JKKI
SCIENTIA EST BASIC VITAE

**Jurnal Kedokteran dan Kesehatan Indonesia**

Indonesian Journal of Medicine and Health

Journal homepage: https://journal.uii.ac.id/JKKI

# Relationship polymorphism exon 17 of insulin receptor (INSR) gene with polycystic ovarian syndrome among Malay ethnic in South Sumatera

Okta Emilia[1], Legiran*[2], Safyuddin[3], Syifa Alkaf[4]

[1]Master of Biomedical studies Program, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia
[2]Department of Anatomy, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia
[3]Department of Biochemistry and Medicinal Chemistry, Faculty of Medicine, Sriwijaya University, Palembang, Indonesia
[4]Department of Obstetric and Gynecology, Dr. Mohammad Hoesin General Hospital, Palembang, Indonesia

Original Article

**ARTICLE INFO**

***Keywords:***
Polymorphism,
INSR gene,
PCOS

***Corresponding author:**
dr.legiran@fk.unsri.ac.id

DOI: 10.20885/JKKI.Vol13.Iss1.art3

***History:***
Received: June 16, 2019
Accepted: January 10, 2020
Online: May 31, 2022



## ABSTRACT

**Background:** A sedentary lifestyle is a risk of obesity. One of the negative effects of obesity is insulin resistance. Insulin resistance is found in 50% - 90% of women with Polycystic Ovary Syndrome (PCOS).
**Objective:** This study aimed to analyse the relationship polymorphism exon 17 of insulin receptor (INSR) gene in Malay ethnicity in South Sumatera.
**Methods:** This is an observational analytic study with a case-control design conducted at the Molecular Biology Laboratory, Faculty of Medicine, Sriwijaya University. This study sample used blood taken from 80 people consisting of 40 cases and 40 controls. Genotyping and allotypic were performed using PCR-RFLP. Analysis was performed using SPSS 16.
**Results:** The statistical analysis using the Chi-Square test on IMT resulting p-value = 0.003 (OR = 4.660 95% CI = 1.764 - 12.311). (Wild type) CC, (Mutant heterozygous) CT, (Homozygous Mutant) TT. The statistical analysis using the Pearson Chi-Square Genotype PCOS resulting p-value = 0.970 and fisher exact test on SOPK allotype resulting p-value = 0.500 (OR = 0.949 95% CI = 0.503 - 1,790).
**Conclusion:** In conclusion, there was a significant relationship between BMI and PCOS among Malay ethnic in South Sumatera. There was no significant relationship between genotypic polymorphism and allotype exon 17 of INSR with PCOS in Malay ethnic groups in South Sumatera.

***Latar Belakang:*** *Pola hidup Sedentary life style beresiko menjadi obesitas. Salah satu dampak negatif dari obesitas adalah resistensi insulin. Resistensi insulin ditemukan pada 50% - 90% perempuan dengan Sindroma Ovarium Polikistik (SOPK).*
***Tujuan:*** *Tujuan penelitian ini untuk menganalisis hubungan polimorfisme gen reseptor insulin (INSR) ekson 17 pada etnis melayu di Sumatera Selatan.*
***Metode:*** *Penelitian ini merupakan penelitian analitik observasional dengan desain kasus kontrol (case-control). Dilaksanakan di Laboratorium Biologi Molekuler Fakultas Kedokteran Universitas Sriwijaya. Sampel penelitian menggunakan sampel darah yang diambil pada 80 sampel yang terdiri dari 40 kasus dan 40 kontrol. Penentuan genotip dan alotip menggunakan PCR-RFLP. Analisis menggunakan SPSS 16.*
***Hasil:*** *Hasil analisis dengan uji Chi-Square diperoleh pada IMT p value = 0,003 (OR = 4,660 95% CI = 1,764 – 12,311). (Wild type) CC, (Mutant Heterozigot) CT, (Mutant Homozigot) TT. Analisa hasil uji statistik* pearson Chi-Square genotip *SOPK diperoleh nilai p* value *= 0,970 dan hasil uji statistik* fisher's exact test alotip *SOPK*

*nilai p value = 0,500 (OR = 0,949 95% CI = 0,503 – 1,790).*
***Kesimpulan:*** *Ada hubungan yang bermakna antara IMT dengan SOPK pada etnis melayu di Sumatera Selatan. Tidak ada hubungan yang bermakna antara polimorfisme genotip dan alotip gen reseptor insulin (INSR) ekson 17 dengan SOPK pada etnis melayu di Sumatera Selatan.*

## INTRODUCTION

Lifestyle changes from traditional to sedentary can increase the risk of being overweight. A sedentary lifestyle (less movement) is usually accompanied by excessive eating patterns, such as a high intake of carbohydrates, fat, protein, and low fibre, leading to overweight and obesity.

One of the negative impacts of obesity is insulin resistance, which is characterised by an increase in fasting insulin levels which then causes increased blood glucose levels. In addition to type 2 diabetes, insulin resistance also underlies polycystic ovary syndrome (PCOS), approximately 50-90% of women with PCOS.[1]

Insulin regulates glucose homeostasis by stimulating glucose binding by insulin-responsive target tissues, fat, and muscle and suppressing glucose production in the liver. Insulin also suppresses lipolysis, which decreases circulating free fatty acid levels, and immediately triggers insulin action on glucose production in the liver. Insulin resistance decreases insulin's ability to mediate metabolic actions during glucose absorption, glucose production, and lipolysis. Thus, insulin resistance is characterised by increased circulating insulin levels in response to a glucose load, essentially.[2]

The Insulin receptor gene-encoded with INSR consists of 22 exons with 120 kb long on chromosome 19. The tyrosine kinase domain in the INSR gene (exons 17-21) gets much attention because this domain mutation is related to hyperinsulinemia and insulin resistance.[3]

The presence of SNPrs1799817 in the INSR gene could be developed as a marker for PCOS accompanied by insulin resistance and metabolic complications in Indian women.[4] The SNP in exon 17 of the INSR gene detected is C/T His 1058 (rs1799817) in the tyrosine kinase domain of the insulin receptor, shown to be related to the PCOS development. The most likely effect is auto-phosphorylation of INSR function in women with PCOS.[5]

A study on Japanese women with PCOS found a significant relationship between the SNP rs1799817 in the INSR gene and non-obese patients.[6] The SNP rs1799817 study among Iranian women with PCOS showed a nonsignificant relationship.[7] The study aimed to analyse the relationship between polymorphism of the exon 17 INSR gene and PCOS.

## METHODS

### Research Design

This is an observational analytic study with a case-control design which studies the relationship between exposure (factor) and disease by comparing the case group, women with PCOS, and the control group, women without PCOS. The study subject was recruited from a mother and child hospital (RSIA Widiyanti Palembang) in collaboration with the Laboratory of Prodia Palembang. Assessment of exon 17 INSR gene polymorphism was carried out using RFLP polymerase chain reaction (PCR) at the Laboratory of Biomolecular of the Faculty of Medicine of Universitas Sriwijaya Palembang (FM UNSRI). This study was conducted from November 2018 to February 2019.

The study subjects were all patients who met the inclusion criteria and then grouped into case and control groups. The case group was patients diagnosed with PCOS by obstetricians (SpOG) following Rotterdam criteria, and the control group was women without PCOS. All study subjects were ethnic Malays aged 20-40 years and willing to participate by signing informed consent.

The number of subjects in each case and control group was 40 people. Sample DNA was amplified by PCR using a forward primer (5 - TCAGGAAAGCCAGCCCATGTC-3) and a reverse

primer (5- CCAAGGATGCTGTGTAGATAAG-3).

The PCR reaction was carried out on the BioRad Thermal Cycler T100 machine. The cycling parameters included denaturation at 94°C for 2 minutes, 29 cycles at 94 °C for 1 minute, 57.5 °C for 1 minute, 72 °C for 1 minute, and 72 °C for 10 minutes. After amplification by PCR technique, DNA was purified using a PCR purification kit and digested with PmlI for 4 hours at 37 °C. Then, the RFLP product was analysed by electrophoresis on 2% agarose gel and observed with Geldoc. For subjects with the C allele, PmlI will recognise the site (CACGTG) and restrict it to form 2 fragments measuring 274 bp and 43 bp. So, a single band of 317 bp indicates homozygosity of the T allele. Two fragments of 274 bp and 43 bp indicate homozygosity of the C allele, and if three fragments are obtained, such as 317 bp, 274 bp, and 43 bp, it indicates heterozygosity for allele C or allele T. The statistical analysis was performed using Chi-Square, Fisher exact, and Pearson's test.

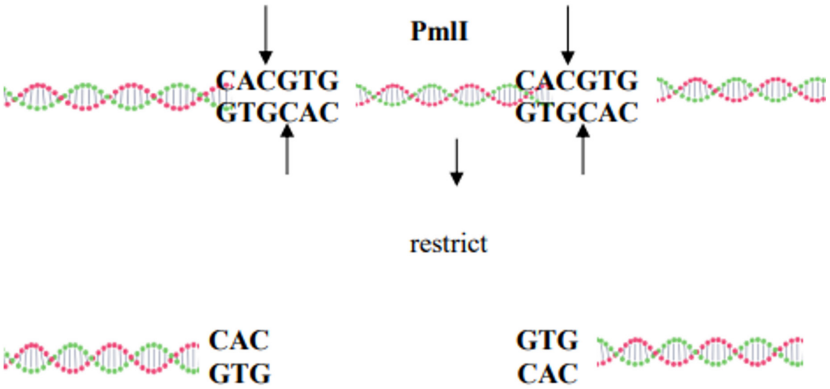


Figure 1. Location of PmlI Enzyme

**RESULTS**

In this study, the characteristics of subjects included age, BMI, and clinical Rotterdam characteristics (oligomenorrhea, polycystic ovaries, hirsutism). Based on subject characteristics in both groups, it was found that reproductive age (≤ 35 years) dominated the study subjects as much as 87.5% in the PCOS group and 77.5% in the non-PCOS group. Based on clinical symptoms, oligomenorrhea and polycystic ovaries were found in all PCOS subjects and not in the non-PCOS group.

Clinical hirsutism between PCOS and non-PCOS is slightly different, 90% in the PCOS group had mild hirsutism, and 10% had moderate hirsutism, but hirsutism was not found in the non-PCOS group. Obesity BMI in the PCOS group was 57.5%. Meanwhile, BMI in the non-PCOS group was 22.5%. As shown in the Chi-Square test of the continuity correction, the results obtained a p-value of 0.003, odds ratio (OR) of 4.660, and 95% confidence interval (95% CI) of 1.764 to 12.311, which means that there is a significant relationship between BMI and PCOS incidence which obesity was 4.6 times likely to have PCOS than not obese.

After all the study subjects on the genotype were distributed by frequency, an analysis of the relationship between exon 17 INSR genotypic polymorphisms and PCOS was carried out.

The number of TT genotypes in the PCOS group was 32.5%, and in the non-PCOS group was 35%. For the CT genotype, 55% were found in the PCOS group and 52.5% in the non-PCOS group. For the CC genotype, which is a wildtype, 12.5% in each PCOS and non-PCOS group, the

Table 1. Frequency Distribution of Study Subjects Based on Age, BMI, Menstrual Cycle, Polycystic Ovary and Hirsutism

| Subjects | PCOS | | Non PCOS | | Total | |
|---|---|---|---|---|---|---|
| | N | % | N | % | N | % |
| Age | | | | | | |
| > 35 years | 5 | 12,5% | 9 | 22,5% | 14 | 17,5% |
| ≤ 35 years | 35 | 87,5% | 31 | 77,5% | 66 | 82,5% |
| BMI | | | | | | |
| Obesity | 23 | 57,5% | 9 | 22,5% | 32 | 40,0% |
| Non Obesity | 17 | 42,5% | 31 | 77,5% | 48 | 60,0% |
| Menstrual Cycle | | | | | | |
| Oligomenorrhea | 40 | 100% | 0 | 0% | 40 | 50% |
| Normal | 0 | 0% | 40 | 100% | 40 | 50% |
| Ovary | | | | | | |
| Polycystic Ovary | 40 | 100% | 0 | 0% | 40 | 50% |
| Normal | 0 | 0% | 40 | 100% | 40 | 50% |
| Hirsutism | | | | | | |
| Moderate | 4 | 10% | 0 | 0% | 4 | 5% |
| Mild | 36 | 90% | 0 | 0% | 36 | 45% |
| Normal | 0 | 0% | 40 | 100% | 40 | 50% |
| Total | 40 | 100% | 40 | 100% | 80 | 100% |

Table 2. Analysis of Relationship between Polymorphisms of Exon 17 INSR Gene and PCOS.

| Genotype Gene | PCOS | | Non PCOS | | P value |
|---|---|---|---|---|---|
| | N | % | N | % | |
| TT | 13 | 32,5% | 14 | 35,0% | 0,970 |
| CT | 22 | 55,0% | 21 | 52,5% | |
| CC | 5 | 12,5% | 5 | 12,5% | |
| Total | 40 | 100 % | 40 | 100,0 % | |

Table 3. Analysis of Relationship between Polymorphisms of Exon 17 INSR Allele Gene and PCOS.

| Allele Gene | PCOS | | Non PCOS | | ρ value | OR |
|---|---|---|---|---|---|---|
| | N | % | N | % | | |
| T | 48 | 60,0 % | 49 | 61,3 % | 0,500 | 0,949 (95%CI) 0,503-1,790 |
| C | 32 | 40,0% | 31 | 38,7% | | |
| Total | 40 | 100% | 40 | 100,0 % | | |

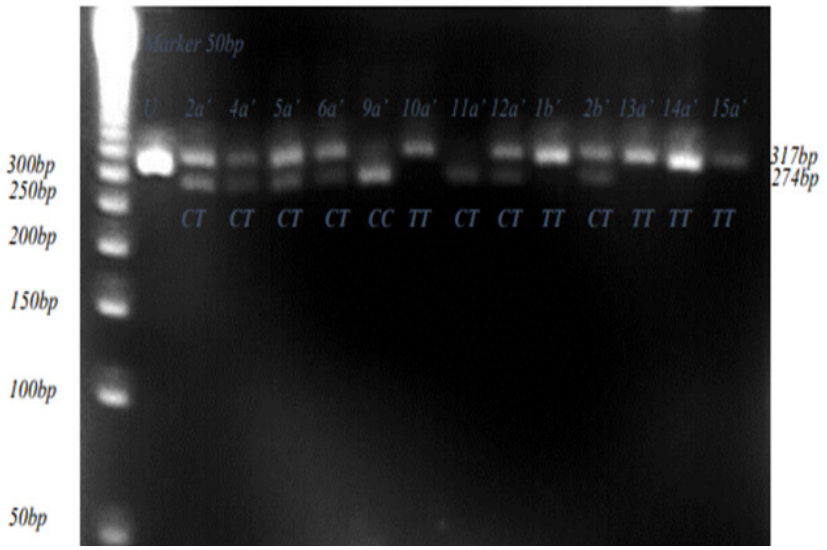


Figure 2. RFLP Visualization Results of Exon 17 of INSR Gene with PmlI Enzyme. Description: wildtype CC genotype (9a'), CT heterozygote genotype (2a',4a'5a',6a',12a',2b) and TT Mutant homozygote (10a',1b',13a',14a',15a')

Pearson Chi-Square test showed a P-value of 0.970 (Table 2).

In the PCOS group, the T allele was 60.0%, and the C allele was 40%, while in the non-PCOS group, the T allele was 61.3%, and the C allele was 38.7%. The Fisher's exact test showed a p-value of 0.500, OR 0.949, and a 95% CI of 0.503 to 1.790 (Table 3).

## DISCUSSION

The characteristics of the subjects were different because this study required a non-PCOS group with different characteristics from the PCOS group. Following the Rotterdam scale for PCOS criteria, they must meet at least 2 of the 3 criteria to diagnose subjects with PCOS.

In this study, all features of polycystic and oligo/anovulatory ovaries were found in the PCOS group, while clinical hirsutism determined by the Ferriman-Galwey score was only 4 out of 40 people in the PCOS group with moderate hirsutism. In the non-PCOS group, there were no features of oligo/anovulation, polycystic ovaries, and hirsutism based on the Ferriman-Galwey score. These results are consistent with Skrgatic's study that women with PCOS do not always have all three characteristics of the Rotterdam criteria, although, in Asian races, patients with clinical features of hyperandrogenism in the form of hirsutism are rarely found.[8]

This study evaluated the association of His 1058 C/T polymorphism in the tyrosine kinase domain of the INSR gene among PCOS patients. In the PCOS group, 57.5% were women with obesity. There is a significant relationship between obesity and BMI > 27 kg/m$^2$ with PCOS, in which women with BMI > 27 kg/m$^2$ were 4.66 times more likely to have PCOS. The frequency of the CC and CT/TT genotypes in both groups was almost similar to the T allele frequency, slightly higher in the PCOS group, which was 60%. The results of this polymorphism showed no significant relationship with PCOS. This study is in line with the study by Lee et al. in Korea that showed similar results, in which the genotype frequency of CC and CT/TT was similar between PCOS and non-PCOS groups.[9] Another study on Deutro-Malay ethnicity showed that the frequency of the CC and CT/TT genotypes was also similar between the PCOS group with insulin resistance and the control group with a slightly higher T allele frequency in the control group.[10]

A study in Japan reported that CC and CT/TT genotypes were almost similar between the PCOS and non-PCOS groups, and insulin resistance was associated with non-obese patients. The plausible reasons are that insulin resistance in PCOS is related to causes other than the INSR gene, the polymorphism screening method used is different from other studies, and ethnic and racial variation factors.[6]

Until now, no precise molecular mechanism has been defining the occurrence of PCOS. However, the serine phosphorylation hypothesis can explain two main features of PCOS, such as androgenemia and insulin resistance. This mechanism may explain molecularly for some PCOS patients but not for all PCOS. Some of the mechanisms why insulin resistance causes androgenemia are: compensatory hyperinsulinemia due to insulin resistance will decrease the hepatic synthesis of SHBG, resulting in increased free androgens in the blood; Excess insulin can bind to IGF-1 receptors in the ovaries, causing increased androgen production by theca cells.[11] All steroid hormone production begins with cholesterol. Steroid enzymes require substrates or cofactors to convert cholesterol into various steroid products. Although the ovaries and adrenals can synthesise cholesterol from acetate, some of the cholesterol used for organ biosynthesis comes from cholesterol esters found in circulating lipids in the blood.[12] The binding of insulin to the insulin receptor occurs at its subunit. The insulin receptor subunit with intrinsic tyrosine kinase activity will undergo a phosphorylation reaction (autophosphorylation) on the protein target. This process will trigger a complex chain of mechanisms. ATP binding will trigger the phosphorylation of the subunit via the tyrosine kinase enzyme. This phosphorylation of tyrosine on intracellular substrates is referred to as IRS. IRS can bind to other signalling molecules, which can activate insulin, whereas serine phosphorylation inhibits the activation of insulin-activated signalling.[13] Phosphorylation of serine, adrenal P450c17 enzymes, and ovarian in PCOS patients increases the activity of 17.20 lyase enzymes which then produce hyperandrogenism.

Impaired insulin sensitivity both in vivo and in vitro led to the hypothesis that genetic lesions of the insulin receptor gene or post-receptor signalling may contribute to the pathogenesis of PCOS. Polymorphism is a natural variation in a DNA sequence gene or chromosome that does not have a negative/harmful effect on individuals and is quite high in incidence in the general population. Molecular studies of the regions encoding insulin receptor genes in women with PCOS have shown many polymorphisms. However, most of them can also be identified in normal subjects and are common polymorphisms that do not show impaired insulin receptor function.[14,15]

The interaction of insulin with the INSR gene triggers an important molecular signalling cascade that actively contributes to the biological activity of the associated target cell. This interaction shows that the INSR gene plays an important role in tyrosine autophosphorylation because it encodes the tyrosine kinase domain on the insulin receptor, although it is partial. Thus, the role of the INSR gene, although it plays an important role, still depends on the performance of other genes such as IRS, INS, EPPN1, PPARγ and Calpain 10, which play a role in insulin signalling.[16]

The differences in polymorphism patterns between race/ethnicity and the mechanism behind insulin resistance in PCOS make the results of this study have few similarities or even differences with other studies.

## CONCLUSION

There was no significant relationship between genotypic and allele polymorphism exon 17 of INSR gene with PCOS among Malay ethnicity in South Sumatera.

## CONFLICT OF INTEREST

None

## ACKNOWLEDGEMENT

None

**REFERENCES**


1. Shaikh N, Dadachanji R, Mukherjee S. Genetic Markers of Polycystic Ovary Syndrome: Emphasis on Insulin Resistance. Int J Med Genet. 2014;2014:1-10. doi:10.1155/2014/478972
2. Diamanti-Kandarakis E, Dunaif A. Insulin resistance and the polycystic ovary syndrome revisited: An update on mechanisms and implications. Endocr Rev. 2012;33(6):981-1030. doi:10.1210/er.2011-1034
3. Kosova G, Urbanek M. Genetics of the polycystic ovary syndrome. Mol Cell Endocrinol. 2013;373(1):29-38. doi:https://doi.org/10.1016/j.mce.2012.10.009
4. Gangopadhyay S, Agrawal N, Batra A, Kabi BC, Gupta A. Single-Nucleotide Polymorphism on Exon 17 of Insulin Receptor Gene Influences Insulin Resistance in PCOS: A Pilot Study on North Indian Women. Biochem Genet. 2016;54(2):158-168. doi:10.1007/s10528-015-9708-7
5. Mutib MT, Diploma H, Hamdan FB, Al-Salihi AR. INSR Gene Variation Is Associated with Decreased Insulin Sensitivity in Iraqi Women with PCOs. Vol 12.; 2014.
6. Kashima K, Yahata T, Fujita K, Tanaka K. Polycystic ovary syndrome: association of a C/T single nucleotide polymorphism at tyrosine kinase domain of insulin receptor gene with pathogenesis among lean Japanese women. J Reprod Med. 2013;58(11-12):491-496.
7. Mohammad NH, Kareem A, Al-Kazaz A, Ad'hiah AH. Detection of Single Nucleotide Polymorphisms (SNPs) in INSR (Insulin Receptor Gene) in Iraqi Women with Polycystic Ovarian Syndrome (PCOS). Vol 3.; 2015. http://crmb.aizeonpublishers.net/content/2015/1/crmb578-585.pdf
8. Skrgatić L, Baldani DP, Gersak K, Cerne JZ, Ferk P, Corić M. Genetic polymorphisms of INS, INSR and IRS-1 genes are not associated with polycystic ovary syndrome in Croatian women. Coll Antropol. 2013;37(1):141-146.
9. Lee EJ, Oh B, Lee JY, Kimm K, Lee SH, Baek KH. A novel single nucleotide polymorphism of INSR gene for polycystic ovary syndrome. Fertil Steril. 2008;89(5):1213-1220. doi:10.1016/j.fertnstert.2007.05.026
10. PUSPITASARI, D., SETYAWAN, A. & THAUFIK S. SINGLE NUCLEOTIDE POLYMORPHISM (SNP) EXON 17 C/T HIS 1058 GEN INSR PADA PENDERITA SOPK DENGAN RESISTENSI INSULIN. Media Med Muda; Vol 2, No 1. Published online 2018. https://ejournal2.undip.ac.id/index.php/mmm/article/view/2622
11. Sperroff L GR. Sperroff L, G.R., kase NG, ed. Clinical gynecologic endocrinology and infertility. 8 ed. 2011. 495-531.
12. Bremer AA, Miller WL. The serine phosphorylation hypothesis of polycystic ovary syndrome: a unifying mechanism for hyperandrogenemia and insulin resistance. Fertil Steril. 2008;89(5):1039-1048. doi:10.1016/j.fertnstert.2008.02.091
13. Dunaif A, Xia J, Book CB, Schenker E, Tang Z. Excessive insulin receptor serine phosphorylation in cultured fibroblasts and in skeletal muscle. A potential mechanism for insulin resistance in the polycystic ovary syndrome. J Clin Invest. 1995;96(2):801-810. doi:10.1172/JCI118126
14. Diamanti-Kandarakis E, Piperi C, Argyrakopoulou G, et al. Polycystic Ovary Syndrome: The Influence of Environmental and Genetic Factors. Vol 5.; 2006.
15. Mukherjee S, Maitra A. Molecular & genetic factors contributing to insulin resistance in polycystic ovary syndrome. Indian J Med Res. 2010;131:743-760.
16. Shaikh N, Dadachanji R, Mukherjee S. Genetic Markers of Polycystic Ovary Syndrome: Emphasis on Insulin Resistance. Goulielmos GN, ed. Int J Med Genet. 2014;2014:478972. doi:10.1155/2014/478972