International Journal for Development of Science and Technology (IJDST)
Vol. 1, Issue 16, March 2021 PP.01-15
www.ijdst.com
ISSN: 2456-8503

A COMBINED ANALYSIS ON *IL7RA* RS6897932 AND *KRAS* RS61764370 GENE POLYMORPHISM AND ITS EFFECT ON DEVELOPMENT OF BREAST CANCER AMONG WOMEN IN KERALA POPULATION.

Mohthash MT, Neelam Tripathi, Sunil Kumar Shah, Anand Thirupathi

Ph.D. Scholar, Sri Satya Sai University of Technology & Medical Sciences, Madhya Pradesh, India.
Professor, University Teaching Department Sri Satya Sai University of Technology and Medical Sciences, Madhya Pradesh, India.
Associate Professor, College of Pharmacy, Sri Satya Sai University of Technology & Medical Sciences, Madhya Pradesh, India.
Sri Venkateshwara Research Centre, Thanjavur, Tamilnadu, India.

Abstract:

Interleukin-7 (IL-7) has always been found to play an important role in developing and maintaining lymphoid cells. IL-7 promotes the growth of tumor cells in breast cancer (BC) patients. Several studies have recently identified an association of single nucleotide polymorphisms (SNPs) in the lethal-7 (let-7) miRNA binding site within the 3' untranslated region (3'UTR) of the *KRAS* gene with the risk of developing various types of cancer. The current research is a combined analysis of our previous work, which sought to establish whether the *IL7RA* rs6897932 and *KRAS* rs61764370 polymorphisms were correlated with BC susceptibility in a diverse population of Kerala, South India. The results for genetic analysis performed to detect *IL7RA* rs6897932 and *KRAS* rs61764370 employing the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method in our previous publications has been taken through a combined analysis to get overall picture of their combined effect on BC. Odds ratio (OR) with 95% class interval (CI) were used to evaluate the relationship of *IL7RA* (rs6897932) polymorphism with BC susceptibility. Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS; version 21.0) software and Medcalc software (version 16.4.3). The *IL7RA* rs6897932 and *KRAS* rs61764370 polymorphisms were found to differ between case and control groups, confirming our previous findings and indicating that the *IL7RA* and *KRAS* genes may play a role in the pathogenesis of BC in the South Indian population. To validate the current study's findings, more advanced and prospective research with larger sample sizes is needed.

Keywords: Breast cancer, *IL7RA* polymorphism, rs6897932, *KRAS* polymorphism, rs61764370, Combined analysis

Introduction

Breast cancer (BC) is the most common cancer in women around the world, accounting for about a quarter (25%) of all cancer cases and 1.67 million new cases diagnosed each year. [1] According to recent estimates, the number of BC cases in developing countries has risen (883000 cases) while the number of BC cases in developed countries has decreased (794 000). [1-2] India has a lower age-adjusted BC incidence rate (25.8 per 100 000) than the United States. According to global and Indian reports, the incidence rate of cancer and cancer-related morbidity and mortality rates have risen dramatically in the Indian subcontinent over the last few years. [3–7]. Previous studies have shown that cervical cancer is the most common cancer in Indian women. The most recent studies (the last five years) show that breast cancer is more common than cervical cancer, even though cervical cancer is still the most common cancer in rural India.[8] Currently, multiple genetic studies have been undertaken in different populations to learn more about the genetic factors associated with breast cancer that could assist in early detection in addition to traditional screening techniques such as breast ultrasound, magnetic resonance imaging (MRI), diagnostic mammogram, and biopsy in the quest for new methods for screening different cancers. Despite the fact that several epidemiologic studies have been performed in cancers such as prostate, oral gastric, and BC, [9-12] studies that focus on the genetic factors associated with BC in people of South Indian ethnicity are rare. *BRCA1* and *BRCA2* are the genes that have been tested the most for BC with hereditary breast and/or ovarian cancer syndrome susceptibility. [1-3] However, because of the polygyny, polygenic nature of BC, which would require screening of non-*BRCA* genes that are often overlooked in standard genetic screening procedures.[13] In BC development, the interaction of growth factors, extracellular matrix, cytokines, fibroblasts with tumor cells, and immune and tumor cells was discovered to be extremely significant. [13]. Interleukin-7 (IL-7) has recently been discovered to play a key role in BC pathogenesis, promoting tumor cell growth and survival. The expression of its signaling pathway molecules has been linked to the worst prognosis in human BC samples. [14] Furthermore, BC cancer patients' peripheral blood mononuclear cells have lower expression of the IL-7 receptor alpha chain (IL-7RA; CD127) and impaired IL-7 response and cytokine production compared to healthy controls [15], meaning that IL-7 deficiencies are related to BC. IL-7 is a type 1 cytokine

that belongs to the hematopoietin family. These are primarily formed by non-immune stromal cells, which play an important role in immune system advancement and homeostasis by encouraging lymphoid cell growth and survival. The IL-7 receptor (IL-7R) [composed of the cytokine receptor gamma chain (c; CD132) and the IL-7RA] activates Janu Kinases 1 and 3, encouraging STAT5 activity and gene expression regulation, as well as phosphatidylinositol 3-kinase (PI3K), resulting in pro-survival and angiogenesis [16]. The *IL7RA* gene is located on chromosome 5 at 5p13.2 [17]. The SNP (rs6897932, Thr244Ile) occurs at exon 6, which results in the conversion of cytosine (C) into thymine (T) at codon 244 (ACC > ATC). This further leads to a change of threonine into isoleucine (Thr> Ile) at the border between the transmembrane and extracellular regions. In individuals with multiple sclerosis, T (Ile) allele for Thr244Ile polymorphism is a marker allele for a protective haplotype [18]. Ithas also been associated with various other immune-related disorders. [19] Moreover, it is also shown in detailed studies that the T allele is associated with decreased expression of soluble IL-7RA (sIL-7RA) isoform [13]. Despite this evidence demonstrating the influence of IL-7 in various cancers, including BC [13], and the documented functional implications of *IL7RA* Thr244Ile polymorphism in autoimmune diseases and immune function [13], no other study is available in the literature which had investigated this polymorphism on BC among South Indian society. Therefore, our group has carried out a study earlier to investigate the association of *IL7RA* Thr244Ile (rs6897932) polymorphism with BC's susceptibility among ethnicity of Kerala [20]. The V-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog is a well-known effector molecule (KRAS). It is involved in various signal transduction pathways, including the Ral (Ras-Like) guanine nucleotide exchange factor, mitogen-activated protein kinase, and phosphatidylinositol 3-kinase. KRAS is a known let-7 target with numerous complementary sites in the mRNA's untranslated (3'UTR) region. A germ-line and functional single-nucleotide polymorphism (SNP) in the KRAS 3'UTR region (rs61764370 T>G) located on the let-7 complementary site 6 has been discovered in recent studies (LCS-6). Further study has discovered that LCS-6 decreases the affinity of let-7 for KRAS. This resulted in enhanced tumour growth and decreased *KRAS* inhibition. [21-22] Several recent studies have looked into the effect of the *KRAS* rs61764370 T>G polymorphism on the risk of developing

different cancers. In inherited BC and ovarian cancer syndrome families, the LCS6 variant allele has been shown to cause non-small cell lung cancer (NSCLC) in moderate smokers, [23] triple-negative BC in premenopausal women [24], and ovarian cancer in BRCA negative female individuals. [25]. In light of this, our group had earlier looked into the possible connection between BC danger and the *KRAS* variant (rs61764370 T>G) in the women of Kerala, South India [26]. Here we did a combined analysis of the findings from our previous publication [20,26] to validate and perform a combined statistical analysis.

Subjects and Methods

The previous published work of our group [20-,26] hada case-control study design that was carried in health care hospitals in Kerala, India. The study involved 112 BC patients and 112 age-adjusted healthy controls. The inclusion criteria for the study were (1) women with age ≥35 years;(2) All study subjects should have undergone breast cancer screening: mammography (i.e., film, digital, tom synthesis) or with other modality; other screening modality (i.e., MRI, ultrasound) and have been confirmed of BC. The exclusion criteria included: women with pre-existing other types of cancer or Li-Fraumeni syndrome, Cowden syndrome, hereditary diffuse gastric cancer, or other familial cancer syndromes. The clinicopathological characteristics, including age, tumor size, nodal metastasis, grade, and BC patients' stages, were collected and documented. Written informed consent was acquired from all the study subjects. The Ethics Committee approved the present study's procedures, Mar Baselios College, Kerala, India(Ref no: IEC/17/2017/MBDC).

DNA extraction, IL7RA rs6897932 and KRAS rs61764370 genotyping

In our previously published studies [20,26]; A 5 ml venous blood was collected from the participants during their hospital visit. Genomic DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Germany). The high-quality DNA isolated from whole blood samples were checked for its quality and quantity using NanoDropTM 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific, United States). The samples' purity was checked based on 260/280nm and 260/230nm ratios. DNA samples' quality was also cross-checked on Agarose gel electrophoresis by preparing 1% gel to avoid any major contaminations. Extracted DNA samples were stored at -20^{o}C for future use. The genotyping has been performed for *IL7RA* rs6897932, and *KRAS* rs61764370 gene polymorphism as previously reported [20,26]. In both

groups, deviations from Hardy–Weinberg equilibrium (HWE) were evaluated using the Chi-square (2 test). Using MedCalc Statistical Tools, the relative risk associated with genotype and alleles was measured as an odds ratio (OR) with a 95 percent confidence interval (CI) (version 16.4.3). A p-value of less than 0.05 was considered statistically significant.

Results

The demographic data (age) and clinical details of the case and control groups are shown in Table 1 [20,26]. The present study did combined analysis of our previous work [20,26]which included 112 BC patients with a mean age of 47.1 ±11.3 years and 112healthy women with a mean age of 47.9 ±11.1 years. The PCR-RFLP results showed that *KRAS* (rs61764370) T>G polymorphism contained three genotypes: TT as a wild-type, TG as a heterozygous mutant and GG as a homozygous mutant. The genotype and allele frequencies for the *KRAS* (rs61764370) polymorphism in all studied groups is illustrated in Figure 1. The differences between genotypes and alleles frequencies of *KRAS* (rs61764370) were remarkable between the case and control group (Figure 2-3). The genotype distribution of all three genotypes was significantly different with p-value <0.05 between cases (TT = 50%, TG = 30.3% and GG=19.6%) vs controls (TT = 71.4%, TG =21.4% and GG=7.1%) respectively. The genotype frequency of both homozygous mutations (GG) and heterozygous mutations (TG) was higher in the case group when compared with the control group with statistical significance. The corresponding allele frequencies showed a difference in the levels as well; cases had T = 65.1% and G = 34.8%, whereas controls had T = 82.1% and G = 17.8% with statistical significance (p=.004 for both T and G allele). Individuals with the TG and GG genotype had a significantly elevated risk of developing BC compared with TT. In addition, we also found that the minor allele frequency for the G allele in the case group was higher than that of the control group, which indicated the risk for BC to be increased by 2.45 folds in the presence of G allele. The *IL7RA* (rs6897932) T>C polymorphism contained three genotypes: TT as a wild-type, TC as a heterozygous mutant and CC as a homozygous mutant. The genotype and allele frequencies for the *IL7RA* (rs6897932) polymorphism in all studied groups are illustrated in Figure 1. The differences between genotypes and alleles frequencies of *IL7RA* (rs6897932) were remarkable between the case and control group (Figure 2-3). The genotype distribution of two of the genotypes was significantly different with p-value <0.05

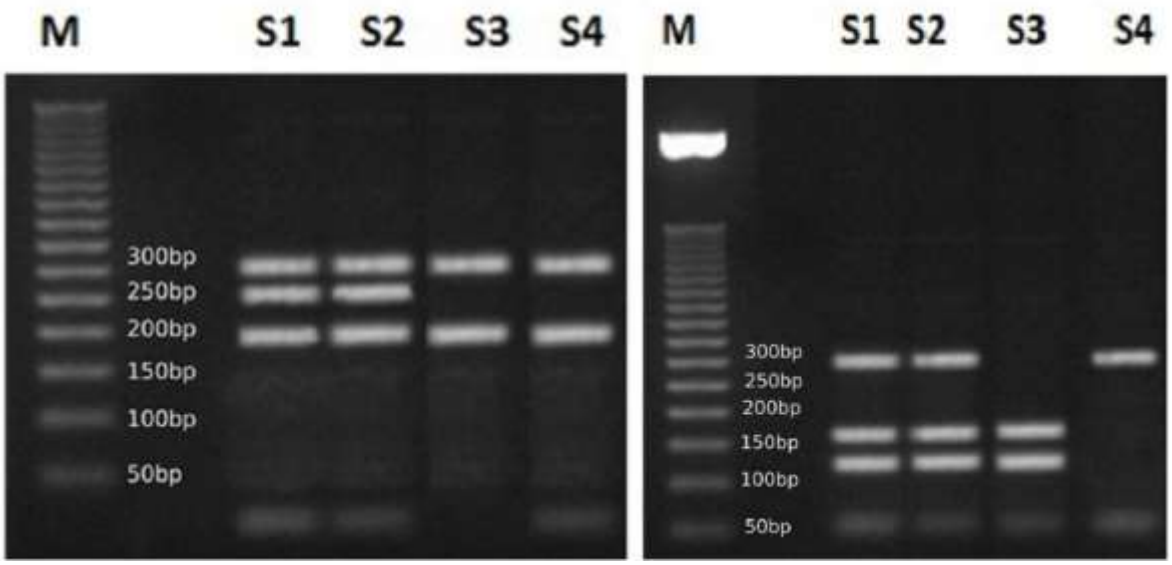
between cases (TT = 46.4% and CC=26.7%) vs controls (TT = 49.1%, and CC=10.71) respectively. However, the differences between genotype TC *IL7RA* (rs6897932) between the case and control remained insignificant (p>0.05). The genotype frequency of homozygous mutations (CC) was higher in the case group when compared with the control group with statistical significance. The corresponding allele frequencies were also showing a difference in the levels as well; cases had T = 59.8% and C = 40.1% whereas controls had T= 79.4% and C = 20.5% with statistical significance (p=0.001 for both T and C allele). Individuals with CC genotype had a significantly elevated risk of developing BC than TT, with an OR (95%CI) of 3.04 vs 0.38, respectively. In addition, we also found that the minor allele frequency for the C allele in the case group was higher than that of the control group, which indicated the risk for BC to be increased by 2.5 folds in the presence of C allele.

Characteristics	**Cases n (%)**
Age	
Mean±SD	47.1 ±11.3 (47.9 ±11.1 healthy controls)
Tumor Size (cm)	
≤2	23(20.5%)
>2	67 (59.8%)
Unknown	22(19.6%)
Nodal metastasis	
No	26 (23.2%)
Yes	72(64.2%)
Unknown	14 (12.5%)
Grade	
I	18 (16 %)
II	54 (48.2%)
III	18(16%)
IV	1 (0.8)
Unknown	21(18.7)
Stage	
I	13(11.6%)
II	36 (32.1%)

III	28(25%)
IV	28(25%)
Unknown	7 (6.25%)
Histology	
Ductal carcinoma	38(33.9%)
Other	74 (66%)

Table 1: The clinicopathological characteristics of BC patients (Extracted from previously published [20,26]

Figure 1: Polymerase chain reaction–restriction fragment length polymorphism genotyping of *KRAS* rs61764370 T>G and *IL7RA* rs6897932 polymorphism extracted from previously published works [20,26]



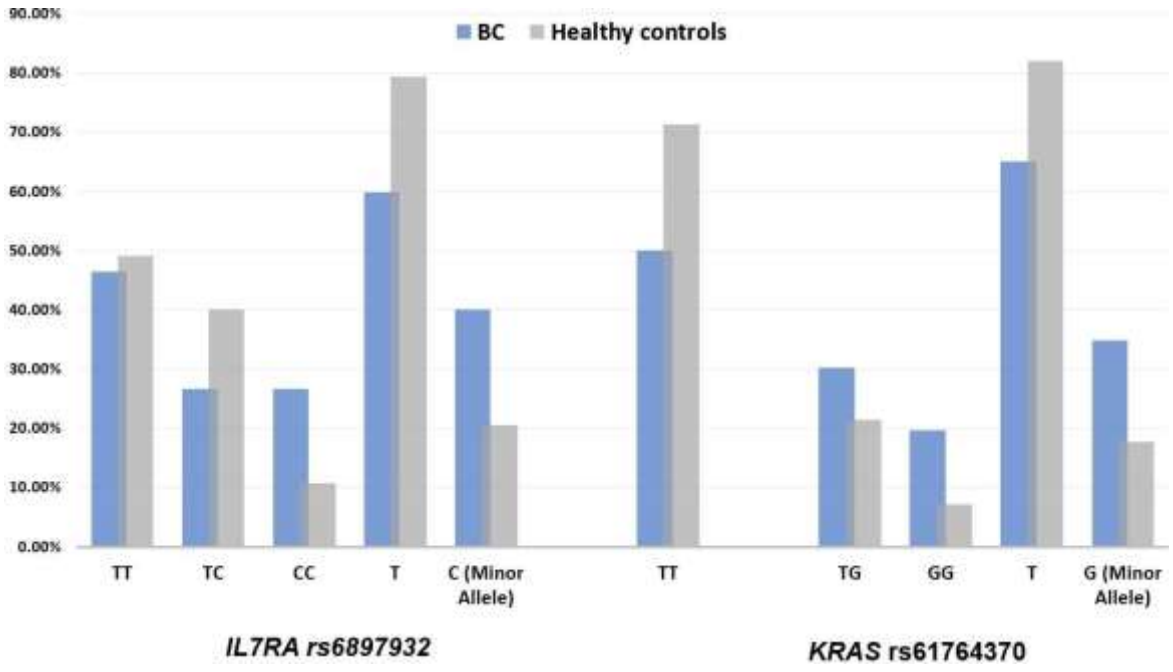


Figure 2: Distribution *IL7RA* rs6897932 and *KRAS* rs61764370 gene polymorphism in BC patients (cases) and BC controls

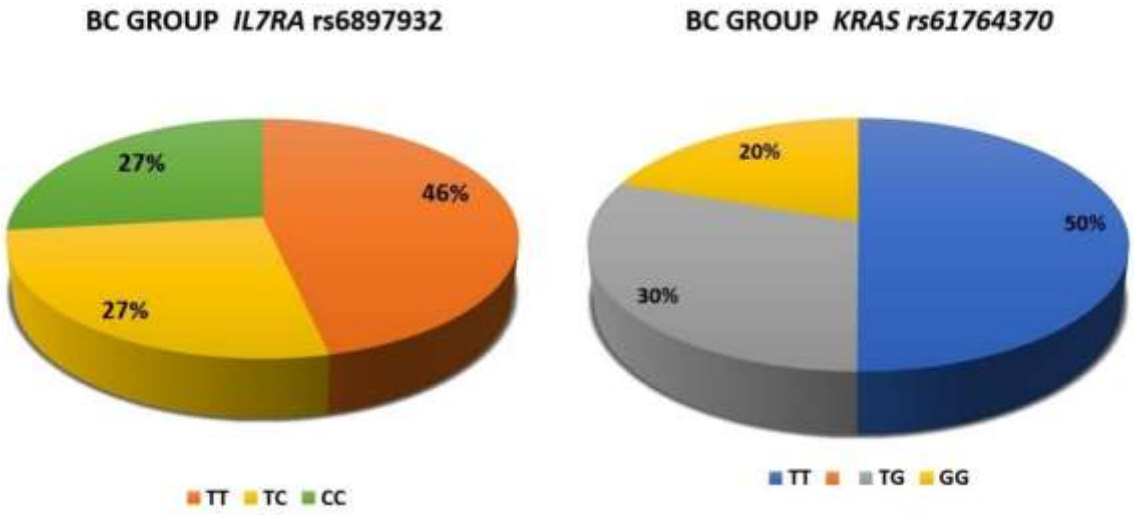


Figure 3: Differential distribution *IL7RA* rs6897932 and *KRAS* rs61764370 polymorphism genotype in BC patients

Discussion:

In Kerala, South India, researchers discovered a strong connection between *KRAS* (rs61764370) polymorphisms and the risk of developing BC [26]. The

rs61764370 TG vs TT and G vs T alleles were found to be risk factors for BC in our study. Johnson et al., 2007 and Chung et al., 2014, respectively, were the first to record a germline and functional SNP in the *KRAS* 3'UTR region (rs61764370 T>G) located in the let-7 complementary site 6 (LCS-6) [21-22]. LCS-6 disrupts the let-7 binding affinity for *KRAS*, resulting in decreased KRAS inhibition and increased tumor development, according to further research. [24-25] Many other studies have recently been effective in determining the effect of the *KRAS* (rs61764370) polymorphism on the risk of various other cancers, including non-small cell lung cancer (NSCLC) in moderate [23], triple-negative breast cancer in premenopausal women [24], and ovarian cancer in BRCA negative females from hereditary breast cancer and ovarian cancer syndrome families. [25] A recent study looked into *KRAS* gene polymorphisms and the risk of developing breast cancer in the Iranian population and discovered a clear correlation between the KRAS gene (rs61764370) mutation and an increased risk breast cancer. [27] This study's findings are consistent with those of a previous study that linked the *KRAS* rs61764370 polymorphism to the risk of double primary breast and ovarian cancer. [28] The findings of this study are consistent with those of a previous study that linked the *KRAS* rs61764370 polymorphism to the risk of double primary breast and ovarian cancer [25] Furthermore, a study by Paranjape et al. [24] found that the *KRAS* variant is a risk factor for triple-negative BC in premenopausal women, emphasizing its importance as a genetic marker for BC. Uvirova et al. [29] found no correlation between the *KRAS* rs61764370 variant and the risk of BC, but they did indicate that the *KRAS* rs61764370 TG genotype could affect HER2 gene expression profile. Cerne et al. [30] found no evidence of a correlation between the *KRAS* rs61764370 variant and the risk of sporadic and familial BC. The results of these studies [29-30] support the findings of a meta-analysis published in 2016 [31] that concluded the *KRAS* genotype GT/GG of rs61764370 has no connection with the risk of breast, ovarian, NSCLC, colorectal, or head-neck cancer. Let-7 is thought to be essential in the progression of BC. Recent evidence also indicates that the let-7 miRNA family plays a key role in the development of BC by altering the expression of *KRAS*, an unusual target of activating mutations in breast tumors. [32] In the *KRAS* 3'-UTR mRNA, LCS6 induced an increase in *KRAS* expression in vitro and decreased inlet-7 levels in vivo. [23] Another study [33] found that targeted knock-in of the polymorphism rs61764370

did not reduce let-7 expression but did not affect KRAS levels. According to recent research, the KRAS LCS6 variant is inconsistent, uncommon in East Asians and Native Americans, and highly rare in Africans, with a minor allele frequency of 7% in European populations 19. In the present study, the *KRAS* rs61764370 variant allele was 34.8% in BC patients and 17.8% in healthy women.

The *IL7RA* (rs6897932) Thr244Ile polymorphism was correlated with BC susceptibility in South Indian ethnicity (Kerala population) in the current research. Previous research has identified a connection between the *IL7RA* (rs6897932) gene and autoimmune disorders [34]. The IL-7RA gene (IL7R) is located on the 5th chromosome at 5p13.2, and it has multiple single nucleotide polymorphisms (SNPs) [17]. IL-7 has been studied as a pleiotropic cytokine that helps immune function by promoting lymphoid cell growth and homeostasis. IL-7 activates JAK1 and 3, which causes phosphorylation of the signal transducers STAT5 and PI3K pathway, triggering pro-survival responses in lymphoid cells, upon ligation with its receptor complex, which includes the typical cytokine-receptor chain (CD132) and the IL-7RA (CD127). The ability of IL-7 to promote cell growth and survival has been demonstrated in cell culture experiments with BC cells, and the process is based on both STAT5 and PI3K activation. [36] IL-7 has been shown to function on tumor cells in addition to its role in the immune system. [36] Exon 6 contains the *IL7RA* SNP (rs6897932), which causes a threonine to isoleucine conversion (Thr> Ile) on the extracellular-transmembrane boundary. This polymorphism has also been linked to multiple sclerosis [18-20] and several other immune-related disorders [19]. Despite evidence that IL-7 plays a role in a variety of cancers, including breast cancer and functional effects of the *IL7RA* rs6897932 polymorphism on immune function and autoimmune diseases [36-37], there are only a few studies that look into this polymorphism's impact on breast cancer. Two recent Brazilian studies looked into the relationship between this *IL7RA* rs6897932 and the sensitivity and clinicopathological parameters of breast cancer subgroups in the Brazilian population [35,38]. Compared to other studies conducted around the world [39], our results on the T allele frequency in the stable control group (79.4%) were higher than the majority of Asian and Admixed American ethnicity studies. Our earlier findings [20], on the other hand, are in line with a recent study in the Brazilian population that found a strong link between the *IL7RA* rs6897932 (Thr244Ile)

polymorphism and BC growth.

The current research has a few drawbacks, including the following: (1) The current study only looked at one SNP in the *IL7RA* and *KRAS* gene; (2) the study lacked patient information on established risk factors for BC (e.g., parity, use of oral contraceptives or hormone therapy, breastfeeding, smoking, and alcohol intake); (3) The research did not look into the relationship between clinical characteristics of BC patients such as age, tumor size, nodal metastasis, grade, stage, and histology; (4) Also the study was carried out only at two health carecenters. As a result, the study subjects would not fully reflect the Kerala, South Indian population. For better results, the authors suggest larger studies.

Conclusion:

Overall, the findings of this follow-up study revealed that *IL7RA* rs6897932 and *KRAS* rs61764370 have a combined effect in the development of breast carcinoma in the Kerala population in South India. These gene polymorphisms may be used as a genetic marker to assess high-risk women's breast cancer risk. There is a need for further studies with a greater sample size and a larger number of BC patients.

List of Abbreviation

BC Brest cancer

SNP Single nucleotide polymorphism

PCR-RFLP Polymerase chain reaction-restriction fragment length polymorphism

HWE Hardy–Weinberg equilibrium

IL7 Interleukin-7

IL7RA Interleukin-7 receptor alpha

CI Class interval

SPSS Statistical Package for the Social Sciences

Consent for Publication:

Informed consent was obtained from all the participants.

Funding: none

Conflict of Interest: The authors declare that there is no conflict of interest regarding the publication of this article.

Ethical Clearance: The present study's procedures were approved by the Ethics Committee, Mar Baselios College, Kerala, India

References

[1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D, Bray F. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. International journal of cancer. 2015;136(5):E359-86.

[2] Gupta A, Shridhar K, Dhillon PK. A review of breast cancer awareness among women in India: Cancer literate or awareness deficit?. European Journal of Cancer. 2015 Sep 1;51(14):2058-66.

[3] Porter PL. Global trends in breast cancer incidence and mortality. Saludpublica de Mexico. 2009;51:s141-6.

[4] Babu GR, Lakshmi SB, Thiyagarajan JA. Epidemiological correlates of breast cancer in South India. Asian Pacific Journal of Cancer Prevention. 2013;14(9):5077-83.

[5] Ali I, Wani WA, Saleem K. Cancer Scenario in India with Future Perspectives. Cancer therapy. 2011;8.

[6] Reddy KS, Shah B, Varghese C, Ramadoss A. Responding to the threat of chronic diseases in India. The Lancet. 2005;366(9498):1744-9.

[7] Balasubramaniam SM, Rotti SB, Vivekanandam S. Risk factors of female breast carcinoma: a case control study at Puducherry. Indian journal of cancer. 2013;50(1):65.

[8] Kaarthigeyan K. Cervical cancer in India and HPV vaccination. Indian journal of medical and paediatric oncology: official journal of Indian Society of Medical &Paediatric Oncology. 2012 ;33(1):7.

[9] Jain S, Saxena S, Kumar A. Epidemiology of prostate cancer in India. Meta Gene. 2014;2:596-605.

[10] Dikshit RP, Mathur G, Mhatre S, Yeole BB. Epidemiological review of gastric cancer in India. Indian journal of medical and paediatric oncology: official journal of Indian Society of Medical&Paediatric Oncology. 2011;32(1):3.

[11] Singh MP, Kumar V, Agarwal A, Kumar R, Bhatt ML, Misra S. Clinico-epidemiological study of oral squamous cell carcinoma: A tertiary care centre study in North India. Journal of oral biology and craniofacial research. 2016;6(1):32-5.

[12] Paymaster JC, Gangadharan JC. Epidemiology of breastcancer in India. J Natl Cancer Instit1972; 48: 1021–24.

[13] Singh J, Thota N, Singh S, Padhi S, Mohan P, Deshwal S, Sur S, Ghosh M, Agarwal A, Sarin R, Ahmed R. Screening of over 1000 Indian patients with breast and/or ovarian cancer with a multi-gene panel: prevalence of BRCA1/2 and non-

BRCA mutations. Breast cancer research and treatment. 2018 Jul 1:1-8.

[14] Meltzer PS. Cancer genomics: small RNAs with big impacts. Nature. 2005;435(7043):745.

[15] Valeri N, Gasparini P, Braconi C, Paone A, Lovat F, Fabbri M, Sumani KM, Alder H, Amadori D, Patel T, Nuovo GJ. MicroRNA-21 induces resistance to 5-fluorouracil by down-regulating human DNA MutS homolog 2 (hMSH2). Proceedings of the National Academy of Sciences. 2010;107(49):21098-103.

[16] Su JL, Chen PS, Johansson G, Kuo ML. Function and regulation of let-7 family microRNAs. Microrna. 2012; 1(1):34-9.

[17] Teutsch SM, Booth DR, Bennetts BH, Heard RN, Stewart GJ. Identification of 11 novel and common single nucleotide polymorphisms in the interleukin-7 receptor-α gene and their associations with multiple sclerosis. European journal of human genetics. 2003;11(7):509.

[18] Hoe E, McKay FC, Schibeci SD, Gandhi K, Heard RN, Stewart GJ, Booth DR. Functionally significant differences in expression of disease-associated IL-7 receptor α haplotypes in CD4 T cells and dendritic cells. The Journal of Immunology. 2010;184(5):2512-7.

[19] Mazzucchelli RI, Riva A, Durum SK. The human IL-7 receptor gene: deletions, polymorphisms and mutations. InSeminars in immunology 2012 (Vol. 24, No. 3, pp. 225-230). Academic Press.

[20] Mohthash MT, Shah SK, ThirupathiA.The role of Interleukin 7 receptor alpha (IL7RA) rs6897932 gene polymorphism in the development of breast cancer among women in Kerala population, South India. International Journal of Scientific & Technology Research.2020;9(3): 1067-1071.

[21] Johnson CD, Esquela-Kerscher A, Stefani G, Byrom M, Kelnar K, Ovcharenko D, Wilson M, Wang X, Shelton J, Shingara J, Chin L. The let-7 microRNA represses cell proliferation pathways in human cells. Cancer research. 2007;67(16):7713-22.

[22] Chung CH, Lee JW, Slebos RJ, Howard JD, Perez J, Kang H, Fertig EJ, Considine M, Gilbert J, Murphy BA, Nallur S. A 3′-UTR KRAS-variant is associated with cisplatin resistance in patients with recurrent and/or metastatic head and neck squamous cell carcinoma. Annals of oncology. 2014;25(11):2230-6.

[23] Chin LJ, Ratner E, Leng S, Zhai R, Nallur S, Babar I, Muller RU, Straka E, Su L, Burki EA, Crowell RE. A SNP in a let-7 microRNA complementary site in the KRAS 3′ untranslated region increases non–small cell lung cancer risk. Cancer research. 2008;68(20):8535-40.

[24] Paranjape T, Heneghan H, Lindner R, Keane FK, Hoffman A, Hollestelle A, Dorairaj J, Geyda K, Pelletier C, Nallur S, Martens JW. A 3′-untranslated region KRAS variant and triple-negative breast cancer: a case-control and genetic analysis. The lancet oncology. 2011;12(4):377-86.

[25] Ratner E, Lu L, Boeke M, Barnett R, Nallur S, Chin LJ, Pelletier C, Blitzblau R, Tassi R, Paranjape T, Hui P. A KRAS-variant in ovarian cancer acts as a genetic marker of cancer risk. Cancer research. 2010;70(16):6509-15.

[26] Mohthash MT, Shah SK, Thirupathi A. KRAS gene polymorphism (rs61764370) and its impact on breast cancer risk among women in kerala population, South India. Journal of Natural Science, Biology and Medicine. 2020 Jul 1;11(2):140.

[27] Sanaei S, Hashemi M, Eskandari E, Hashemi SM, Bahari G. KRAS gene polymorphisms and their impact on breast cancer risk in an iranian population. Asian Pacific journal of cancer prevention: APJCP. 2017;18(5):1301.

[28] Pilarski R, Patel DA, Weitzel J, McVeigh T, Dorairaj JJ, Heneghan HM, Miller N, Weidhaas JB, Kerin MJ, McKenna M, Wu X. The KRAS-variant is associated with risk of developing double primary breast and ovarian cancer. PLoS One. 2012;7(5):e37891.

[29] Uvirova M, Simova J, Kubova B, Dvorackova N, Tomaskova H, Sedivcova M, Dite P. Comparison of the prevalence of KRAS-LCS6 polymorphism (rs61764370) within different tumour types (colorectal, breast, non-small cell lung cancer and brain tumours). A study of the Czech population. Biomed Pap Med FacUnivPalacky Olomouc Czech Repub. 2015;159(3):466-71.

[30] Cerne JZ, Stegel V, Gersak K, Novakovic S. KRAS rs61764370 is associated with HER2-overexpressed and poorly-differentiated breast cancer in hormone replacement therapy users: a case control study. BMC cancer. 2012 Dec;12(1):105.

[31] Zhang SY, Shi J. rs61764370 polymorphism of Kras and risk of cancer in Caucasian population: A meta-analysis.

Journal of cancer research and therapeutics. 2016 Apr 1;12(2):699.

[32] Kumar MS, Swanton C. KRAS 3′-UTR variants and stratification of breast-cancer risk. The Lancet Oncology. 2011;12(4):318-9.

[33] Crowley EH, Arena S, Lamba S, Di Nicolantonio F, Bardelli A. Targeted Knock- in of the Polymorphism rs61764370 Does Not Affect KRAS Expression but Reduces let- 7 Levels. Human mutation. 2014;35(2):208-14.

[34] Mazzucchelli RI, Riva A, Durum SK. The human IL-7 receptor gene: deletions, polymorphisms and mutations. InSeminars in immunology 2012 (Vol. 24, No. 3, pp. 225-230). Academic Press.

[35] Jiang Q, Li WQ, Aiello FB, Mazzucchelli R, Asefa B, Khaled AR, Durum SK. Cell biology of IL-7, a key lymphotropic. Cytokine & growth factor reviews. 2005;16(4-5):513-33.

[36] Cierny D, Hanysova S, Michalik J, Kantorova E, Kurca E, Skerenova M, Lehotsky J. Genetic variants in interleukin 7 receptor α chain (IL-7Ra) are associated with multiple sclerosis risk and disability progression in Central European Slovak population. Journal of neuroimmunology. 2015; 282:80-4.

[37] Gregory SG, Schmidt S, Seth P, Oksenberg JR, Hart J, Prokop A, Caillier SJ, Ban M, Goris A, Barcellos LF, Lincoln R. Interleukin 7 receptor α chain (IL7R) shows allelic and functional association with multiple sclerosis. Nature genetics. 2007 Sep;39(9):1083.

[38] Naslavsky MS, Yamamoto GL, de Almeida TF, Ezquina SA, Sunaga DY, Pho N, Bozoklian D, Sandberg TO, Brito LA, Lazar M, Bernardo DV. Exomic variants of an elderly cohort of Brazilians in the ABraOM database. Human mutation. 2017 Jul;38(7):751-63.