Assessing the Prevalence and Impact of *KRAS*-rs61764370 Polymorphism in Breast Cancer Development Among Bangladeshi Women: A Case-Control Study

By

Nissat Khan

22146093

Samia

22146030

Sanzana Azam Lamia

22146024

Sinthia Baroi

22146031

A thesis submitted to the School of Pharmacy in partial fulfilment of the requirements for the degree of Bachelor of Pharmacy (Hons.)

School of Pharmacy

BRAC University

April 2026

Declaration

It is hereby declared that:

1. The thesis we submitted is our own original work while we were getting our degree at BRAC University.

2. The thesis does not include any material that has been published or written by someone else, unless it is properly cited with full and accurate references.

3. The thesis does not include content that has been accepted or submitted for any other degree or diploma at a university or similar institution.

4. We have recognized all of the main sources of help.

Student's Full Name & Signature:

_____

Nissat Khan
22146093

_____

Samia
22146030

_____

Sanzana Azam Lamia
22146024

_____

Sinthia Baroi
22146031

Approval

The thesis titled ‘‘Assessing the Prevalence and Impact of *KRAS* Gene Variants rs61764370 Polymorphism in Breast Cancer Development Among Bangladeshi Women: A Case-Control Study’’ submitted by Nissat Khan (22146093), Samia (22146030), Sanzana Azam Lamia (22146024), and Sinthia Baroi (22146031) of Summer-2026 has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor of Pharmacy.

Supervised By:

_____

Dr. Md. Aminul Haque
Associate Professor
School of Pharmacy
BRAC University

Approved By:

Chairperson:

_____

Sharmind Neelotpol, PhD
Professor and Chairperson
School of Pharmacy
BRAC University

Dean:

_____

Dr. Arshad Mahmud Chowdhury, PhD
Pro Vice-Chancellor and Dean
School of Pharmacy
BRAC University

Ethics Statement

The most important thing in this study was ethics, which meant making sure that everyone's rights, safety, and well-being were protected. The Institutional Review Board (IRB) at BRAC University (IRB No. BRACUIRB120220005) gave its approval after deciding that the research followed the right ethical rules. The IRB conducted a thorough assessment of the research proposal to guarantee compliance with all pertinent ethical standards and regulatory requirements. All individuals gave their informed consent before taking part in the study. They were given a lot of information about the study's goals, methods, possible hazards, and expected benefits.

Participants were told that they might leave the study at any time without any consequences and that their participation was completely optional. This study adhered meticulously to the protocols and standards set out by the Institutional Review Board (IRB). The methodological approach and related processes were executed with accuracy. This included carefully processing biological samples, safely storing data to protect the privacy of participants, and strictly following rules that were meant to reduce any hazards to the participants.

The research took great effort to make sure that all of the data acquired was anonymous to preserve privacy and was only used for this study.

Abstract

Breast cancer has increased pressure on the healthcare system to come up with unconventional preventive measures due to its rise in the number of Bangladeshi women carrying the disease. Already established diagnostic evaluation methods have improved survival rates, but the number of disease carriers has increased, which calls for deeper analysis of the disease. Among these, the presence of SNPs in the regulatory regions that alter the way genes are expressed is one of the factors in the development of breast cancer. The goal of the study is to investigate the link between the *KRAS* rs 6174370 and breast cancer risk in Bangladeshi women, with the intention of finding a potential biomarker for early diagnosis and risk detection. The case study comprised 236 individuals, with 112 confirmed cases of breast cancer identified through histopathology, while the remaining participants served as controls without any disease. The rs61764370 in the *KRAS* gene was genotyped using the PCR-RFLP technique. The results showed that patients with GT genotypes had 55.36% of cases compared to 20.97% in the control group, which increases the risk by 4.5 times (aOR = 4.548, 95% CI: 2.590-7.988, $p < 0.001$). The GG genotype showed the lowest effect as compared to the others. Making the GT genotype a potential biomarker to identify the potential development of breast cancer and risk assessments.

Keywords: Breast cancer, *KRAS, SNP,* Predictive biomarker, Cancer risk.

Dedication

This work is dedicated to God, the real timber of my life, who has been a constant source of strength. To my parents, for their endless love and support, which shaped what I am today. To my respected supervisor, for the guidance and encouragement throughout the journey. To my dear one, who always believed in me, loves to see me shining, and to my friends Mahima and Safuan for your support and lastly to my sweet sister for every little sacrifice - together I stand strong because of you, you are those branches of my tree.

- Sinthia Baroi 22146031

I dedicate this work to people who have supported me and stood by me. First and foremost, to my parents, who have been my constant source of motivation, who have given me the strength to overcome every challenge. To my people from the university who made the most difficult times easy and gave me comfort when I needed it. Finally, to my respected faculty members, I extend my sincere gratitude for your guidance, knowledge, and continuous support.

- Samia 22146030

The dedication of this work is graciously shared: to my mother for her boundless love and unwavering support, to my father for his steadfast strength and selfless sacrifice, to my sisters for their constant warmth and affection making even the farthest miles feel close, to my friends whose loyal companionship lightened the journey and to my esteemed faculty members for their guidance and scholarly mentorship. I remain forever indebted to you all.

- Sanzana Azam Lamia 22146024

This work is dedicated to the Almighty for the grace that turned my dreams into reality. In loving memory of my grandmother and to Pico, who I will always carry in my heart.

- Nissat Khan 22146093

Acknowledgement

We are very thankful to Allah for giving us the chance to work with such great people from the School of Pharmacy who have always been idealistic, inspiring, and supportive along the way. We owe a lot to our supervisor, Dr. Md. Aminul Haque (Associate Professor, School of Pharmacy, Brac University), for letting us work as his thesis students. His support, guidance, dedication, enthusiasm, and knowledge in this area have also helped us move forward with our thesis work and finish our research. We also want to thank all the faculty members of the School of Pharmacy at BRAC University for all the hard work they put in to help us graduate. Lastly, we want to thank Rufaida BioMeds for letting us use their lab to do our research project.

Table of Contents

List of tables

List of figures

List of Acronyms

KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
CI	Confidence intervals
BMI	Body mass index
GWAS	Genome-wide association studies
SNPs	Single Nucleotide Polymorphisms
XRCC1	X-ray Repair Cross-Complementing 1
XPD	Xeroderma Pigmentosum Group D
RAD51	RAD51 Recombinase
ESR1	Estrogen Receptor 1
COMT	Catechol-O-Methyltransferase
MAPK	Mitogen-Activated Protein Kinase
HER2	Human Epidermal Growth Factor Receptor 2
PI3K	Phosphoinositide 3-Kinase
AKT	AKT Serine/Threonine Kinase
MAPK	Mitogen-Activated Protein Kinase
MEK	Mitogen-activated protein kinase kinase (also known as MAP2K)
RAF	Rapidly Accelerated Fibrosarcoma
aOR	Adjusted Odds Ratio
IDC	Invasive Ductal Carcinoma

Chapter 1

Introduction

Breast cancer is the most commonly diagnosed malignancy among women throughout the world and remains a leading cause of cancer-related mortality. The global problems of breast cancer are increasing, with an increasing incidence observed particularly in developing countries. Also, improvements in screening and treatment have raised survival rates; breast cancer now poses great public health obstacles. Not only environmental but also genetic factors contribute to breast cancer development, with genetic susceptibility contributing to determination (Lukasiewicz et al.,2021).

However, the causes of breast cancer have not been determined yet, although they include factors like genetic, environmental, and hormonal factors. Genetic susceptibility remains the subject of particular interest for scientists due to its association with genetic polymorphisms. Genes polymorphisms can be understood as changes in genetic sequences that are found among at least 1% of individuals.

It is known that high-penetrance mutations in genes increase the probability of breast cancer significantly. Nonetheless, most of these genes are polymorphisms, low penetrance mutations. In other words, the effect of each of these polymorphisms is rather modest. Together they contribute to the development of breast cancer to a large extent (Dunning et al., 1999). The current development of molecular genetics and the application of GWAS allow finding various polymorphisms related to breast cancer, among which are SNP, the most common type of genetic variations (Tamimi, 2006).

There are several ways in which genetic polymorphisms can contribute to the development of the disease. Firstly, they might affect important biological processes, including DNA repair, hormone metabolism, and cell cycle regulation. The DNA repair process includes repair of DNA damage induced by various factors.

Polymorphisms in genes involved in DNA repair, such as *XRCC1, XPD*, and *RAD51*, have been linked with decreased repair proficiency. Hence, the low repair proficiency will lead to increased DNA damage, thus predisposing an individual to develop cancer.

For example, polymorphisms in the *XRCC1* gene have been shown to cause impaired base excision repair while polymorphisms in the *XPD* gene affect nucleotide excision repair capacity. This results in genomic instability, indicating cancer development (Smolarz et al., 2019). Several epidemiological studies and meta-analyses have demonstrated a strong relationship between polymorphisms in DNA repair genes and the risk of developing breast cancer. Hormone modulation, especially estrogen, plays an integral role in the development of breast cancer. Estrogen promotes the growth of breast epithelial cells, and prolonged exposure to estrogen is a known risk factor. Genetic variations in the estrogen receptor gene, such as *ESR1*, alter receptor function and cellular response to estrogen. Furthermore, polymorphisms in the genes encoding enzymes involved in estrogen metabolism, such as COMT, affect estrogen concentrations and contribute to increased exposure to active estrogen metabolites, promoting cell proliferation and enhancing malignancy risk (Dai et al., 2019).

There are a number of methods where genetic polymorphisms could result in the development of breast cancer. One such method is the occurrence of genomic instability due to defective DNA repair. Also, alterations in the levels of gene expression due to polymorphisms in regulatory elements could alter the binding of transcription factors and hence influence the functioning of certain genes. Alterations in the hormonal control as a consequence of genetic modifications affecting the processes of estrogen synthesis and metabolism may contribute to the development of tumors. Gene-gene and gene-environmental interactions play a vital role in determining the susceptibility of an individual to breast cancer. This involves the combined effect of various polymorphisms alongside environmental factors such as dietary habits, lifestyle and exposure to carcinogens. The cumulative influence of these factors determines the

predisposition of an individual to breast cancer (Tamimi, 2006). Recent research has pointed to the importance of epigenetics in controlling the influence of genetic polymorphisms on breast cancer risk.

In summary, genetic polymorphisms influence the development of breast cancer risk factors through the disruption of critical physiological processes. While each individual genetic polymorphism carries a minimal risk in itself, their combined effects could lead to an increased likelihood of disease. Genomics has advanced considerably in its contribution to the elucidation of the genetic polymorphisms involved in breast cancer. Moreover, the diagnosis, prognosis and treatment of breast cancer have become more precise. Further studies will be necessary to overcome current limitations and utilize this research.

Genetic markers have received significant attention in the past few years in relation to breast cancer risk. Notably, the *ESR1* gene appears to be one of the best candidates due to the following: Its function in promoting the survival of breast tissues; regulating and differentiating cell proliferation, cell apoptosis and tumor formation (Bartel, 2004).

The *ESR1* gene encodes the estrogen receptor alpha that regulates the activity of estrogen, a substance with strong links with the cause of breast cancer. The normal activities in the body can be hindered when there are changes in the functioning of the *ESR1* gene. This brings about unlimited cell division, leading to tumor formation. Although this topic is very important, there have not been any studies carried out on the correlation between *ESR1* gene polymorphism and the risk of contracting breast cancer.

Cancer development is influenced by a compound interplay between environment and genetic factors. For example, one of the most important oncogenes involved in cancer development is the *KRAS* gene, which is responsible for the MAPK signalling pathway. Mutations in the *KRAS* gene can lead to deviant activation of signalling pathways that promote cell survival, proliferation and tumorigenesis. Particularly, mutations occurring in regulatory regions of the

KRAS gene may increase its function and contribute to the development of various cancers, causing lung cancer (Prior et al.,2012). MicroRNAs (miRNAs) are important regulators of gene expression at the post-transcriptional level. It is a small non-coding RNA consisting of approximately 18-25 nucleotides. These molecules bind to complementary order in the target messenger RNAs (mRNA). This degrades mRNA and inhibits the transition of mRNA. MicroRNAs are believed to influence around 60% of protein-coding genes and participate in various biological processes, including cell proliferation, differentiation, death, and tumorigenesis (Bartel, 2004). Alterations in miRNA expression have been extensively associated with cancer advancement.

By modifying miRNA–mRNA interactions, SNPs found inside miRNA binding sites can have a substantial impact on gene expression. One such polymorphism is rs61764370 (T/G), which is found in the let-7 miRNA binding site of the *KRAS* gene's 3′ untranslated region (3′UTR). A higher risk of ovarian, cervical, breast, colorectal, and lung cancers has been linked to this polymorphism, which has been demonstrated to impact *KRAS* expression (Chin et al., 2008; Ratner et al., 2010). The *KRAS* gene is a proto-oncogene that produces a protein called the *KRAS* protein, which controls cell growth, repair, and cell division. It signals the protein into the cell. Overactivation of this gene leads to the proliferation of cells and eventually cancer. Another gene that has been functionally correlated with the *KRAS* gene to cause cancer, and specifically Breast cancer, is the *HER2* gene. *HER2* is a gene that produces protein receptors, which further control growth signalling. This receptor is present on the cell surface, which, when activated, works by signalling growth, repair, and division of cells. The *KRAS* gene and the *HER2* gene are correlated by their similar mechanisms, where the *KRAS* gene transmits internal signals, and the *HER2* gene receives external signals.

The correlation between the regulation that takes place inside the cell and growth signalling genes that regulate cell growth is an important RNA called MicroRNA.

The MicroRNAs (miRNA) are non-coding RNA which does not code for proteins; they instead work by controlling other genes and specifically reducing the activity of those genes. miRNAs work by attaching themselves to RNA and either don't allow the RNA to produce proteins or directly destroy the RNA based on how perfectly the molecule fits with the RNA. The specific miRNA called let-7 is responsible for controlling the cancer-related genes, including the *KRAS* gene. In regular occurrence, the let-7 miRNA attaches itself to the *KRAS* gene and controls cell growth, but with the presence of a mutation, the let-7 does not bind to the active site of the gene, as the binding site was altered due to the variant. This leads to higher *KRAS* levels and an increase in growth signals and more chances of tumour formation. The oncogene known as the *KRAS* gene (Ki-ras2 Kirsten rat sarcoma virus oncogene homolog) encodes a small GTPase transducer protein known as *KRAS*. Until the *KRAS* protein binds to GTP, it remains inactive. Intracellular signals control the transition from an inactive to an active phase. Once the GTP binds to the *KRAS* protein, two parts of the protein undergo conformational changes that activate the protein. Switch 1 (amino acids 30–38) and Switch 2 (amino acids 59–67) are two crucial areas that create an effector loop and regulate the specificity of this GTPase's binding to its effector. The structural modification in the *KRAS* protein affects its interactions with several downstream transducers, particularly GAPs. Moreover, mutations in the *KRAS* gene significantly influence the process of carcinogenesis. Cancers with a high prevalence rate of *KRAS* mutations frequently express upregulation of *KRAS* signalling, but less than 2% of cases of breast cancer have been found to have *KRAS* mutations. However, it was recently found that a single nucleotide polymorphism in the 3'-untranslated region of the *KRAS* oncogene (*KRAS* variation; rs61764370) acts as a genetic marker for an increased possibility of developing human cancers by disrupting a let-7 miRNA binding. However, for evidence-based results, further insight into the research is importantly known that effector signalling pathways, including the MAPK and PI3K/AKT pathways, can be altered, but the alteration is mainly

found in hormone receptor-positive cancer molecules. Even though there has been a relation between the *rs61764370* and breast cancer but not in all scenarios. As per the initial hypothesis, it was believed that *rs61764370* could be useful as a genetic marker to anticipate the plausible risk of breast cancer or the progression of the disease due to its relation with the *KRAS* gene and the *HER2* gene. Such as, a study published in *BMC Cancer* reported that r*s61764370* was not solely associated with the overall breast cancer, but it was associated with the characteristics of the tumour, more specifically, tumours with *HER2*-positive, poor tumour differentiation, and postmenopausal women taking hormone replacement therapy (Paranjape et al., 2011).

Likewise, other studies also suggest a similar idea that it does not directly cause cancer but may contribute to the risk of breast cancer for a group of premenopausal and postmenopausal women (Hollestelle et al., 2016; Ratner et al., 2012).

Nevertheless, there have been conflicting results about the relationship between the rs61764370 polymorphism and cancer risk. This variant and cancer susceptibility are significantly correlated in some research, but not in others. For instance, Kjersem et al. (2012) and Luong et al. (2012) found no significant correlation with colorectal and uterine malignancies, respectively, while Ratner et al. (2010) identified rs61764370 as a potential genetic marker for ovarian cancer. These contradictory findings underscore the necessity of conducting population-specific research to enhance the understanding of the role of genetic differences in cancer development. It is essential to examine these relationships within specific populations, as genetic factors such as *KRAS* -related regulatory mechanisms in cancer biology and *ESR1* polymorphisms in breast cancer are critically important. Notably, there is a lack of information regarding the hereditary predisposition of Bangladeshi women to breast cancer. With the aim of improving knowledge of genetic risk factors and possibly supporting early identification and individualized treatment plans, this study attempts to assess the relationship

between *ESR1* gene polymorphisms and breast cancer risk. This study was conducted with the aim of investigating the association between genetic polymorphisms (*KRAS* rs61764370) and breast cancer susceptibility, with a specific focus on the impact of such polymorphisms on the development of breast cancer, as well as to evaluate whether such genetic polymorphisms have a significant impact on the risk of developing breast cancer within the Bangladeshi population.

Chapter 2

Methodology

2.1 Materials

TAKARA BIO INC (Japan) sold us the TaKaRa Taq DNA Polymerase Master Mix, restriction enzymes, and DNA ladder. Solarbio Life Sciences (China) sent the Tris-borate-EDTA (TBE) buffer, while Lonza (USA) sent the SeaKem LE Agarose. DNA extraction kit from Favorgen (USA) and a Midori Green stain from Nippon Genetics (Europe).

2.2 Study design and participants

To establish the link between the *KRAS* rs61764370 polymorphism and breast cancer, a hospital-based case-control study was conducted in the Bangladeshi population. For the following study, 112 confirmed breast cancer patients were recruited from the National Institute of Cancer Research and Hospital (NICRH) and 124 healthy patients were taken from the same region. The patient group included confirmed breast cancer female patients who were at least 18 years old and did not have any previous record of any other cancer. Also, patients who received chemotherapy or radiotherapy before blood sample collection or with severe chronic systemic diseases were excluded. Structured questionnaires were used to collect demographic and clinical data, including age, body mass index (BMI), number of children, occupation, education, socio-economic status, residential status and menopausal status.

2.3 DNA Extraction and Genotyping

Peripheral blood samples collected from the volunteers were stored in 15 ml tubes where EDTA was used as an anticoagulant. Then, genomic DNA was extracted from these samples using the commercial DNA extraction kit Favorgen, USA, by the spin extraction column method. To isolate the genomic DNA, the blood samples were subjected to lysis, and then the lysate was sent to the binding column. The DNA was then washed with multiple buffers, after which nuclease-free water was used to wash out the DNA. This DNA was stored at -20°C.

After the completion of DNA extraction, a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was carried out using a thermal cycler (mini PCR, USA) and Emerald AMP GT PCR Master Mix was used to identify SNP at different polymorphic sites. The products of PCR were confirmed by 1% agarose gel electrophoresis. After PCR was completed, the restriction enzyme *MspI* was used at 37°C for 1 hour to digest the products. These digested fragments were separated on 3% agarose gel electrophoresis, stained with Midori Green and then visualized under UV illumination.

The conditions of PCR and the primer sequence are shown in the table below.

Gene, SNP	**Primers' sequence F-Forward, R-Reverse**	**Thermal condition**	**Fragments, BP**
KRAS rs61764370	F 5'-GTGTCAGAGTCTCGCTCTTGTC-3' R 5'-AGACCACACTAGCACTACCTAAGGA-3'	5 min of denaturation at 98°C, then 40 cycles of 98°C for 40s, 63°C for 40s, and 72°C for 50s. The final cycle had a 5 min extension at 72°C	376

Table 1: Primer sequences, thermal conditions and amplified products' size

2.4 Quantitative Analysis

The data were statistically analyzed using R software (version 4.4.1; R Foundation for Statistical Computing, Vienna, Austria). The Hardy-Weinberg principle was used to test the Hardy-Weinberg equilibrium. Chi-squared tests were used to assess the differences in the distribution of single-nucleotide polymorphisms between healthy individuals and individuals with breast cancer. Age and BMI were used as covariates in multivariate logistic regression models to calculate Adjusted Odds Ratios with 95% confidence intervals.

2.5 Ethical considerations

The following study was conducted in Rufaida BioMeds as a final year thesis project by students at the School of Pharmacy, Brac University, under the approval of the Institute Review

Board (IRB), Brac University (IRB No. BRACUIRB120220005). Prior to the beginning of this study, all the participants gave written informed consent. They had the freedom to withdraw at any point without any consequences.

Chapter 3

Result

In this study, genomic DNA was isolated from 236 volunteers, consisting of 112 breast cancer patients (cases) and 124 healthy volunteers (controls). All participants were residents of Dhaka, Bangladesh. We used this data to assess associations between breast cancer and the *KRAS* rs61764370 polymorphism across various genetic models. Genotype distributions for both cases and controls were tested for Hardy-Weinberg Equilibrium (HWE), with p-values > 0.05 indicating the distributions followed HWE.

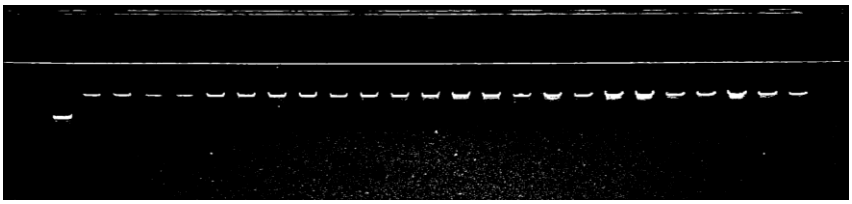


Figure 1: Presentative 1% gel image of extracted DNA by spin column extraction method

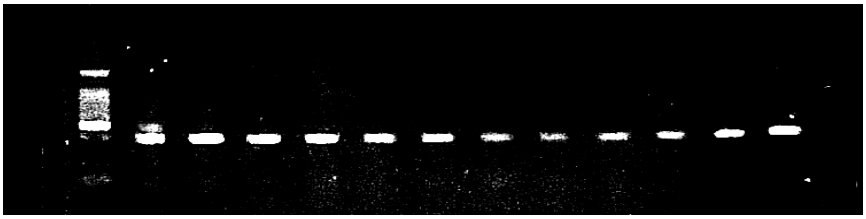
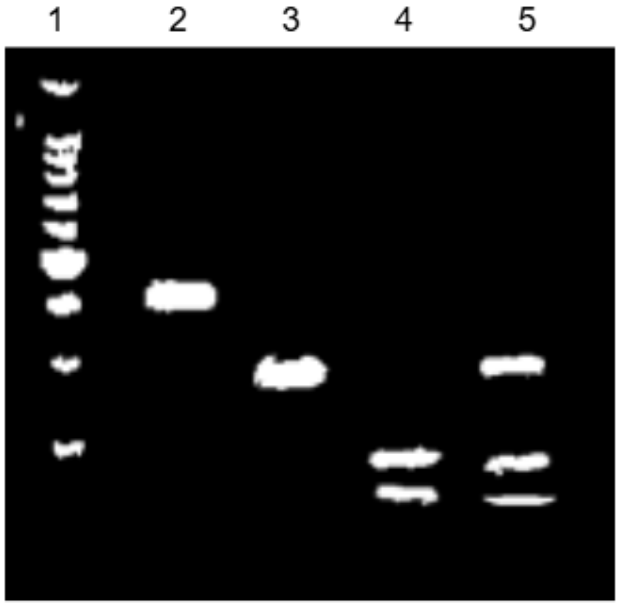


Figure 2: 376 bp DNA ladder after electrophoresis of 2% agarose gel

The DNA ladder after electrophoresis of 2% agarose gel is shown above. The results from gel electrophoresis showed two types of bands: G-type at 296 bp and T-type at 135 bp and 161 bp.



1 = DNA Ladder (100 BP); 2 = PCR Product; 3 = GG Homozygote; 4 = TT Homozygote; 5 = TG Heterozygote

Figure 3: Representative 3% gel image of digested samples by enzyme Bsrl at 37°C for 1 hour.

Based on the bands observed in Figure 2, *KRAS* rs61764370 polymorphism was identified as follows:

TT common homozygote showing bands at 80 bp, 135 bp, and 161 bp

GT heterozygote showing bands at 80 bp, 135 bp, 161 bp, and 296 bp

GG rare homozygote showing bands at 80 bp and 296 bp

For these three genotypes, adjusted odds ratios (aOR), 95% confidence intervals (CI), and p-values were calculated using multivariate logistic regression analysis in R, adjusted for age and BMI.

Allele	**Cases (n = 112)**	**HWE**		**Controls (n = 124)**	**HWE**		**Genetic models**	**aOR**	**95% CI**	***P*-value**
		X^2	***P***		**X^2**	***P***				
TT Common homozygote (80 bp, 135 bp and 161 bp)	n=48; (42.85 %)	0.153	0.696	n=96; (77.42 %)	0.015	0.903	Additive model 1 (GT vs. TT)	4.548	2.590 – 7.988	<0.001
							Additive model 2 (GG vs. TT)	1.970	0.039 – 100.763	0.736
GT Heterozygote (80 bp, 135 bp, 161 bp and 296 bp)	n=62; (55.36 %)			n=26; (20.97 %)			Dominant model (GT+GG vs. TT)	4.548	2.590 – 7.988	<0.001
GG Rare homozygote (80 bp and 296 bp)	n=2; (1.79%)			n=2; (1.61%)			Recessive model (GG vs. GT+TT)	1.107	0.022 – 56.240	0.960
							Over-dominant model (GT vs. GG+TT)	2.566	1.461 – 4.506	0.001

Note: Genotype frequencies were tested for Hardy-Weinberg equilibrium (HWE) using the Chi-square test (X^2). Adjusted Odds Ratios (aOR), 95% Confidence Intervals (CI), and p-values were calculated using multivariate logistic regression in R. All models used the TT genotype (common homozygote) as the reference group.

$p > 0.05$ indicates that genotype distribution follows the Hardy-Weinberg equilibrium.

Abbreviations: HWE – Hardy-Weinberg equilibrium, OR – Odds Ratio, CI – Confidence Interval, X^2 – Chi-square value

Table 2: Association between Breast Cancer and *KRAS* rs61764370 polymorphism

3.1 Association of the rs61764370 polymorphism with BC

As shown in Table 1, the association between the rs61764370 polymorphism and breast cancer risk was evaluated by comparing genotype distributions between cases and controls under different genetic models.

The genotype frequencies among cases were 42.85% (TT), 55.36% (GT), and 1.79% (GG), whereas in controls they were 77.42% (TT), 20.97% (GT), and 1.61% (GG). Genotype distributions in both groups were consistent with the Hardy–Weinberg equilibrium ($p > 0.05$).

Using the TT genotype as the reference, logistic regression analysis demonstrated a significant association between rs61764370 and breast cancer risk. In the additive model 1, individuals with the GT genotype (GT vs. TT) showed a significantly increased risk (aOR = 4.548; 95% CI: 2.590–7.988; $p < 0.001$), whereas Additive Model 2 (GG vs. TT) did not show a statistically significant association (aOR = 1.970; 95% CI: 0.039–100.763; $p = 0.736$).

Under the dominant model (GT + GG vs. TT), a significantly increased risk was observed (aOR = 4.548; 95% CI: 2.590–7.988; $p < 0.001$), suggesting that carriers of at least one G allele have

a higher susceptibility to breast cancer. In contrast, the recessive model (GG vs. GT + TT) did not show a significant association (aOR = 1.107; 95% CI: 0.022–56.240; p = 0.960).

Furthermore, the over-dominant model (GT vs. GG + TT) with an odds ratio of 2.566 (95% CI: 1.461 – 4.506) suggests an increased risk of developing BC in GT genotype carriers compared to GG and TT genotype carriers, with a p-value of 0.001 (Figure 4). In contrast, the GG genotype does not show a statistically significant association with BC risk, both in the additive model 2 and the recessive model, when compared to the TT and GT + TT, respectively (Figure 4).

The forest plot association between breast cancer and the *KRAS* rs61764370 polymorphism is given below:

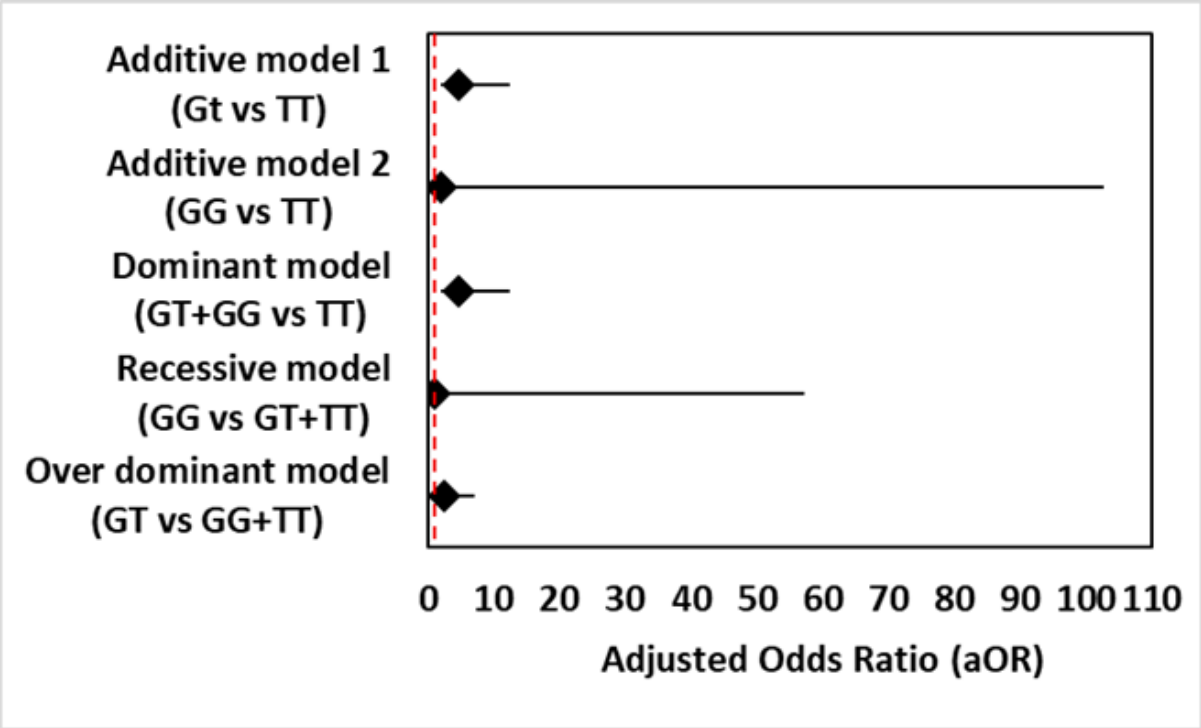


Figure 4: Forest plot illustrating the connection between breast cancer and the *KRAS* rs61764370 polymorphism

3.2 Socio-demographic History

The demographic profiles of the 112 cases and 124 controls were closely matched to ensure study reliability. The mean age was 46.9 ± 12.5 years for cases and 47.8 ± 13.1 years for controls ($p = 0.58$) as per analysis of age distribution. The mean BMI was 25.7 ± 4.9 kg/m² and 25.2 ± 5.1 kg/m², respectively ($p = 0.32$). With cases having an average of 2.13 ± 0.91 children compared to 2.09 ± 0.87 children in the control group ($p = 0.45$), reproductive history was similarly consistent between the cohorts. With respect to socioeconomics and occupation, the majority of participants in both groups were working women (60.7% of cases; 58.9% of controls), followed by housewives (33.9% vs. 35.5%) and a small percentage of students (5.4% vs. 5.6%; $p = 0.46$). Educational background showed no significant disparity ($p = 0.71$); although just over half of the cases were illiterate (53.57%), 35.71% had completed less than ten years of schooling, and 10.71% had ten or more years of education. These figures closely mirrored the distribution seen in the control group (48.38%, 38.71%, and 12.9%, respectively) In economic status, the majority of cases belonged to the lower socio-economic class (52.68%), 40.19% were middle class and 7.14% were higher class, which was statistically comparable to the control group distribution of 59.68%, 30.66%, and 9.68% ($p = 0.29$). A well balanced has been noticed in Residential and menopausal statuses. 67.86% of cases resided in rural areas compared to 68.55% of controls ($p = 1.0$), and 56.25% of cases were postmenopausal compared to 58.06% of controls ($p = 0.881$). Overall, none of the assessed demographic or socioeconomic variables showed statistically significant differences ($p > 0.05$), suggesting that the case and control groups were appropriately matched for meaningful comparative analysis.

3.3 Histopathological Characteristics of BC Patients

Comprehensive histopathological examinations were conducted for all 112 cases to evaluate the clinical and pathological profiles of the patients. 100% of the patients presented with a breast lump in terms of clinical presentation. In physical findings, 40.19% of cases were found weight loss and 39.29% for peau d'orange. Nipple-related symptoms were also documented, with nipple discharge affecting 18.75% and 14.29% of retraction of the nipple, and deviation of the nipple in 8.92% of the cohort. Moreover, Ulceration involving the overlying skin or nipple–areola complex was observed in 9.82% of the patients. In histological classification, invasive ductal carcinoma was the most prevalent type, which is identified in 75% of the cases. Ductal carcinoma in situ was found in 14.29% of the cases and invasive lobular carcinoma in 10.71%. Tumor grading analysis showed that 46.43% of patients had Grade I tumors, 37.5% had Grade II tumors, and 16.07% were diagnosed with Grade III tumors. A small minority of cases (5.36%) had tumors measuring less than 2 cm was revealed in an analysis of tumor size. Most tumors were between 2.1 and 5.0 cm, of which 55.36% and substantial portions of 39.29% exceeded 5.0 cm. The majority of the patients were receptor- positive in hormone receptor status with ER+ and PR+ status observed in 65.19% and 71.43% of cases, respectively. The patient of 28.57% was detected in ER-2+ expression. Lastly, in terms of lymphatic spread, 73.21% showed no lymph node involvement and whereas 26.79% had positive lymph node involvement.

Chapter 4

Discussion

The current case-control study investigated the association between the *KRAS* polymorphisms (rs61764370) and BC risk in the Bangladeshi community, involving 112 BC patients and 124 healthy volunteers. The association between *KRAS* genotypes and BC risk was evaluated in this study using logistic regression methods to calculate aOR, CI, and p-values. Our results indicate that *KRAS* rs712 and rs61764370 polymorphisms may be associated with BC susceptibility in this population after analyzing various genetic models; however, these findings should be considered with caution. BC is the combination of all malignancies, particularly those that impact women globally. According to Al-Haddad et al., frequent genetic modifications that primarily regulate cell growth and proliferation can cause BC to develop. Most significantly, 30% of all human malignancies, including BC, are caused by mutations in the *KRAS* gene (Uprety et al., 2020). To be more specific, the human oncogene *KRAS* primarily codes for the *KRAS* protein. To control cell division, this protein transmits specialized signals to the cell nucleus. Recent research has shown that SNPs located in the 3'-UTR of *KRAS* contain possible tumour suppressor miRNAs like let-7. These SNPs impact *KRAS* activity by inhibiting let-7 miRNAs and changing *KRAS* protein expression, which eventually results in the development of BC (Al-Haddad et al.,2019).

The *KRAS* rs61764370 polymorphism, particularly disrupts the let-7 binding affinity for the *KRAS* gene, is located in the let-7 complementary site 6 and is categorized as both a functional and germline polymorphism in the *KRAS* 3'UTR. In the end, it enhances tumour growth triggered by the G allele and inhibits *KRAS*. For instance, Gallegos-Arreola et al. observed a significant correlation between Mexican women's BC risk and *KRAS* rs61764370 (Gallegos-Arreola et al., 2021). In the exact same way, Sanaei et al. (2017) found that the G allele of rs61764370 and the

TG genotype were linked to an elevated risk of BC in Iranian women. According to a different study by Pilarski et al., the rs61764370 polymorphism, a *KRAS* variant, has a strong correlation with ovarian cancer and double primary BC (Pilarski et al., 2012). Our results remain consistent with other research, which indicates that the rs61764370 polymorphism might cause Bangladeshi women more susceptible to breast cancer. Despite this, other studies, including the one investigated by Uvirova et al. (2015), did not find a significant correlation between the *KRAS* rs61764370 polymorphism and the risk of BC in the Czech population, suggesting that further research is needed to fully understand the impact of this variant in other populations.

Overall, our findings indicate a possible correlation between the *KRAS* gene (rs61764370) polymorphism and BC susceptibility in the Bangladeshi community. Additional research on people in Southeast Iran, Iraq, and Mexico showed that the *KRAS* gene polymorphisms (rs61764370) had been correlated with an increased risk of breast cancer. According to research findings, *KRAS* SNPs such as rs61764370 might be responsible for an increased risk of breast cancer.

According to our demographic study, age, BMI, occupation, number of children, education, socioeconomic class, residency status, and menopausal state did not significantly differ between BC patients and HCs. The multivariate models did not properly compensate for the assessed demographic variables, despite the fact that no statistically significant differences were found between the cases and controls. Consequently, residual conflation cannot be ruled out. Although studies elsewhere (particularly among postmenopausal women) have shown greater BMI as a risk factor, indicating the need for more investigation, our study's lack of a BMI–BC connection is consistent with earlier findings in Bangladeshi women. Since the groups were reported to have a comparable number of children, reproductive history also seemed to have little bearing. Overall, by lowering demographic confounding, these findings improve the validity of our genetic results.

According to worldwide trends, invasive ductal carcinoma was the most prevalent tumour type. It is promising that the majority of malignancies were hormone receptor positive (ER and PR), because they are linked to improved outcomes and frequently react favorably to hormone therapy.The 29% HER2-positive prevalence, which is similar to other Asian populations, indicates many patients may benefit from targeted therapy. Nearly 40% of tumors were larger than 5 cm at diagnosis, despite the fact that the vast majority of tumors were medium-sized (2.1–5.0 cm), emphasizing the need for more extensive screening programs and early detection. Lymph node involvement was present in about 25% of patients, indicating its crucial role in diagnosis, treatment planning, and prognosis.

One crucial methodological consideration in this research is the potential for residual confounding. Although age and BMI were appropriately taken into consideration in the regression models, other relevant factors such as reproductive history, hormonal influences, and lifestyle traits were excluded from the final models. Risk estimations could still be influenced by their exclusion even if there was no statistically significant difference between the groups. Further research with larger sample sizes must include comprehensive multivariable adjustment.

Many of the limitations of the study should be taken into consideration when evaluating the results. First, the very small sample size could limit the study's statistical power. Second, while several demographic and reproductive information were collected, additional significant clinical and lifestyle factors, such as smoking status, the use of oral contraceptives, and breastfeeding history, were not collected. Finally, because this was a single-center study conducted primarily in a Bangladeshi community, the findings might not be completely applicable to other ethnicities. Additionally, many genetic models were evaluated without explicit modification for multiple comparisons, which increases the probability of type I error. Because they may not be significant

after adjustment, some of the statistically important correlations discovered in this study should be considered merely exploratory findings.

Despite these limitations, the study presents new insights on *KRAS* polymorphisms in a Bangladeshi population, where genetic epidemiology studies on BC are still limited. Future meta-analyses and systematic reviews examining the impact of *KRAS* mutations in BC susceptibility may benefit greatly from the findings.

Overall, the current study indicates that *KRAS* rs712 and rs61764370 polymorphisms may affect Bangladeshi women's susceptibility to BC; however, additional research with larger multi-center datasets and functional analysis is required to confirm these results.

Chapter 5

Conclusion

In conclusion, the findings indicate a link between the *KRAS rs61764370* polymorphism and breast cancer risk. Across all the models, the genotype GT showed a significant genetic risk factor in developing breast cancer compared to the TT genotype. On the other hand, the GG genotype barely shows any effect. Overall, the study shows that the presence of one G allele can significantly increase the risk factor in an individual. Besides the genetic factors, the study also focused on socio- demographic and medical history. Participants from rural backgrounds and with limited educational exposure were more prone to the disease than others. Further evaluation showed hormonal involvement with breast cancer, as the majority of the tumours were hormone receptor-positive (ER+ and PR+) (Yersal & Barutca, 2014). A fraction of the patients were also found to be HER-2 positive, indicating the need for targeted therapy. The methodology of the study proves maximum accuracy. The PCR-RFLP technique was used for genotyping. The results were as expected and eliminated the presence of error. Other variables were validated using multivariate logistic regression analysis. Identifying the association of the *KRAS* gene and breast cancer allows doctors to use it as a potential genetic biomarker. This gives a scope for developing targeted screening strategies to detect cancer at an earlier stage and take necessary preventive measures, which improves the survival rates of patients. Furthermore, this gives an idea for more routine genetic screening and allows scope for developing personalized treatments.

Reference

Al-Haddad, R. H., Jasim, H. M., & Saleh, W. A. (2019). Association between *KRAS* rs712 G>T polymorphism with the incidence of breast tumors in Iraqi women. *Iraqi Journal of Biotechnology, 18*(3).

Bartel, D. P. (2004). MicroRNAs: Genomics, biogenesis, mechanism, and function. *Cell, 116*(2), 281–297. https://doi.org/10.1016/S0092-8674(04)00045-5

Cerne, J. Z., Stegel, V., Gersak, K., & Novakovic, S. (2012). *KRAS* rs61764370 is associated with HER2-overexpressed and poorly differentiated breast cancer in hormone replacement therapy users: A case-control study. *BMC Cancer, 12*, Article 105. https://doi.org/10.1186/1471-2407-12-105

Chin, L. J., Ratner, E., Leng, S., Zhai, R., Nallur, S., Babar, I., Müller, R. U., Straka, E., Su, L., Burki, E. A., Crowell, R. E., Patel, R., Kulkarni, T., Homer, R., Zelterman, D., Kidd, K. K., Zhu, Y., Christiani, D. C., & Slack, F. J. (2008). A SNP in a let-7 microRNA complementary site in the *KRAS* 3′UTR increases non-small cell lung cancer risk. *Cancer Research, 68*(20), 8535–8540. https://doi.org/10.1158/0008-5472.CAN-08-2129

Chin, L. J., et al. (2010). Let-7 microRNA binding site polymorphism in *KRAS* 3′UTR and cancer risk. *Journal of Clinical Oncology, 28*(14), 2388–2395. https://doi.org/10.1200/JCO.2009.26.2105

Crowley, E. H., Arena, S., Lamba, S., Di Nicolantonio, F., & Bardelli, A. (2014). Targeted knock-in of the polymorphism rs61764370 does not affect *KRAS* expression but reduces let-7 levels. *Human Mutation, 35*(2), 208–214. https://doi.org/10.1002/humu.22490

Dai, Z., Chen, Z., Yang, S., & Zhang, L. (2019). Estrogen receptor gene polymorphisms and breast cancer susceptibility. *Cancer Cell International, 19*, Article 73. https://doi.org/10.1186/s12935-019-0727-z

Dunning, A. M., Healey, C. S., Pharoah, P. D., Teare, M. D., Ponder, B. A., & Easton, D. F. (1999). A systematic review of genetic polymorphisms and breast cancer risk. *Cancer Epidemiology, Biomarkers & Prevention, 8*(10), 843–854.

Gallegos-Arreola, M. P., Figuera-Villanueva, L. E., Ramos-Silva, A., Zúñiga-González, G. M., Puebla-Pérez, A. M., & Delgado-Saucedo, F. (2021). Association between rs61764370, rs9266, and rs140080026 polymorphisms of the *KRAS* gene and breast cancer risk in a Mexican population. *European Review for Medical & Pharmacological Sciences, 25*(21), 6571–6583. https://doi.org/10.26355/eurrev_202111_27103

Hollestelle, A., Pelletier, C., Hooning, M., Crepin, E., Schutte, M., Look, M., et al. (2011). Prevalence of the variant allele rs61764370 T>G in the 3′UTR of *KRAS* among Dutch BRCA1, BRCA2 and non-BRCA1/BRCA2 breast cancer families. *Breast Cancer Research and Treatment, 128*(1), 79–84. https://doi.org/10.1007/s10549-010-1080-z

Hossain, A., Murshid, G. M., Zilani, M. N. H., Islam, F., Sultana, R., Sultana, T., et al. (2017). TP53 codon 72 polymorphism and breast cancer risk in the Bangladeshi population. *Breast Cancer, 24*(4), 571–578. https://doi.org/10.1007/s12282-016-0740-1

Jancík, S., Drábek, J., Radzioch, D., & Hajdúch, M. (2010). Clinical relevance of *KRAS* in human cancers. *Journal of Biomedicine and Biotechnology, 2010*, Article 150960. https://doi.org/10.1155/2010/150960

Łukasiewicz, S., Czeczelewski, M., Forma, A., Baj, J., Sitarz, R., & Stanisławek, A. (2021). Breast cancer—Epidemiology, risk factors, classification, prognostic markers, and current treatment strategies. *Cancers, 13*(17), Article 4287. https://doi.org/10.3390/cancers13174287

Masuda, H., Zhang, D., Bartholomeusz, C., Doihara, H., Hortobagyi, G. N., & Ueno, N. T. (2012). Role of epidermal growth factor receptor in breast cancer. *Breast Cancer Research and Treatment, 136*(2), 331–345. https://doi.org/10.1007/s10549-012-2289-9

Nasrollahzadeh-Khakiani, M., et al. (2016). *KRAS* rs61764370 polymorphism and breast cancer risk in an Iranian population. *Gene, 587*(1), 10–15. https://doi.org/10.1016/j.gene.2016.04.026

Paranjape, T., Heneghan, H., Lindner, R., Keane, F. K., Hoffman, A., Hollestelle, A., et al. (2011). A 3′-untranslated region *KRAS* variant and triple-negative breast cancer: A case–control and genetic analysis. *Cancer Research, 71*(17), 5501–5510. https://doi.org/10.1158/0008-5472.CAN-11-0563

Peng, S., Lü, B., Ruan, W., Zhu, Y., Sheng, H., & Lai, M. (2011). Genetic polymorphisms and breast cancer risk: Evidence from meta-analyses and genome-wide association studies. *Breast Cancer Research and Treatment, 127*(2), 309–317. https://doi.org/10.1007/s10549-010-1191-3

Pilarski, R., Patel, D. A., Weitzel, J., McVeigh, T., Dorairaj, J., Heneghan, H., et al. (2012). The *KRAS* variant is associated with the risk of developing double primary breast and ovarian cancer. *PLoS ONE, 7*(5), Article e37891. https://doi.org/10.1371/journal.pone.0037891

Prior, I. A., Lewis, P. D., & Mattos, C. (2012). A comprehensive survey of *Ras* mutations in cancer. *Cancer Research, 72*(10), 2457–2467. https://doi.org/10.1158/0008-5472.CAN-11-2612

Ratner, E., Lu, L., Boeke, M., et al. (2010). A *KRAS* variant in ovarian cancer acts as a genetic marker of cancer risk. *Cancer Research, 70*(16), 6509–6515. https://doi.org/10.1158/0008-5472.CAN-10-0324

Sanaei, S., Hashemi, M., Eskandari, E., Hashemi, S. M., & Bahari, G. (2017). *KRAS* gene polymorphisms and their impact on breast cancer risk in an Iranian population. *Asian Pacific Journal of Cancer Prevention, 18*(5), 1301–1305. https://doi.org/10.22034/APJCP.2017.18.5.1301

Siegel, R. L., Miller, K. D., & Jemal, A. (2020). Cancer statistics, 2020. *CA: A Cancer Journal for Clinicians, 70*(1), 7–30. https://doi.org/10.3322/caac.21590

Smolarz, B., Michalska, M. M., Samulak, D., & Romanowicz, H. (2019). Polymorphisms of DNA repair genes and breast cancer risk. *Oncotarget, 10*(4), 527–536. https://doi.org/10.18632/oncotarget.26571

Talseth-Palmer, B. A., & Scott, R. J. (2011). Genetic variation and its role in malignancy. *International Journal of Biomedical Science, 7*(3), 158–171.

Tamimi, R. M. (2006). Single-nucleotide polymorphisms and breast cancer risk. *Breast Cancer Research, 8*(4), Article 214. https://doi.org/10.1186/bcr1529

Tokumaru, Y., Oshi, M., Katsuta, E., Yan, L., Satyananda, V., Matsuhashi, N., Futamura, M., Akao, Y., Yoshida, K., & Takabe, K. (2020). *KRAS* signalling-enriched triple-negative breast cancer is associated with favorable tumor immune microenvironment and better survival. *American Journal of Cancer Research, 10*(3), 897–970.

Uprety, D., & Adjei, A. A. (2020). *KRAS*: From undruggable to a druggable cancer target. *Cancer Treatment Reviews, 89*, Article 102070. https://doi.org/10.1016/j.ctrv.2020.102070

Uribe, M. L., Marrocco, I., & Yarden, Y. (2021). EGFR in cancer: Signalling mechanisms, drugs, and acquired resistance. *Cancers, 13*(11), Article 2748. https://doi.org/10.3390/cancers13112748

Uvirova, M., Machackova, T., Savarese, M., Brychtova, S., Speranza, A., Srovnal, J., et al. (2015). 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. *Biomedical Papers of the Medical Faculty of the University Palacky, Olomouc, Czech Republic, 159*(3), 466–471. https://doi.org/10.5507/bp.2014.041

Wilkinson, L., & Gathani, T. (2022). Understanding breast cancer as a global health concern. *The British Journal of Radiology, 95*(1130), Article 20211033. https://doi.org/10.1259/bjr.20211033

Yersal, O., & Barutca, S. (2014). Biological subtypes of breast cancer. *World Journal of Clinical Oncology, 5*(3), 412–424. https://doi.org/10.5306/wjco.v5.i3.412

Ying, H. Q., Wang, F., He, B. S., Pan, Y. Q., Wang, S. K., & Chen, J. (2014). The involvement of *KRAS* gene 3′-UTR polymorphisms in risk of cancer: A meta-analysis. *OncoTargets and Therapy, 7*, 1487–1496. https://doi.org/10.2147/OTT.S65496

Zhang, K., Zhou, B., Wang, Y., Rao, L., & Zhang, L. (2013). The TLR4 gene polymorphisms and susceptibility to cancer: A systematic review and meta-analysis. *Infection, Genetics and Evolution, 19*, 331–338. https://doi.org/10.1016/j.meegid.2013.07.021