



Analysis of Genetic Studies in Association of α and β Estrogen Receptor Gene Polymorphisms Against Endometriosis

Eldafira^{1*}; Novitasari²

^{1*)} Department of Biology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

²⁾ Research Center of Testing Technology, The National Research and Innovation Agency, Jakarta, Indonesia

ARTICLE INFO

Article history:

Received 11 March 2021

Accepted 21 June 2022

Published 10 July 2022

Keyword:

IDS
DDoS
ZigBee
Naïve Bayes
Classification

**) corresponding author*

Eldafira

Email: eldafira96@yahoo.com

DOI: 10.30604/jika.v7iS1.1269

ABSTRACT

Endometriosis is a common cause of morbidity in women of unknown etiology. Several studies have shown that endometriosis with a polygenic/multifactorial trait. To evaluate α and β estrogen receptor (ER) gene polymorphisms against endometriosis, this study was analyzed from endometriosis outpatient's clinic which compared 83 women with endometriosis and 76 women without endometriosis. The ER α gene rs9340799 A/G and ER β gene rs4986938 G/A polymorphism was identified by restriction fragment length polymorphism-polymerase chain reaction. Genotype distribution and allele frequency of the +1730 G/A polymorphism in the ER α gene rs9340799 A/G and ER β gene rs4986938 G/A. The genetic model on the ER α rs2234693 SNP analysis that there is no significant difference in the four genetic models, but on the ER β rs4986938 it appears that the genetic model dominant (GG vs (GA + AA) and co-dominant (GA vs GG + AA) showed significant differences with $p < 0.05$, i.e. 0.013 and 0.06 respectively and with OR (95% CI) 0.350 and 3.258, respectively. For other genetic models, this SNP did not show significant differences. The data suggest that the ER β gene rs4986938 G/A polymorphism can be associated with the risk of endometriosis development, regardless of the stage of the disease.

This open access article is under the [CC-BY-SA](https://creativecommons.org/licenses/by-sa/4.0/) license.



INTRODUCTION

Endometriosis is a form of endometrial-like tissue that grows and develops outside the uterine cavity, especially in the peritoneum of the ovaries.¹ This tissue grows abnormally and is common in women of reproductive age. The main symptoms of the clinical picture of this incident are pain around the waist during menstruation (dysmenorrhea), pain during intercourse (dyspareunia) and infertility.^{1,2} This is because endometriosis is multifactorial, namely a disease caused by the interaction of several factors: environmental, immunological and genetic. Locations where endometriosis tissue develops are the pelvis and the abdominal cavity.³ Based on clinical data obtained from various hospitals in Indonesia, the incidence of endometriosis reached 13.6-69.5% from the infertile group and data from the Immuno-Endocrinology Polyclinic of the Department of Obstetrics and Gynecology FKUI-RSCM in 2006-2010 recorded as much as approximately 10%. endometriosis in women of reproductive age⁴

Indications for endometriosis are characterized by high concentrations of estrogen levels in local tissues. Estrogen is the main candidate gene associated with endometriosis. The action of estrogen is mediated by its specific receptor, the estrogen receptor (ER).⁵ The estrogen receptor gene consists of two isoforms, namely ER α and ER β which belong to the nuclear gene receptor family and play a role in intracellular transcription factors by activating ligands. Several previous studies suggest that there is an association of the estrogen receptor gene polymorphism with endometriosis.^{6,7}

According to Jakimiuk et al (2007), the results of the study of ER α gene polymorphisms are still experiencing a lot of controversy, while the molecular mechanism of polymorphism influencing ER activity in endometriosis is still unclear.⁸ The ER α gene polymorphism is located in the intronic gene area which is a non-functional gene region. Genetic abnormalities in humans are more than 50% known to be caused by disruption of the normal splicing pattern of intron regions. SnRNA and protein malfunctions can result

from the splicing process that has a detrimental effect on cells.⁹

According to Zhao et al (2016) that the incidence of genetic mutations in the ER α gene tends to indicate the expression of aberrant genes or abnormalities involved in the pathogenesis and development of endometriosis. The ER α gene variants with restriction sites PvuII (rs2234693 position 397 T/C) and XbaI (rs9340799 position 351 A/G) were the most studied. This is the main reason to further study the role of polymorphisms in the diagnosis and incidence of recurrent endometriosis.¹⁰ According to Bulun (2012), the etiology and pathogenesis of endometriosis are still not fully understood, but it is known that estrogen can stimulate the growth of endometrial tissue.¹¹

Two ER α gene polymorphism sites separated by 50 base pairs in the polymorphism study conducted by Cai (2003) showed that the ER α gene polymorphism in the intron one region was also thought to be influenced by linkage disequilibrium (LD).^{8,12} LD is the correlation between adjacent variants such as alleles in neighboring polymorphisms observed on the same chromosome that are associated in the population more often than unlinked. The results of the study stated that three ER α gene polymorphisms in the intron one region, namely RFLP PvuII, XbaI and repeating TA dinucleotide polymorphisms were stated to have strong LDs between polymorphism sites in intron one and microsatellite TA dinucleotide polymorphisms with high coincidence levels. from the short TA allele and the PvuII and XbaI restriction sites.^{13,14}

Linkage and association studies are the most commonly used methods for complex disease investigations. Fundamentally, these methods follow the same principles, such as determining the co-inheritance of adjacent DNA. For generations between cases and controls in haplotypic linkage identification studies suggest that a trait that is inherited for several generations in association study investigations, is retention of adjacent versions of DNA.¹⁵ Many studies have succeeded in revealing that SNPs and alleles are associated with disease. The association between SNPs and disease indicates that there are genes in that area that contribute to disease. If two haploid genomes are compared randomly there are approximately three million differences.¹⁶ The number of variations of DNA sites containing SNPs in humans is not fully known, but between 10 and 30 million SNPs is approximately one every 100 to 300 bases. SNPs generally have a frequency above 20%.¹⁷

We conducted research on ER gene polymorphisms against endometrial disease represented by several hospitals in Jakarta. The results of this study are expected to be able to determine the ER gene variants and the association that cause endometriosis in the Indonesian population considering that until now there has been no comprehensive study of the ER gene polymorphisms and there is no data base for ER gene polymorphisms and their effects on endometriosis.

METHOD

The design of this study is a case control study which compares women with endometriosis (cases) and normal women (controls). The sample that will be used in this study is peripheral blood from 159 patients. The total number of samples used for polymorphism examination in this study were 159 samples consisting of two groups, namely 83 people with endometriosis (cases) and 76 people without endometriosis (control II). Clinical data and peripheral blood

samples were collected only after explaining the objectives of the study and obtaining signed informed consent, as approved by the Research Ethics Committee of the RSCM-UI school of medicine.

Peripheral blood was collected from each patient and control in an EDTA-containing tube. Genomic DNA was extracted from peripheral blood lymphocytes with salting out method using an DNeasy Blood and Tissue (Qiagen, USA), according to the manufacturer's instructions. Molecular analysis of the ER α gene rs9340799 A/G and ER β gene rs4986938 G/A polymorphism was performed according to the protocol of Lee et al. (2012) with modifications.¹⁸ The primers ER α used were: 5 α -CAGGGTTATGTGGCAATGAC-3 α (*forward*) dan 5 α -TACCTATAAAAATGACAAAA-3 α (*reverse*) and ER β 5 β -CGGCAGAGGACAGTAAAAGC-3 β (*forward*) dan 5 β -AGGCCATTGAGTGTGGAAAC-3 β (*reverse*). The PCR reaction was carried out in a final volume of 25 μ L, containing 5 μ L genome DNA, 12,5 μ L master mix (*kappa, Bioline*), 0,5 μ L primer *forward*, 0,5 μ L primer *reverse* and 6,5 μ L *aqua bidestillata* (ddH₂O). Amplification was performed with an initial denaturation step at 94 $^{\circ}$ C for 6 min, followed by 35 cycles of: denaturation at 94 $^{\circ}$ C for 60 s, annealing at 56 $^{\circ}$ C for 40 s, elongation at 72 $^{\circ}$ C for 90 s and a final extension step at 54 $^{\circ}$ C for 5 min.

The PCR products were analyzed for RFLP by using 5 U of AluI restriction enzyme at 37 $^{\circ}$ C overnight and visualized in 2% agarose gel stained with ethidium bromide under UV light. Allele and genotype frequencies were compared between groups using the χ^2 test. All p values were two-tailed and 95% confidence intervals (CIs) were calculated. A p value < 0.05 was considered to be significant.

RESULT AND DISCUSSION

Case and control studies generally compare disease-specific prevalence of individuals with normal alleles and variant alleles in individuals resulting in an odd ratio (OR). The most common type of allele variation to the SNP distinguishes between homozygous major allele (MM), heterozygous (Mm) and homozygous minor allele (mm). Odds are given for each genotype and the pair is OR. The data is displayed using the 2x2 contingency method in determining the OR. There are several genetic models that are often used to determine OR: dominant, multiplicative, recessive and over dominant.

The association of dominant, recessive, additive, co-dominant genetic models is in the ER α gene in intron 1 rs9340799 (XbaI) and rs2234693 (PvuII) and ER β exon rs4986938 (AluI). For the genetic model with 3 SNPs from the ER (Table 1), it can be seen in the table that the ER α rs9340799 SNP with the dominant genetic model (AA vs AG + GG) did not show significant differences as well as the co-dominant genetic model (AG vs AA + GG); recessive (GG vs GA + AA) and additive (GG vs AA) allele frequencies showed significant differences with p<0.05, respectively, were 0.038 and 0.024 with OR values were 2.017 and 2.545 (95% CI), respectively, while the dominant and co-dominant genetic models did not show significant differences.

The genetic model on the ER α rs2234693 SNP, it can be seen from the analysis results that there is no significant difference in the four genetic models, but on the ER β rs4986938 SNP it appears that the genetic model dominant (GG vs (GA + AA) and co-dominant (GA vs GG + AA) showed significant differences with p<0.05, i.e. 0.013 and 0.06 respectively and with OR (95% CI) 0.350 and 3.258, respectively. For other genetic models, this SNP did not show significant differences.

Table 1.
Association of Allele Frequency with Pearson Chi-Square Test

	p<0,05	OR (IK 95%)	min	maks
ERα rs9340799 A/G				
Dominan:				
AA vs(GA+GG)	0,074	1,909	0,934	3,902
Resesif:				
GG vs(AA+GA)	0,038	2,017	1,035	3,929
Additive:				
GG vs AA	0,024	2,545	1,121	5,778
Co-dominan:				
AG vs(AA+GG)	0,706	1,131	0,596	2,147
ERα rs2234693 T/C				
Dominan:				
TT vs(TC+CC)	0,626	0,830	0,392	1,759
Resesif:				
CC vs(TT+TC)	0,192	0,591	0,267	1,308
Additive:				
TT vs CC	0,252	0,567	0,214	1,503
Co-dominan:				
TC vs(TT+CC)	0,515	0,811	0,431	1,525
ERβ rs4986938 G/A				
Dominan:				
GG vs(GA+AA)	0,013	0,350	0,150	0,816
Resesif:				
AA vs(GG+GA)	0,294	-	-	-
Additive:				
GG vs AA	0,346	-	-	-
Co-dominan:				
GA vs(GG+AA)	0,006	3,258	1,357	7,826

Notes: Test the significance of genotypic frequencies from the genetic model on three ER SNPs. The results of the Chi-Square test on SNP rs9340799 showed that the recessive genetic model (GG) was significantly different from the other genotypes (p=0.038) with a risk of endometriosis of 2.017 times. Likewise, the additive genetic model (GGvsAA) showed that the frequency of the GG genotype was significantly different from that of the AA genotype (p=0.024) with 2.545 times the risk of endometriosis. The results of the Chi-Square test on SNP rs2234693 showed that the genotype frequencies in the four genetic models were not significantly different p>0.05.

DISCUSSION

Classical Mendelian genetic methods in studying genes cannot be used to find out information about individual genes because the effects of each gene will not be visible. Lack of knowledge about individual genes makes many unrealistic assumptions such as the frequency of the gene at all loci is approximately the same as well as the effect of the gene and its dominance relationship. The table above shows the association test on four genetic models of genotypic frequency for the SNPs of the ER α and ER β genes by looking at the strength of the association and determining the p value of the OR value.^{13,19}

The results of studies of estrogen receptor (ER) polymorphisms associated with the risk of endometriosis in Asia and Europe show that polymorphisms can be used to predict an increase or decrease in the risk of endometriosis. Potentially useful in the preventive diagnosis, treatment or prognosis of endometriosis.²⁰ A number of studies have linked genetic polymorphisms as a contributing factor to the development of endometriosis. Endometriosis is a disease with a polygenic model and multiple loci inherited traits and

there are several chromosomal regions associated with the endometriosis phenotype.²¹

The results of the association of estrogen receptor polymorphisms with endometriosis were inconsistent. This inconsistency may be due, among other things, to ethnic differences and the small sample size of the association study. Previously, several studies on the association of steroid receptor gene SNPs and genes involved in steroid metabolism and endometriosis have been carried out. Several studies have shown the association of polymorphisms with specific enzymes PvuII and ER α in endometriosis.^{22,23} The results of other studies did not show any association.

Genomic wide association analysis (GWAs) is the development of gene mapping technology whose analysis requires genotypic and phenotypic data. GWAs are also one of the new methods that can provide insights including environmental interactions and identification of candidate genes associated with endometriosis. It is also a potential new technology in finding association models in polygenic diseases such as endometriosis.¹⁹

The etiology of endometriosis in the understanding of the disease has evolved through linkage analysis and association studies, although the pathophysiology of endometriosis and genetics is unclear. The genetic basis of endometriosis is to determine the early detection of associated disease. Especially in improving the treatment of endometriosis associated symptoms: infertility, and pelvic pain.^{24,25}

Association studies in the complex disease of endometriosis have been identified based on high-density genetic markers. The result is that quantitative trait susceptibility statistically shows "valid associations" with: common diseases, hypotheses of common genomic variants in the population. The studies carried out are "direct" (associative studies of functional variance analysis, changes in amino acids in coding regions) or show expression in regulatory regions of candidate genes in a disease. In contrast, "indirect" studies (investigations of genetic marker setting in disease expect loci through LD (linkage disequilibrium) analysis between disease alleles and genetic markers.^{26,27}

When detecting gene susceptibility, especially to multifactorial diseases such as endometriosis, it is necessary to assign a PAR (population-attributable risk) risk level to the population picture. The genetic strength will be reduced and the PAR level and OD ratio (Odd Ratio, OR) will be low. This is if a "rare cause" variant is found in "common disease".^{13,15}

Such conditions to detect susceptibility can be done through an "indirect" approach depending on the level of linkage disequilibrium (LD) between disease variants and genetic markers. Determination of genotyping is relatively risky, where factors that influence genetic power are also included in association studies. Although it is generally recognized that allele frequencies and LD strengths vary widely in different populations and chromosomal regions.^{13,28}

From the four genetic models, it appears that for the SNP gene ER α rs9340799 A/G that the additive genetic model (AA vs. GG) is the best genetic model by showing a significant difference with p value = 0.024 meaning that the frequency of the additive model genotype wild type AA is associated with lower risk (risk factor) for endometriosis cases with an OR value of 2.55 times at a minimum limit of 1.12 and a maximum limit of 5.78 which is higher than other genetic models. It means that individuals with the AA genotype at SNP rs9340799 can reduce their susceptibility to sporadic endometriosis cases 2.55 times compared to the GG variant genotype. Meanwhile, the SNP ER α rs2234693 T/C showed that the four genetic models did not show a significant value.

SNP ER β rs4986938 G/A showed a co-dominant genetic model (GA vs GG+AA) from the statistical test results appeared to show a significant difference with p value = 0.006 and it means that the GA genotype was associated with a lower risk of endometriosis cases with an OR value of 3.258 times at the limit a minimum of 1,357 and a maximum limit of 7,826. It means that individuals with the GA genotype on SNP ER β rs4986938 can reduce their susceptibility to cases of sporadic endometriosis by 3,258 times compared to other genotypes.

SNP ER β rs4986938 G/A showed the dominant genetic model (GG vs AA+AG) from the statistical test results appeared to show a significant difference with p value = 0.038 and it means that the GG genotype was associated with a lower risk of endometriosis cases with an OR value of 2.012 times at a minimum limit of 0.15 and a maximum limit of 0.82. It means that individuals with the GG genotype at SNP ER β rs4986938 can reduce their susceptibility to cases of sporadic endometriosis by 2.02 times compared to other genotypes.

In conclusion, an association between the ER α gene rs9340799 A/G and ER β gene rs4986938 G/A was clear that the ER β gene rs4986938 G/A polymorphism can be associated with the risk of endometriosis development. Even though, due to the limitations shown above, it is important to carry out larger and well-designed multi-center studies to confirm the results.

CONCLUSION

From the results of the analysis of genotype frequencies, allele distributions and association studies conducted in this study on three SNPs (ER α : rs9340799, rs2234693 and ER β : rs4986938) comprehensively, it can be concluded that at the ER α locus rs9340799, the effect of mutant allele frequency (G) appears to have an effect. to an increased risk of endometriosis.

Conflict of Interest statement

The author declares that there is no potential conflict of interest in relation to the authorship and publication of this article.

Funding

Not Applicable

Kontribusi Penulis

Seluruh penulis memiliki kontribusi yang sama dalam penulisan laporan penelitian ini baik dari tahap penyusunan kerangka berpikir hingga interpretasi hasil dalam laporan penelitian.

REFERENCES

[1] V. S. Baranov, T. E. Ivaschenko, T. Liehr, and M. I. Yarmolinskaya, "Systems genetics view of endometriosis: A common complex disorder," *Eur. J. Obstet. Gynecol. Reprod. Biol.*, vol. 185, pp. 59–65, 2015, doi: 10.1016/j.ejogrb.2014.11.036.

- [2] H. S. Wang *et al.*, "Functional analyses of endometriosis-related polymorphisms in the estrogen synthesis and metabolism-related genes," *PLoS One*, vol. 7, no. 11 November, 2012, doi: 10.1371/journal.pone.0047374.
- [3] S. J. Han and B. W. O'Malley, "The dynamics of nuclear receptors and nuclear receptor coregulators in the pathogenesis of endometriosis," *Hum. Reprod. Update*, vol. 20, no. 4, pp. 467–484, 2014, doi: 10.1093/humupd/dmu002.
- [4] Badan Penelitian dan Pengembangan Kesehatan, "Riset Kesehatan Dasar 2013," *Ris. Kesehat. Dasar 2013*, 2013.
- [5] E. Chantalat *et al.*, "Estrogen receptors and endometriosis," *Int. J. Mol. Sci.*, vol. 21, no. 8, pp. 1–17, 2020, doi: 10.3390/ijms21082815.
- [6] F. Zhang, Y. Yang, and Y. Wang, "Association between TGF- β 1-509C/T polymorphism and endometriosis: A systematic review and meta-analysis," *Eur. J. Obstet. Gynecol. Reprod. Biol.*, vol. 164, no. 2, pp. 121–126, 2012, doi: 10.1016/j.ejogrb.2012.05.004.
- [7] S. Govindan, N. A. Shaik, B. Vedicherla, V. Kodati, K. P. Rao, and Q. Hasan, "Estrogen receptor- α gene (T/C) Pvu II polymorphism in endometriosis and uterine fibroids," *Dis. Markers*, vol. 26, no. 4, pp. 149–154, 2009, doi: 10.3233/DMA-2009-0625.
- [8] A. J. Jakimiuk *et al.*, "Prevalence of estrogen receptor α PvuII and XbaI polymorphism in population of Polish postmenopausal women," *Folia Histochem. Cytobiol.*, vol. 45, no. 4, pp. 331–338, 2007, doi: 10.5603/4504.
- [9] J. Bouquet De Jolinière, J. M. B. Ayoubi, L. Gianaroli, J. B. Dubuisson, J. Gogusev, and A. Feki, "Endometriosis: A New Cellular and Molecular Genetic Approach for Understanding the Pathogenesis and Evolutivity," *Front. Surg.*, vol. 1, no. May, 2014, doi: 10.3389/fsurg.2014.00016.
- [10] C. Zhao, K. Dahlman-Wright, and J. A. Gustafsson, "Estrogen receptor beta: an overview and update," *Nucl. Recept. Signal.*, vol. 6, pp. 1–10, 2008, doi: 10.1621/nrs.06003.
- [11] S. E. Bulun *et al.*, "Role of estrogen receptor- β in endometriosis," *Semin. Reprod. Med.*, vol. 30, no. 1, pp. 39–45, 2012, doi: 10.1055/s-0031-1299596.
- [12] Q. Cai *et al.*, "Genetic polymorphisms in the estrogen receptor α gene and risk of breast cancer: Results from the Shanghai breast cancer study," *Cancer Epidemiol. Biomarkers Prev.*, vol. 12, no. 9, pp. 853–859, 2003.
- [13] L. Becherini *et al.*, "Evidence of a linkage disequilibrium between polymorphisms in the human estrogen receptor α gene and their relationship to bone mass variation in postmenopausal Italian women," *Hum. Mol. Genet.*, vol. 9, no. 13, pp. 2043–2050, 2000, doi: 10.1093/hmg/9.13.2043.
- [14] H. Lu *et al.*, "Association of estrogen receptor- α gene polymorphisms with coronary artery disease in patients with familial hypercholesterolemia," *Arterioscler. Thromb. Vasc. Biol.*, vol. 22, no. 5, pp. 821–823, 2002, doi: 10.1161/01.atv.0000014424.18209.21.
- [15] M. W. Pfaffl, "Relative quantification," *Real-time PCR*, pp. 64–82, 2007.
- [16] E. Weiderpass, I. Persson, H. Melhus, S. Wedrén, A. Kindmark, and J. A. Baron, "Estrogen receptor α gene polymorphisms and endometrial cancer risk," *Carcinogenesis*, vol. 21, no. 4, pp. 623–627, 2000, doi: 10.1093/carcin/21.4.623.
- [17] K. L. Lunetta, "Genetic association studies," *Circulation*, vol. 118, no. 1, pp. 96–101, 2008, doi: 10.1161/CIRCULATIONAHA.107.700401.

- [18] D. H. Lee *et al.*, "Effects of 17 β -estradiol on the release of monocyte chemoattractant protein-1 and MAPK activity in monocytes stimulated with peritoneal fluid from endometriosis patients," *J. Obstet. Gynaecol. Res.*, vol. 38, no. 3, pp. 516–525, 2012, doi: 10.1111/j.1447-0756.2011.01734.x.
- [19] H. T. T. Luong *et al.*, "Fine mapping of variants associated with endometriosis in the WNT4 region on chromosome 1p36," *Int. J. Mol. Epidemiol. Genet.*, vol. 4, no. 4, pp. 193–206, 2013.
- [20] D. K. Hapangama, A. M. Kamal, and J. N. Bulmer, "Estrogen receptor b: The guardian of the endometrium," *Hum. Reprod. Update*, vol. 21, no. 2, pp. 174–193, 2015, doi: 10.1093/humupd/dmu053.
- [21] P. Viganò, F. Parazzini, E. Somigliana, and P. Vercellini, "Endometriosis: Epidemiology and aetiological factors," *Best Pract. Res. Clin. Obstet. Gynaecol.*, vol. 18, no. 2, pp. 177–200, 2004, doi: 10.1016/j.bpobgyn.2004.01.007.
- [22] C. S. De Mattos *et al.*, "ESR1 and ESR2 gene polymorphisms are associated with human reproduction outcomes in Brazilian women," *J. Ovarian Res.*, vol. 7, no. 1, 2014, doi: 10.1186/s13048-014-0114-2.
- [23] C. A. Anderson, F. H. Pettersson, G. M. Clarke, L. R. Cardon, A. P. Morris, and K. T. Zondervan, "Data quality control in genetic case-control association studies," *Nat. Protoc.*, vol. 5, no. 9, pp. 1564–1573, 2010, doi: 10.1038/nprot.2010.116.
- [24] G. D. Adamson and D. J. Pasta, "Endometriosis fertility index: The new, validated endometriosis staging system," *Fertil. Steril.*, vol. 94, no. 5, pp. 1609–1615, 2010, doi: 10.1016/j.fertnstert.2009.09.035.
- [25] J. Cui, Y. Shen, and R. Li, "Estrogen synthesis and signaling pathways during aging: From periphery to brain," *Trends Mol. Med.*, vol. 19, no. 3, pp. 197–209, 2013, doi: 10.1016/j.molmed.2012.12.007.
- [26] C. A. Witz and W. N. Burns, "Endometriosis and infertility: Is there a cause and effect relationship?," *Gynecol. Obstet. Invest.*, vol. 53, no. SUPPL. 1, pp. 2–11, 2002, doi: 10.1159/000049418.
- [27] N. K. Mungreiphy, S. Kapoor, and R. Sinha, "Association between BMI, Blood Pressure, and Age: Study among Tangkhul Naga Tribal Males of Northeast India," *J. Anthropol.*, vol. 2011, no. September, pp. 1–6, 2011, doi: 10.1155/2011/748147.
- [28] S. E. Bulun *et al.*, "Estrogen receptor- β , Estrogen receptor- α , and progesterone resistance in endometriosis," *Semin. Reprod. Med.*, vol. 28, no. 1, pp. 36–43, 2010, doi: 10.1055/s-0029-1242991.

