood.<sup>7</sup> It has aided in the rise in PCOS instances.<sup>6</sup> Data sources estimate that 38-88% of women with PCOS are overweight.<sup>8</sup>

Ovulatory dysfunction is caused by hyperandrogenemia in PCOS.<sup>9</sup> The ovaries create extra androgens in response to elevated LH (Luteinizing Hormone), which inhibits follicular growth and maturation.<sup>6</sup> Elevated androgen levels in 20-30% of PCOS women have been documented, probably due to cortisol metabolism abnormalities or defective biosynthetic enzymes in steroidogenesis. This disorder is linked to the possibility of hereditary causes causing hyperandrogenism in PCOS. Human genetic disorders connected with steroidogenesis still require research into steroid biosynthesis and physiology.<sup>10</sup>

CYP17 (Cytochrome P450c17) is

an enzyme that regulates the rate of androgen production in the gonads and adrenal cortex.<sup>11,12</sup> Its expression is strongly influenced by tropic hormone stimulation, specifically LH in the ovaries and ACTH in the adrenal cortex.<sup>12</sup> CYP17 is a microsomal enzyme that catalyzes two different activities, 17-hydroxylase and 17,20-lyase, both of which are required for the production of glucocorticoids and steroid precursors. Specifically, CYP17 mediates the 17-hydroxylation of DHE or progesterone to produce 17-OH DHE or 17-OH progesterone, as well as the cleavage of these compounds' c17,20 bond to yield dehydroepiandrosterone (DHEA) or androstenedione.<sup>13</sup> Cytochrome P450c17 (CYP17) located at 10q27.3 is expressed to have activity in ovarian theca cells, especially in women with PCOS.<sup>14</sup> The existence of polymorphisms in the steroidogenesis pathway will change the activity of important enzymes. For example, there is an increase in the activity of the enzymes 17 $\alpha$ -hydroxylase and 17,20-lyase, resulting in hyperandrogen in PCOS.<sup>15</sup> The study of polymorphism in CYP17 in Iraqi women in 2015 showed two genotypes: TT wild type and TC heterozygous mutant. The final result was no association of CYP17 polymorphism with the incidence of PCOS in Iraqi women.<sup>16</sup>

There are still mixed results shown in studies related to CYP17 gene polymorphisms with steroid levels, and diverse sample characteristics, so researchers are interested in conducting research with the title analysis of CYP17 gene polymorphisms, with increased androstenedione levels in PCOS women with obesity.

## MATERIAL AND METHODS

### Study design

This type of research is non-experimental with a cross-sectional study approach in obese PCOS women and non-obese PCOS women at Kendangsari Hospital and El Shafi Clinic Surabaya. Non-PCOS obese subjects as a control in this study.

### Study subjects

The population in this study were all women of childbearing age who suffered from PCOS with obesity as a case group,

healthy obese women of childbearing age as a control group, all populations in the last 3 months were not on medication, did not take hormonal drugs, and had never been diagnosed with one of the diseases (endometriosis, hypothyroidism, hyperprolactinemia, Cushing's syndrome, diabetes mellitus) or felt signs of these diseases who checked at Kendangsari hospital and El Shafi clinic Surabaya.

There were two groups of research subjects: PCOS women with obesity and non-PCOS women with obesity. Inclusion criteria for the case group were: (1) Obese women (BMI=  $\geq$  25 Kg/m<sup>2</sup>), (2) 20-40 years old, (3) Meet two of three clinical symptoms according to Rotterdam criteria in 2003 (Anovulation /oligoovulation /amenorrhea, Clinical Examination Results there is excessive hair (on the face, and body, acne, large voice) that refer to hyperandrogenous conditions with Ferriman-Gallway Score  $\geq$  8, Ultrasound examination obtained polycystic ovaries (minimum number of 12 follicles) with a diameter of 2-9 mm, (4) agree to participate in the study by signing an inform consent. While the inclusion criteria for the control group were: (1) Obese women (BMI=  $\geq$  25 Kg/m<sup>2</sup>), (2) Aged 20-40 years old, (3) Regular menstruation with an interval of 21-35 days, (4) No secondary signs of hyperandrogen (Ferriman-Gallway Score  $<$  8), (5) Ultrasound examination was not found polycystic ovaries, (6) agreed to participate in the study by signing an inform consent.

### Data collection

The research was conducted after obtaining approval from the ethics commission of the Faculty of Medicine, Airlangga University. Sample data information was obtained from Kendangsari Surabaya Hospital and El Shafi Clinic. Then, researchers took samples at the home of each research subject or the hospital/clinic. The case group was sampled during and the control group when getting menstruation on days 2-5 and had obtained Informed Consent. Blood was collected through the cubital vein as much as 3 ml, Put into a container that does not contain EDTA (ELISA examination) and which contains EDTA anti-coagulant to isolate peripheral blood mononuclear cells (PBMC), then put into

an Eppendorf tube and stored at minus 800 C at the Institute of Tropical Disease (ITD) Universitas Airlangga which is then carried out DNA isolation examination for PCR-RFLP (Polymerase Chain Reaction- Restriction Fragment Length Polymorphism) and ELISA (Enzyme-linked immunosorbent assay). ELISA examination required serum by means of blood samples obtained were allowed to stand for 0.5 hours at room temperature and then centrifuged at 3000 rpm for 10 minutes.

### Genotype determination

Genomic DNA was extracted from PBMCs and then PCR was performed. PCR of CYP17 gene SNP -34 T/C (rs743572-34), used.

Forward primer: 5'CATTCGCACTCTGGAGTC-3', Reverse primer: 5'-AGGCTCTTGGGGTACTTG-3'. This PCR examination used a Top Taq master mixed reagent kit. According to the kit instructions, the PCR product was then electrophoresed. The results of PCR of the CYP17 gene, when on electrophoresis, the PCR product is obtained in the form of a 414 bp DNA fragment that appears as a band on a 2% agarose gel, which is then documented. This band is then compared with the marker.

Electrophoresis results that provide a positive band then continued with RFLP examination by means of the remaining PCR products incubated in a water bath (water heater) at a certain temperature, and previously added restriction endonuclease enzyme MspAI (New England Biolabs) Restriction Enzym after incubation at a time that has been in accordance with the incubation procedure on the then performed electrophoresis again to see if there is cutting PCR products in certain places by restriction endonuclease. The results of RFLP were: CYP17 gene is TC heterozygote indicated by 414-,290-,124 bp band, 414 bp T homozygote, C homozygote indicated by 290-, 124 bp band.

### Elisa Examination

Androstenedione Elisa Kit REF. CAN-AD-208 LOT. 222850 was used for the Elisa examination. The examination

process follows the instructions in the kit. All reagents should be at room temperature before use. Calibrators, controls, and specimen samples should all be evaluated in pairs. Once the treatment has begun, all steps should be performed without interruption within 120 minutes.

**Data Analysis**

Descriptive analysis is a data processing procedure that describes and summarizes data scientifically and systematically in graphs or tables. The data that has been collected from the results of the study will be processed using the SPSS version 26 program. A 2x2 table is used for the odds ratio with 95% CI in bivariate data. Test the difference by using the Chi-Square test. Bivariate relationship test using Eta Coefficient because of nominal and ratio data. The analysis of CYP polymorphism with the incidence of PCOS with regression analysis.

**RESULTS**

**Subject Characteristics**

Table 1 shows that the overall TVS of the case group research subjects was polycystic, and most experienced hirsutism ( $FG \geq 8$ ) were 19 people (83%), and all experienced secondary amenorrhea, namely 23 people (100%).

Tables 2 and 3 show that the research subjects were aged 20-30 years from the case and control groups, namely 56.5% each. Most of the research subjects had a BMI  $\geq 25$  with obesity category grade 1 in the control group, namely 21 people (91.3%).

Table 4 shows that the mean on these variables is no different based on the t-test results. It is known that the characteristics of respondents include age, weight, height, BMI (Body Mass Index), WC (Waist Circumference), LPg (Pelvic Circumference), and Waist-Hip Ratio (WHR) produces a p-value greater than  $\alpha 0.05$ . So, it is stated that there is no significant difference. So it can be stated that there is no significant difference in the variables of age, weight, height, BMI, WC, LPg, and WHR in PCOS women and controls.

**Table 1. Distribution of clinical symptoms and TVS of study subjects**

Variable	PCOS	
	Frequency	Percentage
Secondary amenorrhea	23	100
Hirsutism/ $FG \geq 8$	19	83
Acne	3	13
Hair Fall	13	56
TVS	23	100

**Table 2. Age distribution of research subjects**

Age	PCOS		Control	
	Frequency	Percentage	Frequency	Percentage
20-30 Years Old	13	56.5	13	56.5
31-40 Years Old	10	43.5	10	43.5
<b>Total</b>	<b>23</b>	<b>100</b>	<b>23</b>	<b>100</b>

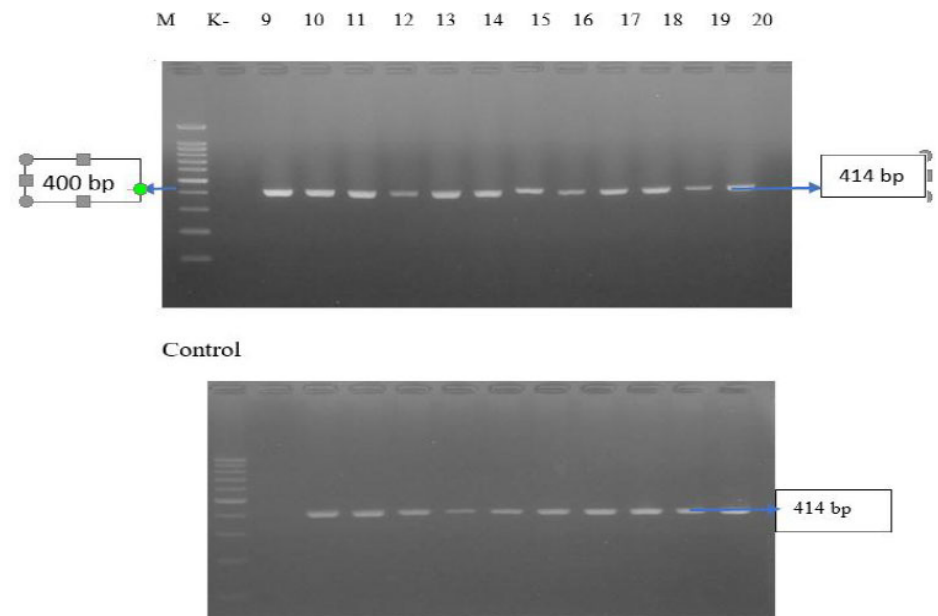
**Table 3. BMI distribution of research subjects**

BMI	PCOS		Control	
	Frequency	Percentage	Frequency	Percentage
$\geq 25$	17	73,9	21	91,3
$\geq 30$	6	26,1	2	8,7
<b>Total</b>	<b>23</b>	<b>100</b>	<b>30</b>	<b>100</b>

**Table 4. Distribution of age, BW, BH, BMI and WHR of study subjects**

Characteristics	PCOS	Control	p-value
Age (years)	29.0870 $\pm$ 3.95328	29.2609 $\pm$ 3.97969	0.882 <sup>a</sup>
BW (kg)	69.8261 $\pm$ 12.1567	64.6522 $\pm$ 7.18337	0.086 <sup>a</sup>
BH (M)	1.5465 $\pm$ 0.05875	1.5465 $\pm$ .04313	1.000 <sup>a</sup>
BMI	29.0870 $\pm$ 4.31143	27.2000 $\pm$ 2.05249	0.222 <sup>b</sup>
LP	84.4783 $\pm$ 11.09410	79.8696 $\pm$ 8.42779	0.123 <sup>b</sup>
LPg	102.9565 $\pm$ 10.2313	100.0000 $\pm$ 7.57388	0.587 <sup>b</sup>
WHR	0.8135 $\pm$ 0.06117	0.8013 $\pm$ 0.05841	0.271 <sup>a</sup>

Caption: <sup>a</sup> chi-square test <sup>b</sup>independent t test <sup>c</sup>Mann Whitney test. BMI (Body Mass Index), WC (Waist Circumference), LPg (Pelvic Circumference), WHR (Waist-Hip Ratio)



**Figure 1.** Electrophoresis image of CYP17 gene in case and control group. Lane M= marker 100 bp; lane K-= control.

**Overview of CYP17 gene PCR and RFLP products**

RFLP Gen CYP17 Result  
CYP17 gene SNP -34 T/C (rs743572-34) with restriction enzyme MsA11 NEB. PCR results of study subjects in lanes 9-20 cases and controls obtained PCR

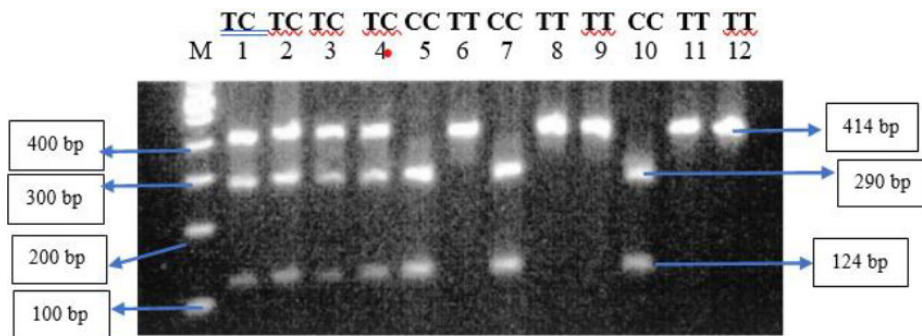
product of CYP 17 gene 414 pb (Figure 1). Figure 2 shows RFLP results of CYP 17.414,290,124 bp TC heterozygous 290-, 124 bp C homozygous, 414 T homozygous. Figure 3 shows CYP17 gene RFLP results in normal samples observed through 1% agarose electrophoresis. CYP17 gene

measurement results showed 414 bp T homozygote. Distribution of genotypes and alleles of the CYP 17 gene in the study subjects.

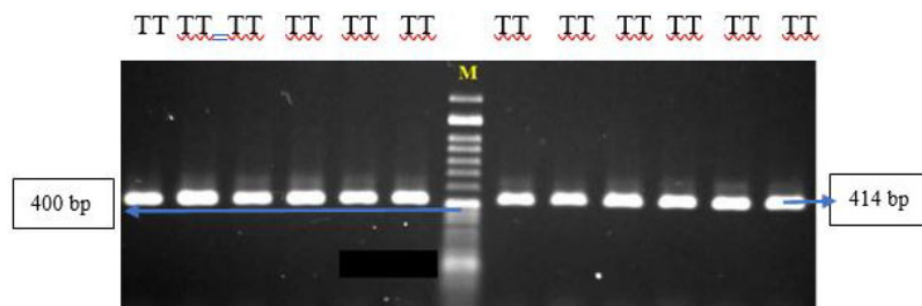
Table 5 shows that the CYP17 gene mostly has the TC genotype in the case group, 47.8%, while in control, all genotypes are TT (100%). The dominant allele in cases is the C allele, indicating that the C allele is a risk factor for PCOS. In comparison, the T allele is a protective factor for PCOS.

Based on Table 6, it can be seen that the results of statistical test analysis with chi-square resulted in a value of 33.370 and an allele value of 38.215 with a significance value of 0.000. This value is < alpha (5% or 0.05). Therefore, it can be stated that there is a significant difference in CYP17 gene polymorphisms and alleles in obese PCOS women with normal obese women.

Table 7 shows that the value of p=0.000 is smaller than  $\alpha$  0.05. It can be concluded that there is an association between the CYP17 allele and the incidence of PCOS. Likewise, the CYP19 allele has a p-value of 0.002, indicating a relationship between the CYP19 allele and the incidence of PCOS.



**Figure 2.** RFLP results of CYP17 gene in PCOS group



**Figure 3.** RFLP results of the CYP17 gene in the control group.

**Table 5. Distribution of genotypes and alleles of CYP 17 gene in study subjects**

Genotypes	PCOS		Control	
	Frequency	Percentage	Frequency	Percentage
TT	4	17,4	23	100
TC	11	47,8	0	0
CC	8	34,8	0	0
Total	23	100	23	100
<b>Alleles</b>				
T	19	41,3	46	100
C	27	58,7	0	0

**Table 6. Difference test of CYP 17 gene polymorphisms (genotypes and alleles) in PCOS women and controls**

Variable	Chi-Square	
	Value	Sig
gen CYP 17	32,370	0,000
Allel		
T	38,215	0,000
C		

**Table 7. Association of CYP17 alleles with PCOS incidence**

Nominal by Nominal	Value	Asymptotic Standard Error	Approximate T	Approximate Significance
Lambda Allele CYP17 vs. PCOS	0,587	0,113	3,574	0,000

**Table 8. Results of the Androstenedione t-test in the study subjects**

Levels	Group	$\bar{x} \pm SD$	Statistics	p-value
Androstenedione	PCOS	2.9078 $\pm$ 5.21929	-4.592	0,000
	Control	0.8897 $\pm$ 0.37306		

**Table 9. Test Results of the Association of CYP17 Gene Polymorphisms with Increased Androstenedione Levels in Obese SPOK Women in Surabaya**

Relationship	Correlation Coefficient	Sig.	Description
CYP17 Gene with Androstenedione Levels	-0.261	0.061	Not Significant

**Table 10. Hypothesis Testing Results based on partial least square analysis**

Relationship	Original Sample (O)	T Statistics ( O/STDEV )	p-values
Androstenedione hormone $\oplus$ PCOS occurrence	-0.062	0.376	0.707
CYP17 $\oplus$ Androstenedione hormone	0.407	1.730	0.084
CYP17 $\oplus$ PCOS occurrence	-0.428	3.566	0.000

can be stated that there is no significant relationship between the CYP17 gene and increased androstenedione levels in obese SPOK women. Table 10 shows that only CYP17 has a significant correlation with the incidence of PCOS.

## DISCUSSION

CYP17 polymorphism in the study's results was mostly in the TC genotype, which was 47.8%, while the control group was all TT genotypes. Statistical test dg Chi Square p value 0.00 is smaller than  $\alpha$  0.05, so there is a difference in CYP17 gene polymorphism in the case and control groups.

In this study, the C allele has a greater frequency in cases, indicating that the C allele is a risk factor for SOPK. In contrast, the T allele is mostly present in controls, indicating that the T allele is a protective factor for SOPK. This result does not agree with research on Iraqi women in 2015 showing the existence of two types of genotypes, TT wild type and TC mutant heterozygote and mentioning no difference between the 2 groups. In Indian women, there is a similar frequency comparison in genotyping. There is an increase in the C allele in SOPK compared to the control  $P < 0.05$ , indicating that there is an association of genotyping with SOPK. At the same time, the CC genotype also appears relatively high in SOPK but not significant.<sup>17</sup>

The results of the T genotype seen in SOPK and controls differ from Pulsakar's research, which states that the T genotype appears in SOPK and does not appear in controls.<sup>17</sup> In a Turkish study, it was mentioned that polymorphisms were found in the regulatory region of the 17  $\alpha$  Hydroxylase gene (CYP17) substitution of T to C -34 bp.<sup>18</sup> In the results of SNP -34 TC (rs 743572-34), TC appears at 414-,290-,124 bp and homozygote C appears at 290-,124 bp.

The TT genotype is thought to have no risk association with SOPK, wherein the study results showed all controls had the TT genotype. This is similar to a study in China in 2012. There were no significant results in the homozygous model (TT vs CC: OR = 0.72, 95% CI = 0.56, 0.94), indicating that TT homozygotes have no association with SOPK risk compared to CC homozygotes.<sup>18</sup>

The differences in the results of each study indicate the diversity of polymorphisms that can be influenced by race or ethnicity, where there are differences in genetics and lifestyle, including diet, physical activity, BMI or WHR, as well as environmental factors, including temperature, pollutants, stress levels, and others. Interestingly, the subjects of the study were both obese and there was a difference in the mean even though statistically, there was no difference. In Martinez's study, the body

weight and BMI were higher in the SOPK group than in the normal group.<sup>19</sup> People who are obese in some studies tend to develop SOPK.<sup>3</sup>

Androstenedione hormone levels in obese SOPK women with obese normal women. The average value in the Obese SOPK group is higher than the Obese Normal group, indicating that Androstenedione Hormone Levels in Obese SOPK Women are significantly higher compared to Obese Normal Women.

In Lerchbaum's 2014 study<sup>20</sup> hyperandrogenism occurred in 85.6% of respondents, and SOPK women with high androstenedione levels had an adverse metabolic phenotype. Obesity can be a factor in the occurrence of hyperandrogenism. In this study, most had a BMI  $\geq 25\%$ . This is in accordance with the opinion that the common occurrence of hypogonadotropic hyperandrogenism in obese women is an exception to this model because it is caused by polycystic ovary syndrome (SOPK), a condition in which insulin-resistant hyperinsulinism plays a role in both androgen excess and adiposity. This condition's frequency affects 5-10% of women of reproductive age.<sup>11</sup> A study in India showed an increase in androstenedione in the case group, associated with a polymorphism in CYP17.<sup>19</sup>

The Chinese study also mentioned that androgen levels were higher in cases than in controls with both testosterone and androstenedione levels. Androgens are produced in the ovaries and adrenal glands as end products of enzymatic reactions that begin with converting cholesterol into dehydroepiandrosterone and androstenedione. Changes in enzymatic activity can affect androgen synthesis, and a decrease in the affinity of the aromatase enzyme results in a decrease in affinity and estradiol levels.<sup>15</sup>

The test of the effect of the CYP17 gene on the incidence of SOPK resulted in a p-value of 0.000  $< 0.05$ . This means that there is a significant influence of the CYP17 gene on the incidence of SOPK. The resulting coefficient value is negative, -0.428. Thus, the higher the healthy CYP17 gene, the lower the risk of SOPK.

The allele in the RFLP examination

was mostly the T allele, which was present entirely in the control while the C allele was mostly present in the case group. Wickernheisser's study showed that there was an increase in CYP17 expression in theca cells in SOPK ovaries compared to non-SOPK19). AT>C polymorphism appeared in the promoter region of the gene, 34 bp translational initiation site, which was found in patients with SOPK with hyperandrogen.<sup>19,21-24</sup>

The limitation of this case study is women of childbearing age with PCOS/infertility problems who visit health clinics are relatively few, and research on polymorphism requires substantial funds. A recommendation for the future is follow-up research on CYP17 gene polymorphism and other steroidogenesis-related genes in obese adolescents who experience menstrual disorders as a risk factor for PCOS.

## CONCLUSION

There are differences in CYP17 gene polymorphisms in obese SOPK women and obese normal women. Androstenedione levels are higher in SOPK women compared to obese normal women. There is no association of CYP17 gene polymorphisms with increased androstenedione levels in obese SOPK women. There is an association of CYP17 gene polymorphisms with the incidence of SOPK.

## CONFLICT OF INTEREST

The authors report no conflict of interest.

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## ETHICAL APPROVAL

This study protocol was reviewed by the health research ethics committee at Universitas Airlangga School of Medicine in Surabaya and was given the ethical clearance certificate No. 172/EC/KEPK/FKUA/2023.

## AUTHOR CONTRIBUTION

All authors contributed to this research. SNK and EGD: Designing the research concepts and formulating research objectives. SNK: Coordinating all data collection and management, data validation, visualization, and analysis, and writing the initial draft of the article for publication. EGD: coordinating technical and supervising the conduct of the research, GS: supervising the research, editing and correcting the final article for publication.

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