Original Article
Running Title: ER Pathway Genes in Breast Cancer Risk
Received: November 16, 2024; Accepted: December 7, 2025

Breast Cancer Susceptibility Genes in Estrogen Receptor Signaling Pathway in a Southern Indian Population

Andrea Mary F[*♦],PhD, Devi Krishna RB[*],MSc, Nandini Krishnamurthy[*],MSc,Sanjana Murali[*],MSc, Ramya R[**], MD,Nalini Ganesan[***], PhD

[*]*Department of Human Genetics, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India*

[**]*Department of Surgery, Appollo Institute of Medical Sciences and Research, Chittor, Andhra Pradesh, India*

[***]*Department of Biochemistry, Sri Ramachandra Institute of Higher Education and Research, Chennai, Tamil Nadu, India*

♦Corresponding Author
Andrea Mary F, PhD
Department of Human Genetics,
Sri Ramachandra Institute of Higher Education and Research,
Chennai, Tamil Nadu, India.
Email:andreamary@sriramachandra.edu.in

Abstract

Background: Ovarian cancer starts in the ovaries and is hard to detect early due to vague symptoms. The present study aimed to investigate whether the *ESR1* gene is linked to breast cancer. Also, we aimed to examine whether genetic changes in the *ESR1* gene affect breast cancer risk through estrogen binding.

Method: A hospital‐based case–control study was conducted with 200 breast cancer patients and 200 age‐matched healthy controls. Peripheral blood samples were collected, and genomic DNA was extracted using standard protocols. *ESR1* gene variants were genotyped by real‐time PCR. Allele and genotype frequencies were compared, and risk was estimated by statically.

Results: The C/C genotype of the PvuII site in the *ESR1* gene showed a significant link to breast cancer risk. No association was found for the AG/GG genotype of theXbaI polymorphism. The TG and GG genotypes of *rs2881766* showed a weak risk increase.

Conclusion: The *rs2234693* variation is linked to breast cancer risk, suggesting its potential for genetic screening and personalized therapy. No significant link was found for other variations, highlighting *rs2234693* for future research.

Keywords: Breast neoplasm, Estrogen receptor, Polymorphism, Genetic, Genotype

1. Introduction

The most common cancer in women is breast cancer worldwide.[1] Millions of people in the world are still impacted by breast cancer, a complicated and common health issue.[2] In both developed and developing countries, breast cancer is the most common cancer. It is the second most common cancer in the world. Breast cancer is the common gynecological malignancy in western countries with lifetime prevalence of 1 in 12 and 1 in 8 for United Kingdom and U.S.A. In the United States, women have an approximate 13% chance of getting breast cancer at some point in their lives. This indicates that her chances of getting breast cancer are 1 in 8. Epidemiological studies expect the global burden of breast cancer to cross 2 million by 2030.[3] Like other cancers genetic factors has a central role in the pathogenesis of breast cancer. Studies have reported an association between high levels of exogenous estrogen in the development, progression and alteration in breast tumors[4]. Estrogen plays a vital role in the proliferation of the mammary gland. Estrogens exhibit their effects through the receptors *ESR1*and *Estrogen Receptor beta (ER β).* The ESR *1* gene is a large gene located at the position 6q25.1 spanning 473Kb and it consists of eight coding exons and nine non coding exons.[5] The *ESR1* gene is found to be tightly associated with breast cancer risk as it regulates the cellular proliferation and differentiation of the mammary gland through paracrine mechanisms. The transcription factor encoded by the *ESR1* gene has estrogen binding domain, estrogen response element and an activation domain.[6,7] Genetic alterations in the *ESR 1* gene result in altered estrogen activity having the ability to modulate the breast cancer risk. Numerous studies conducted on the single-nucleotide polymorphisms (SNPs) of *ESR1* have shown an association with 2 SNPs in the first intron of the *ESR 1* gene.[8] The PvuII and Xba I single nucleotide polymorphism have found to be associated with carcinogenesis and other diseases. The polymorphism is generally described by the respective reference ID numbers *rs2234693* and *rs9340799* or by the restriction enzyme named PvuII and XbaI which is used for the detection of the variant C454A – 397 T → C and C454 A → G, located 397 and 351 bp upstream of exon 2.[9] The two polymorphisms are separated by just 50bps and are expected to be in strong linkage disequilibrium and located in the intronic region of the gene. Various studies have shown these polymorphisms to be associated with several pathological conditions like prostate cancer, breast cancer, cardiovascular diseases, Alzheimers disease and osteoporosis.[10]Allelic variants in the genes encoding the estrogen receptor (ER) influence the activity of the receptor, and also alter the expression of sex steroid response pathways; Thus, polymorphism in *ESR1* gene are implicated as candidate risk markers for breast cancer.[11,12] Therefore, the present study aimed to determine if breast cancer is associated with the *ESR1* genes *rs9340799, rs2234693, and rs2881766.*

2. Materials and Methods

2.1. Participants

The present case-control study comprised of 200 histological confirmed breast cancer patients from South India. The patients were from the oncology department of Sri Ramachandra Institute of Higher Education and Research India, from January 2013 to December 2017. Relevant clinical and pathological data were collected from all the patients. Pathological grading of the tumors was obtained by the histopathological examination. The inclusion criteria for the healthy volunteers were: no previous diagnosis of any benign breast diseases; no history of mastectomy, hysterectomy or oophorectomy; no family history of ovarian,

breast, endometrial and prostate cancer, no mental or physical disability. The patients and controls were similar in ethnicity and nutritional habits and they were age matched. The criteria for breast cancer patients were no previous treatment for cancer and confirmation of breast malignancy with histological diagnosis. Informed consent was mandatory for both groups. The study was authorized by the institutional Ethics Committee at the Sri Ramachandra Institute of Higher Education and Research, Chennai, India. (REF: IEC-NI/13/APR/33/28) (ICMR guidelines), for control and breast cancer affected groups. Written informed consent was collected from all the participants enrolled for the study.

2.2. Molecular analysis by Taqman allelic discrimination assay

Peripheral blood samples about 3ml were collected from the participant by veinpuncture and collected into K2 - EDTA vacutainers. DNA was isolated from peripheral blood using salting-out method. DNA sample analyzed for selected SNP's by Taqman SNP methodology with real-time polymerase chain reaction technology (Taqman SNP Genotyping Assay, Applied Biosystems, Carlsbad, USA Version 2). The DNA isolated was amplified using Taq Gold Polymerase of Taqman polymerase chain reaction (PCR) master mix in ABI machine ABI 7900 using sequence specific primers (Table 7). The reaction volume was set to 5µL, consisting of 2.50µL of Taqman genotyping master mix (2X), 0.25µL of Taqman genotyping assay mix (20X) and 2.25µL of the genomic DNA of 10ng concentration obtained on diluting the DNA with distilled water. Thermal cycle reaction condition is programmed to initial denaturation at 95°C for 10minutes followed by denaturation at 95°C for 15s for 40 cycles followed by annealing and extension at 60 C for 1 minute in 384 wells in ABI 7900 machine. Taqman probes will provide a fluorescence signal for the amplification of each allele. After PCR amplification, an end plate read will be performed in ABI machine. The sequence detection system software will use the fluorescence measurements made during the plate read to plot fluorescence (Rn) values based on the signal from each well. The plotted fluorescence signals will indicate which alleles are in each sample One fluorescent dye detector is a perfect match to the wild type (allele 1) and the other fluorescent dye detector is a perfect match to the variant allele (allele 2). The alleles were labelled with VIC-dye and FAM dye.

2.3. Statistical analysis

All statistical analyses were carried out using the SPSS statistical software version 19.0. $\chi 2$ goodness-of-fit test was used for comparison of expected and observed genotype frequencies for performing Hardy Weinberg equilibrium. $\chi 2$ test was performed for comparing the genotype frequencies of the polymorphisms between the controls and subjects with breast cancer. Using the wild type genotypes as the reference groups the odds ratio and 95% confidence interval were calculated. P <0.05 was considered to be statistically significant. Pairwise linkage disequilibrium (Table 7) was computed as both D' and r2 using Haploview v.4.1.The study participants were stratified into two groups based on their menopausal status as premenopausal and menopausal, based on the aggressiveness of the cancer as low grade and high grade, based on their receptor status as estrogen positive, progesterone positive and Her 2 positive and the risk was determined for each group by calculating the odds ratio with 95% confidence interval (CI). In order to further analyze SNPs, ORs and 95% CIs were used in three logistic regression models: additive, dominant, and recessive. The criterion for statistical significance was less than 0.05. We used data from HapMap to our research findings with those of other ethnic groups.

The allele frequencies of *ESR1* gene polymorphisms between the communities being studied and other groups were compared using HapMap data (Figure 1). The results demonstrated that the variant allele of the *ESR1rs2234693* polymorphism was positively associated with the South Indian population when compared with other groups.

4. Results

Three polymorphisms *rs9340799, rs2234693 and rs2881766* of *ESR1* gene were studied to determine the association with breast cancer risk. The demographic data of the participants are represented in Table 1. A significant association of the CC genotype of the PvuII site in the *ESR1* gene (odds ratio, 2.18; 95% CI, 1.14–4.16; *P*=0.02), with breast carcinoma risk, was observed (Table 2). The variant *rs2234693* showed a positive association with breast cancer risk. For *ESR1* gene *rs9340799* (A to G) polymorphism the frequency of homozygous wild type AA is 40.5%, heterozygous mutant AG is 43.5% and homozygous mutant GG is 16% in the control group. The frequency of homozygous wild type AA is 41% heterozygous mutant AG is 42% and homozygous mutant GG is 17% in the breast cancer patients (Table 2). For *ESR1*gene *rs2234693* (T to C) polymorphism the frequency of Homozygous wild type TT is 44%, Heterozygous mutant TC is 46.5% and Homozygous mutant CC is 9.5% in the control group. The frequency of homozygous wild type TT is 35% heterozygous mutant TC is 48% and Homozygous mutant CC is 16.5% in the breast cancer patients (Table 2). For ESR Gene *rs2881766* (T to G) polymorphism the frequency of homozygous wild type TT is 54%, heterozygous mutant TG is 36% and homozygous mutant GG is 10% in the control group. The frequency of homozygous wild type TT is 51% heterozygous mutant TG is 37.5% and Homozygous mutant GG is 11.5% in the breast cancer patients (Table 2). The association of the genotypes for the three polymorphisms with menopausal status was analyzed *rs2234693* showed an association with premenopausal breast cancer cases (Table 3). The association analysis of the genotypes with tumor grade revealed association with high grade tumor for *rs2234693* (Table 4). The association analysis with receptor status did not show an association with receptor status for all the three polymorphisms studied. Haplotype analysis was not informative Table 5 and Figure 2.

4. Discussion

The study investigated the association of three polymorphisms in the *ESR1* gene (*rs9340799, rs2234693,* and *rs2881766*) with breast cancer risk. It found that the *rs2234693* polymorphism showed a significant association with an increased risk of breast cancer, particularly in premenopausal women and those with high-grade tumors. Specifically, the CC genotype of this polymorphism at the PvuII site had an odds ratio of 2.18, indicating a higher likelihood of developing breast cancer. In contrast, the *rs9340799* and *rs2881766* polymorphisms did not show a significant association with breast cancer risk or receptor status. Overall, the findings highlight the potential role of the *rs2234693* variant in contributing to breast cancer susceptibility.

ERs belong to a group of proteins classified as nuclear hormone receptors and are members of ligand-activated transcription factors. The action of the hormone estrogen on its targets is mediated by ER.[13] The PvuII and XbaI are intronic variants expected not to be involved in protein modification, but they have been suggested as genetic biomarkers for hormone-related pathological conditions, as they work by modulating other functional regions with which they interact, thereby altering the function of the *ESR1* gene or its

expression.[14] There are two forms of ERs, namely ERs α and β, each coded by two genes, *ESR1* and ESR2. These receptors are expressed in many organs and tissues like the mammary gland, uterus, hypothalamus, ovary, and pituitary gland. Estradiol performs several functions: it stimulates the growth of granulosa cells, increases the production of granulosa cell insulin-like growth factor 1, upholds the Follicle-Stimulating Hormone receptor, increases the activity of the enzyme aromatase and thereby the production of estradiol, and stops the apoptosis of granulosa cells. Estrogen binds to receptors that are members of nuclear receptors and performs ligand-regulated transcription factor activities, activating the transcription of other genes as well. The receptor estrogen and its ligand 17 beta-estradiol play a vital role in the development and growth of secondary sexual characteristics in females, the female reproductive tract, and the maintenance of pregnancy. The *ESR1* gene has several promoters responsible for tissue-specific expression of genes. The encoded protein has three domains similar to other nuclear hormone receptors: an amino-terminal domain for transcription regulation, a central domain for binding to DNA, and a carboxy-terminal hormone-binding domain. When the estrogen receptor is bound to the principal ligand, compound estradiol, the complex transforms to a form that has high affinity for the components in the nucleus, thus activating or repressing the expression of several genes involved in organizational and developmental functions.

The findings suggest that genetic variation in the genes coding for proteins participating in hormone-mediated signal transduction has the ability to modulate the potential of steroid hormones, the concentration of the hormones, and their carcinogenic metabolites. The binding of estradiol to the estrogen receptor triggers DNA synthesis, division, and growth factor production.[15] Estrogen is essential for the normal development of the mammary gland, but genetic variation induces breast cell proliferation, which may be procarcinogenic. Identified factors like lifetime exposure to estrogen and other hormones explain the involvement of estrogen levels in the pathogenesis of malignancy.[16] Estrogens are implicated in the regulation of bone metabolism, the cardiovascular system, the central nervous system, and the reproductive system. They are also reported to be associated with the pathogenesis of breast and endometrial cancers.[17]

To gain insight into the functional role of the polymorphism, several hypotheses have been put forward. It may be that the *ESR1* gene *rs2234693* with the C allele may have increased responsiveness towards estrogen, resulting in higher estrogen-regulated activities of growth proliferation, increasing estrogenic diseases. The C allele has a potential binding site for the myeloblastosis (myb) transcription factor that, in the presence of B-myb, is capable of augmenting in vitro transcription of a downstream reporter construct tenfold. Thus, in some settings, the presence of the C allele might amplify ERα transcription. Genes related to estrogen metabolism, like endothelial nitric oxide synthase and vascular endothelial growth factors, are also activated, promoting the survival of cells and suppressing apoptosis. Studies assessing the cell response to estradiol for proliferation on myometrial cell lines with different genotypes of ER alpha showed that, compared with other genotypes of ER α, cell lines with the CC genotype had a significant increase in proliferation, correlating to in-vivo conditions for estrogenic diseases.[18]

While some studies reported the association of the T allele of the PvuII polymorphism with increased breast cancer risk,[19] other studies did not show an association between the PvuII polymorphism of the *ESR1* gene

and breast cancer risk. The inconsistent results may be due to variable factors such as ethnicity, environment, age, and other factors contributing to genetic heterogeneity.[13] The results of the study were in accordance with some studies that reported an increased risk of breast cancer for the PvuII CT and CC genotypes.[18] A meta-analysis conducted in Asian women showed an association of the *rs2234693* of the *ESR1* gene.[20] The results also correlated with the findings of a study conducted in Asia, where an association of the CC allele of the *rs2234693* of the *ESR1* gene with breast cancer risk in premenopausal women was reported.[21] The polymorphism *rs2881766*, studied for the first time in the South Indian population, failed to show an association with breast cancer risk in South Indian women. The *rs2881766* was found to be strongly associated with breast cancer risk in two studies in Korean and Chinese populations.[22,23]

Recent studies have continued to explore the role of estrogen receptors and their polymorphisms in various conditions. For example, a study discusses recent strategies targeting estrogen receptor alpha for the treatment of breast cancer, highlighting the importance of ER-α in breast cancer development and treatment strategies.[18] Another study by Chen et al. in 2024 investigates the role of G protein-coupled estrogen receptor in non-small cell lung cancer, providing insights into the mechanisms by which estrogen receptors influence cancer progression. These recent studies underscore the ongoing relevance and importance of understanding estrogen receptor polymorphisms in the context of cancer research and treatment.

Since ethnic/race specific associations of *ESR1* gene have been reported contradiction among different investigations could be attributed to variations in ethnic groups. Indeed, the specific influence of gene polymorphisms and environmental factors could be different in various populations. Estrogenic endocrine disruptors or Xenoestrogens are widely distributed in the environment such as pesticides, polychlorinated biphenyl congeners, food related toxic chemicals, metals influence hormonal response by binding to estrogen receptors and the presence of polymorphic allele results in high estrogen receptor mediated activity. Thus, the polymorphism *rs2234693* of *ESR 1* gene showed an association with breast cancer risk in premenopausal women and an association with tumor grade.

Our findings suggest a significant association between the *rs2234693* variation in the *ESR1* gene and breast cancer risk, while no association was observed for the XbaI*rs9340799* and *rs2881766* polymorphisms. This highlights the potential for genetic screening and personalized therapy targeting the *rs2234693* variation. However, this study has certain limitations. The sample size was relatively small, which may affect the generalizability of the results. Additionally, the study population was limited to a specific geographic region, and further research with a more diverse population is needed to validate these findings. Despite these limitations, our results provide valuable insights for future research and clinical applications in breast cancer genetics.

5. Conclusion

The analysis of the parameters suggested the involvement of genetic polymorphism in estrogen receptor regulating genes with breast cancer risk. The genotype analysis aids the assessment of risk individual for early detection and for the selection of candidates for chemoprevention and hormonal more effectively. Our study clarifies previous in consistent results shedding light on the genes for future research focus.

Acknowledgements
We express our gratitude to every individual who volunteered to take part in this study. We also appreciate the nurses and employees from SRIHER's Department of Oncology for supplying the samples and the data.

Authors' Contribution
AMF: Study design, data collection, analysis, interpretation, drafting, reviewing, and validation of results; **DK:** Drafting the manuscript, designing tables and figures; **NK:** Drafting the manuscript, designing tables and figures; **SM:** Drafting the manuscript, designing tables and figures; **SFDP:** Drafting and reviewing the manuscript; **NG:** Data provision and validation of results; **RR:** Data provision and validation of results. All authors read and approved the final version of the manuscript.

Funding
None declared.

Conflict of Interest
None declared.

Data Availability
Data sharing not applicable to this article as no datasets were generated or analyzed during the present study.

References

1. Wilkinson L, Gathani T. Understanding breast cancer as a global health concern. *Br J Radiol.* 2022;95(1130):20211033. doi: 10.1259/bjr.20211033. PMID: 34905391; PMCID: PMC8822551.
2. Obeagu EI, Obeagu GU. Breast cancer: A review of risk factors and diagnosis. *Medicine (Baltimore).* 2024;103(3):e36905. doi: 10.1097/MD.0000000000036905. PMID: 38241592; PMCID: PMC10798762.
3. Mehrotra R, Yadav K. Breast cancer in India: Present scenario and the challenges ahead. *World J Clin Oncol.* 2022;13(3):209-18. doi: 10.5306/wjco.v13.i3.209. PMID: 35433294; PMCID: PMC8966510.
4. Crooke PS, Justenhoven C, Brauch H; GENICA Consortium; Dawling S, Roodi N, et al. Estrogen metabolism and exposure in a genotypic-phenotypic model for breast cancer risk prediction. *Cancer Epidemiol Biomarkers Prev.* 2011;20(7):1502-15. doi: 10.1158/1055-9965.EPI-11-0060. PMID: 21610218; PMCID: PMC3472969.
5. Yoshidome K, Shibata MA, Couldrey C, Korach KS, Green JE. Estrogen promotes mammary tumor development in C3(1)/SV40 large T-antigen transgenic mice: paradoxical loss of estrogen receptoralpha expression during tumor progression. *Cancer Res.* 2000;60(24):6901-10. PMID: 11156389.
6. Mallepell S, Krust A, Chambon P, Brisken C. Paracrine signaling through the epithelial estrogen receptor alpha is required for proliferation and morphogenesis in the mammary gland. *Proc Natl Acad Sci U S A.* 2006;103(7):2196-201. doi: 10.1073/pnas.0510974103. PMID: 16452162; PMCID: PMC1413744.
7. Rusidzé M, Adlanmérini M, Chantalat E, Raymond-Letron I, Cayre S, Arnal JF, et al. Estrogen receptor-α signaling in post-natal mammary development and breast cancers. *Cell Mol Life Sci.*

2021;78(15):5681-705. doi: 10.1007/s00018-021-03860-4. PMID: 34156490; PMCID: PMC8316234.
8. Karsono R, Haryono SJ, Karsono B, Harahap WA, Pratiwi Y, Aryandono T. ESR1 PvuII polymorphism: from risk factor to prognostic and predictive factor of the success of primary systemic therapy in advanced breast cancer. *BMC Cancer.* 2021;21(1):1348. doi: 10.1186/s12885-021-09083-x. PMID: 34930150; PMCID: PMC8686387.
9. Eldafira E, Prasasty VD, Abinawanto A, Syahfirdi L, Pujianto DA. Polymorphisms of estrogen receptor-α and estrogen receptor-β genes and its expression in endometriosis. *Turk J Pharm Sci.* 2021;18(1):91-5. doi: 10.4274/tjps.galenos.2019.94914. PMID: 33634683; PMCID: PMC7957307.
10. Dunning AM, Healey CS, Baynes C, Maia AT, Scollen S, Vega A, et al. Association of ESR1 gene tagging SNPs with breast cancer risk. *Hum Mol Genet.* 2009;18(6):1131-9. doi: 10.1093/hmg/ddn429. PMID: 19126777; PMCID: PMC2722230.
11. Wieser F, Schneeberger C, Tong D, Tempfer C, Huber JC, Wenzl R. PROGINS receptor gene polymorphism is associated with endometriosis. *Fertil Steril.* 2002;77:309-12. doi: 10.1016/S0015-0282(01)02975-4.
12. Tan GC, Chu C, Lee YT, Tan CCK, Ashburner J, Wood NW, et al. The influence of microsatellite polymorphisms in sex steroid receptor genes ESR1, ESR2 and AR on sex differences in brain structure. *Neuroimage.* 2020;221:117087. doi: 10.1016/j.neuroimage.2020.117087. PMID: 32593802; PMCID: PMC8960998.
13. Yaşar P, Ayaz G, User SD, Güpür G, Muyan M. Molecular mechanism of estrogen-estrogen receptor signaling. *Reprod Med Biol.* 2016;16(1):4-20. doi: 10.1002/rmb2.12006. PMID: 29259445; PMCID: PMC5715874.
14. Montazeri-Najafabady N, Dabbaghmanesh MH. Association of estrogen receptor alpha gene PvuII and XbaI polymorphisms with obesity-related phenotypes and body composition in Iranian children and adolescents: a cross-sectional study. *Egypt J Med Hum Genet.*2025;26(48). doi: 10.1186/s43042-025-00678-8.
15. Ganguly S, Naik D, Muskara A, Mian OY. The nexus of endocrine signaling and cancer: How steroid hormones influence genomic stability. *Endocrinology.* 2021;162(1):bqaa177. doi: 10.1210/endocr/bqaa177. PMID: 33260197; PMCID: PMC7707372.
16. Dall GV, Britt KL. Estrogen effects on the mammary gland in early and late life and breast cancer risk. *Front Oncol.* 2017;7:110. doi: 10.3389/fonc.2017.00110. PMID: 28603694; PMCID: PMC5445118.
17. Zhu J, Zhou Y, Jin B, Shu J. Role of estrogen in the regulation of central and peripheral energy homeostasis: from a menopausal perspective. *Ther Adv Endocrinol Metab.* 2023;14:20420188231199359. doi: 10.1177/20420188231199359.PMID: 37719789; PMCID: PMC10504839.
18. Khan NU, Khan H, Alanzi AR, Chen T. Association of ESR1, HER1, and HER2 polymorphisms with breast cancer risk in the KP population: a case-control study. *J Mammary*

Gland Biol Neoplasia. 2025;30(1):6. doi: 10.1007/s10911-025-09500-3.
19. Zhang ZL, Zhang CZ, Li Y, Zhao ZH, Yang SE. Association between ERα gene Pvu II polymorphism and breast cancer susceptibility: A meta-analysis. *Medicine (Baltimore).* 2018;97(17):e0317. doi: 10.1097/MD.0000000000010317.PMID: 29702977; PMCID: PMC5944501.
20. Smolarz B, Nowak AZ, Bryś M, Forma E, Łukasiewicz H, Samulak D, et al. Analysis of single nucleotide polymorphisms (SNPs) rs2234693 and rs9340799 of the ESR1 gene and the risk of breast cancer. *In Vivo.* 2024;38(5):2134-43. doi: 10.21873/invivo.14237.PMID: 39187368; PMCID: PMC11363788.
21. Al-Amri RJ, Alotibi MK, Al-Raddadi RI, Shebli WT, Fallatah EI, Alhujaily AS, et al. Estrogen receptor 1 gene (ESR1) rs2234693 polymorphism and breast cancer risk in Saudi women. *Asian Pac J Cancer Prev.* 2020;21(11):3235-40. doi: 10.31557/APJCP.2020.21.11.3235.PMID: 33247680; PMCID: PMC8033134.
22. Son BH, Kim MK, Yun YM, Kim HJ, Yu JH, Ko BS, et al. Genetic polymorphism of ESR1 rs2881766 increases breast cancer risk in Korean women. *J Cancer Res Clin Oncol.* 2015;141(4):633-45. doi: 10.1007/s00432-014-1849-2.PMID: 25323936; PMCID: PMC11823984.
23. Dai Z, Tian T, Wang M, Yang T, Li H, Lin S, et al. Genetic polymorphisms of estrogen receptor genes are associated with breast cancer susceptibility in Chinese women. *Cancer Cell Int.* 2019;19:11. doi: 10.1186/s12935-019-0727-z.PMID: 30636932; PMCID: PMC6325673.

Table 1. Factors associated with breast cancer risk

Factors	**Cases (n=200)**	**Controls (n=200)**	**χ2**	***P***	**OR (95% CI)***	***P*-value**
Menarcheal age						
>14	14	16	1.629	0.44	1	
12-14	175	178			1.12(0.53-2.37)	0.75
<12	11	6			2.09(0.61-7.14)	0.23
Parity						
Parous	178	181	0.108	0.74	1	
Nulliparous	22	19			1.17(0.61-2.25)	0.62
Age at first birth						
<20	20	23	1.831	0.60	1	
20–24	78	88			1.01(0.52-1.99)	0.95
25–29	50	46			1.25(0.60-2.56)	0.54
>29	52	43			1.39(0.67-2.86)	0.37
Lactational history						
Yes	164	169	0.286	0.59	1	
No	36	31			1.19(0.70-2.02)	0.50
BMI						
Normal (18–25)	80	91	1.517	0.67	1	
Underweight (<18)	20	19			1.19(0.59-2.40)	0.61
Overweight (25–30)	62	59			1.19(0.75-1.90)	0.45
Obesity (>30)	38	31			1.39(0.79 2.44)	0.24
Family history						
No	171	200	29.14	6.707e-08	1	
Yes	29	0			68.97(4.18-1137.33)	0.003**
O.C						
No	182	194	5.36	0.020*	1	
Yes	18	6			3.19(1.24-8.23)	0.01*
H.R.T						
No	188	193	0.88	0.347	1	
Yes	12	7			1.75(0.67-4.56)	0.24

*Odds ratios and 95% CI was calculated by logistic regression analysis showed association with oral contraceptive. OR: Odds ratio; CI: Confidence interval; BMI: Body mass index; OC: Oral contraceptives; HRT: Hormone replacement therapy

Table 2. Genotype and allele frequencies of studied SNP's in estrogen receptor signaling

Gene	Genotype	Control (%)	Case (%)	OR (95% CI)	*P* value
***ESR1*XbaI (*rs9340799*) A>G, Genotype frequency**					
ESR 1	AA	81(40.50%)	84(42%)	Ref	
rs9340799	AG	87(43.50%)	82(41%)	0.91(0.59-1.39)	0.66
	GG	32(16%)	34(17%)	1.02(0.58-1.81)	0.93
	AG+GG	119(59.50%)	116(58%)	0.94(0.63-1.39)	0.76
	A	249(62.25%)	250(62.50%)	Ref	
	G	151(37.75%)	150(37.50%)	0.98(0.74-1.32)	0.94
	MAF	0.38	0.38		
	HWP	0.29	0.07		
***ESR1*PvuII (*rs2234693*) T>C, Genotype frequency**					
ESR 1	TT	88(44%)	70(35%)	Reference	
rs2234693	TC	93(46.50%)	97(48.5%)	1.31(0.85-2.00)	0.21
	CC	19(9.50%)	33(16.50%)	2.18(1.14-4.16)	0.02*
	TC+CC	112(56%)	130(65%)	1.46(0.97-2.18)	0.06
	T	269(67.25%)	237(59.25%)	Reference	
	C	131(32.75%)	163(40.75%)	1.41(1.06-1.88)	0.02*
	MAF	0.33	0.41		
	HWP	0.43	0.95		
***ESR 1* Gene *rs2881766* T>G, Genotype frequency**					
ESR 1	TT	108 (54%)	102 (51%)	Ref	
rs2881766	TG	72 (36%)	75 (37.5%)	1.10(0.72-1.68)	0.65
	GG	20 (10%)	23 (11.5%)	1.22(0.63-2.35)	0.56
	TG+GG	92 (46%)	98 (49%)	1.13(0.76-1.67)	0.55
	T	288 (72%)	279 (69.7%)	Ref	
	G	112 (28%)	121 (30.25%)	1.12(0.82-1.51)	0.48
	MAF	0.28	0.30		
	HWP	0.13	0.11		

A $\chi 2$ test was performed to evaluate the association between SNP and breast cancer cases. The genotypes were verified to comply with the HW; OR and 95% CI were calculated to assess the relative risk; $p < 0.05$ was considered to be statistically significant. A significant association of the CC genotype of the PvuII site in the *ESR1* gene (odds ratio, 2.18; 95% confidence interval, 1.14–4.16; *P*=0.02), with breast cancer risk, was observed. The variant *rs2234693* showed a positive association with breast cancer risk.OR: Odds ratio; CI: Confidence interval; ref: Reference

Table 3. Stratified analysis of the genotypes of the estrogen receptor signaling pathway with clinical characteristics like menopausal status

Genotype	Controls (%)	Patients (%)	OR	95% CI	*P* value
***ESR1*Xba I (*rs9340799*) genotype among breast cancer patients and control stratified by age at diagnosis**					
Premenopausal	**N=(86No)**	**N=(86No)**			
AA	43(50%)	36(41.86%)	Ref		
AG	27 (31.3%)	31(36%)	1.37	0.69-2.70	0.36
GG	16 (18.6%)	19(22%)	1.41	0.638-3.15	0.39
Menopausal	**N=(114No)**	**N=(114No)**			
AA	38 (33.33%)	48(42.10%)	Ref		
AG	60 (52.6%)	51(44.7%)	0.672	0.382-1.185	0.17
GG	16 (14%)	15(13.15%)	0.742	0.32-1.69	0.47
ESR Pvu II (*rs2234693*) genotype among breast cancer patients and control stratified by age at diagnosis					
Premenopausal	**N=(86No)**	**N=(86No)**			
TT	46(53.48%)	26(30.2%0	Ref		
TC	31(36%)	42(48.8%)	2.39	1.22- 4.67	0.01*
CC	9 (10.46%)	18(20.9%)	3.53	1.39-9.00	0.008**
Menopausal	**N=(114No)**	**N=(114No)**			
TT	42(36.8%)	44(38.5%)	Ref		
TC	62(54.38%)	55(48.2%)	0.84	0.48-1.478	0.55
CC	10(8.7%)	15(13.1%)	1.43	0.57-3.53	0.43
***ESR1* (*rs2881766*) genotype among breast cancer patients and control stratified by age at diagnosis**					
Premenopausal	**N=(86No)**	**N=(86No)**			
TT	56 (65.1%)	46(53.4%)	Ref		
GT	25 (29.06%)	31(36.4%)	1.50	0.78-2.9	0.21
GG	5(5.8%)	9(10.4%)	2.19	0.68-6.99	0.18
Menopausal	**N=(114No)**	**N=(114No)**			
TT	52 (45.6%)	56(49.1%)	Ref		
GT	47 (41.2%)	44(38.5%)	0.86	0.497-1.519	0.62
GG	15(13.1%)	14(12.2%)	0.866	0.381-1.96	0.73

Association between premenopausal, menopausal and SNP was evaluated. The relative risk was accessed by calculating the OR and 95% CI, $P<0.05$ level was considered to be significant. The *rs2234693* of *ESR1* the TC; OR; 2.3, $P=0.01^{*}$ and CC; OR;3.5, $P=0.008^{**}$ was found to be associated with premenopausal breast cancer. OR: Odds ratio; CI: Confidence interval; N: Number of samples

Table 4. Estrogen receptor signaling pathway genotype among breast cancer patients stratified by grade of the tumor

Genotype	Low grade Tumors% (76No)	High Grade Tumors % (124No)	OR	95% CI	*P* value
Association between *ESR1*Xba I and tumor grade					
AA	41 (53.9%)	43 (34.6%)			
AG	25 (32.8%)	57 (45.9%)	2.174	1.15-4.10	0.01*
GG	10(13.1%)	24(18.5%)	2.288	0.975-5.36	0.05
Association between *ESR1*PvuII (*rs2234693*) and tumor grade					
TT	33 (43.4%)	37 (29.8%)	Ref		
TC	33 (43.4%)	64 (51.6%)	1.729	0.921-3.24	0.08
CC	10(13.1%)	23(18.5%)	2.05	0.85-4.93	0.10
Association between *ESR1rs2881766* and tumor grade					
TT	38 (50%)	64 (51.6%)			
GT	27 (35.52%)	48 (38.7%)	1.055	0.56-1.960	0.86
GG	11 (14.47%)	12 (9.67%)	0.64	0.26-1.611	0.35

Association between tumor grade and SNP was evaluated. The relative risk was accessed by calculating the OR and 95% (CI), P<0.05 level was considered to be significant. The *rs9340799* polymorphism of *ESR1* gene AG; OR=2.1 *P* value 0.01* was found to be associated with high grade tumors.

Stratified analysis of the genotypes of *rs9340799*, *rs2234693*, and *rs2881766* of *ESR1* gene with clinical characteristic like receptor status did not show an association with estrogen, Progesterone and Her 2-receptor status.

OR: Odds ratio; CI: Confidence interval; SNP: Single nucleotide polymorphism

Table 5. Association between *ESR1* gene AND ER, PR AND HER2 *Receptor* Status

Genotype	**Patient %**	**OR**	**95% CI**	***P* value**	
Association between *ESR1* (*rs9340799*) and *ER, PR* and *Her2 Receptor* Status					
ESR1 rs9340799	**ER Positive (92)**	**ER Negative (108)**			
AA	46 (50%)	38 (35.18%)	Ref		
AG	28 (30.43%)	54 (50%)	0.42	0.22-0.80	0.008**
GG	18 (19.56%)	16 (14.81%)	0.92	0.41-2.06	0.85
	PR Positive (84)	**PR negative (116)**			
AA	41(48.8%)	43(37%)	Ref		
AG	30 (35.7%)	52 (44.8%)	0.749	0.52-1.07	0.11
GG	13(15.47%)	21 (18.10%)	0.78	0.48-1.26	031
	Her 2 Positive (68)	**Her 2 Negative (132)**			
AA	32 (47.05%)	52 (39.39%)	Ref		
AG	28 (41.17%)	54 (40.90%)	0.896	0.59 - 1.34	0.59
GG	8 (11.76%)	26 (19.69%)	0.61	0.31-1.20	0.15
Association between *ESR1* gene (*rs2234693*) and *ER, PR* and *Her2 Receptor* Status					
***ESR1* gene (*rs2234693*)**	**ER Positive (92)**	**ER Negative (108)**			
TT	26 (28.26%)	44 (40.74%)	Ref		
TC	50 (54.34%)	47 (43.51%)	1.8	0.96-3.3	0.06
CC	16 (17.39%)	17 (15.74%)	1.59	0.68-3.67	0.27

	PR Positive (84)	PR Negative (116)			
TT	32 (38.09%)	38 (32.57%)	Ref		
TC	37 (44.04%)	60 (51.7%)	0.73	0.39-1.36	0.32
CC	15 (17.85%)	18 (15.5%)	0.98	0.43 – 2.27	0.98
	Her 2 Positive (68)	Her 2 Negative (132)			
TT	27 (39.70%)	43 (32.57%)	Ref		
TC	31 (45.58%)	66 (50%)	0.748	0.39-1.42	0.37
CC	10 (14.70%)	23 (17.42%)	0.692	0.28-1.67	0.41
Association between *ESR1* gene (*rs2881766*) and *ER, PR* and *Her2 Receptor* Status					
ESR1 rs2881766	**ER Positive (92)**	**ER Negative (108)**			
TT	45 (48.91%)	57 (52.77%)	Ref		
TG	36 (39.13%)	39 (36.11%)	1.16	0.64-2.12	0.60
GG	11 (11.95%)	12 (11.11%)	1.16	0.46-2.87	0.74
	PR Positive (84)	PR Negative (116)			
TT	40 (47.6%)	62 (53.4%)	Ref		
TG	35 (41.6%)	40 (34.4%)	1.35	0.74-2.47	0.32
GG	9(10.7%)	14 (12.06%)	0.99	0.39-2.51	0.99
	Her 2 Positive (68)	Her 2 Negative (132)			
TT	31 (45.58%)	71 (53.78%)	Ref		
TG	28 (41.17%)	47 (35.60%)	1.36	0.72-2.56	0.33

GG	9 (13.23%)	14 (10.60%)	1.472	0.57-3.76	0.41

ER: Estrogen receptor; PR: Progesterone receptor; OR: Odds ratio; CI: Confidence interval

Table 6. Pair-wise Linkage Disequilibrium Values D' and r2 values for *ESR 1*variants

SNPs	*rs2881766*	*rs2234693*	*rs9340799*
rs2881766		0.118	0.075
rs2234693	0.003		0.208
rs9340799	0.004	0.043	

D' values are located above the diagonal; r^2 values are below the diagonal; Note: Measures of pairwise linkage disequilibrium for the *ESR1*gene polymorphism was presented in figure 2 and table6. The low r^2 values showed that the *ESR1*variants were not in LD and failed to LD blocks (Figure 2). Haplotype analysis was not informative.

Table 7. Sequence specific primers for Taqman assay

Gene*ESR1*	**Primer**
rs2881766	GATGCATTCCTATAAACTGCAGACT [G/T]AAATTAAGACCTTGAGCAAACTGGC
rs2234693	TCATCTGAGTTCCAAATGTCCCAGC [C / T] GTTTTATGCTTTGTCTCTGTTTCCC
rs9340799	TTCCCAGAGACCCTGAGTGTGGTCT [A/G]GAGTTGGGATGAGCATTGGTCTCTA

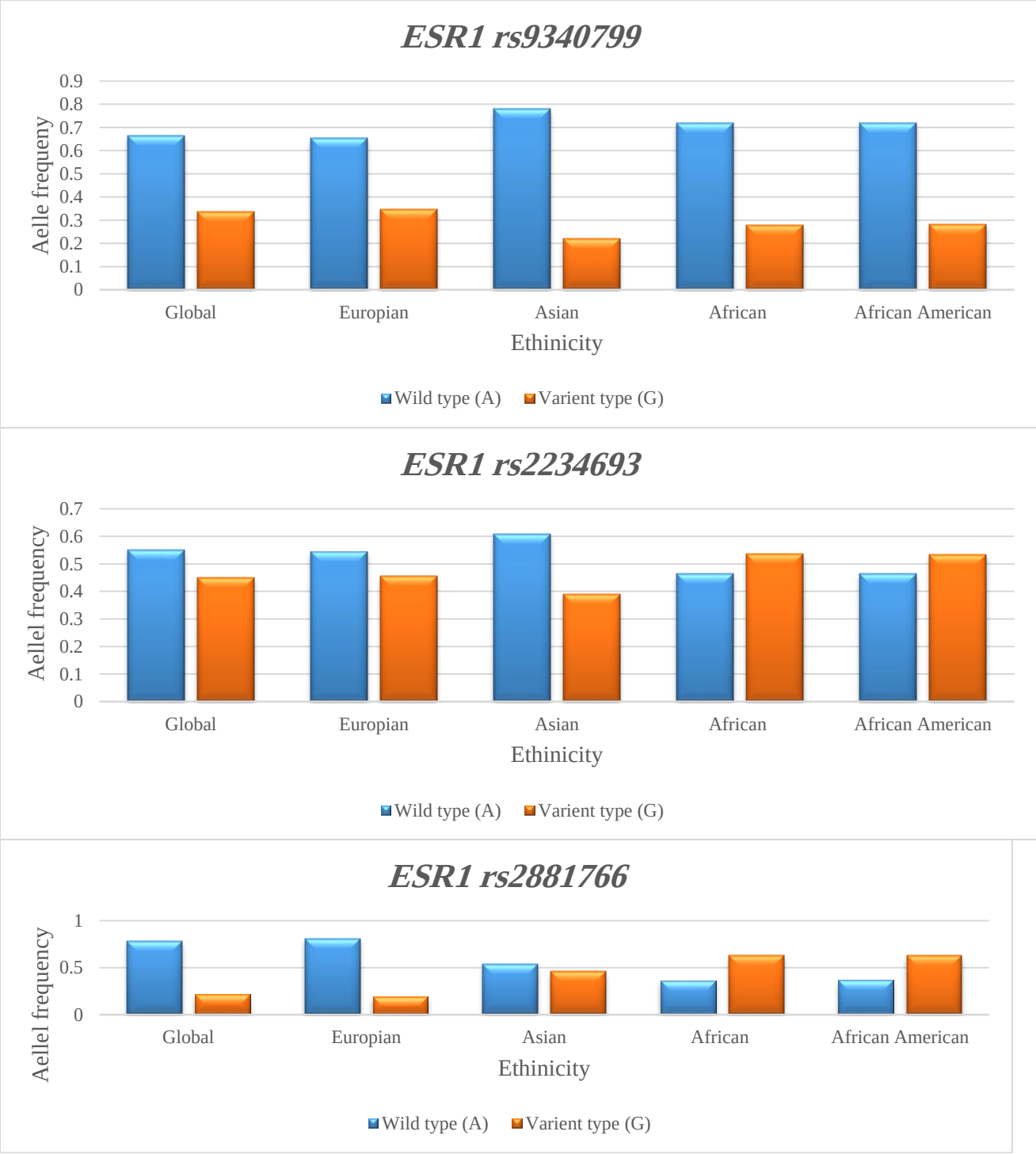

Figure 1. HapMap data for the current study's allele frequency compared to other ethnic groups.
ESR1: Estrogen receptor 1

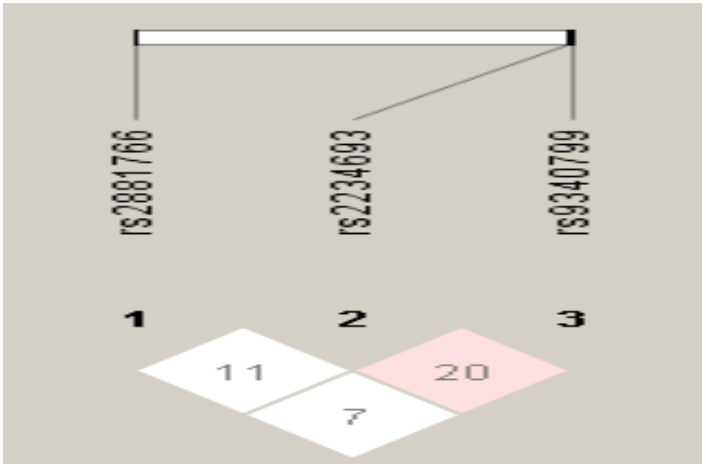

Figure 2. An LD map showing the pair-wise LD between the variants (SNPs) in the gene *ESR1* in the cases and normal controls. The colour coding represents the r^2 values and the values inside the squares indicating the D' values.