Assessing the Prevalence and Impact of *HER2*-rs1136201 Polymorphism in Breast Cancer Development Among Bangladeshi Women: A Case-Control Study

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

School of Pharmacy
Brac University
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Approval

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Ethics Statement

The Institutional Review Board (IRB) of BRAC University granted approval for the study (Approval No. BRACUIRB120220005). All participants gave their written, informed permission after being informed of their freedom to leave the study at any moment and without consequence. Every procedure was carried out in compliance with the rules and specifications specified in the authorised study protocol.

Abstract

Breast cancer is one of the most malignant diseases in Bangladesh, and it is on the rise among women. The rs1136201 (Ile655Val) single-nucleotide polymorphism (SNP) and the human epidermal growth factor receptor 2 (*HER2*) gene have been proven to be involved in the development of breast cancer across various populations. The purpose of the study was to determine the relationship between the *HER2* rs1136201 polymorphism and the susceptibility of breast cancer in the Bangladesh population among the women of this country on a case-control basis. 236 people, including 112 histopathologically-proven breast cancer patients and 124 age-matched controls, took part in the study. Multivariate logistic regression was used to perform statistical testing under various genetic model assumptions, including additive, dominant, recessive, and overdominant models. The outcomes showed that there was no association with significant results among the *HER2* rs1136201 SNP and breast cancer in an additive, dominant, or recessive model ($p > 0.05$). Nevertheless, an overdominant model was significant (OR = 1.83, 95 % CI = 1.02-3.28; $p = 0.041$), suggesting that the risk of breast cancer was higher in individuals with AG heterozygote. Considering such an outcome, there may be an overdominance effect of the *HER2* polymorphism among the Bangladeshi people. These associations should be confirmed by further research on larger sample sizes and of functional significance to determine their biological roles in the pathogenesis of breast cancer.

Keywords: Breast cancer, *HER2* gene, Single nucleotide polymorphism (SNP), Overdominant model, AG genotype.

Dedication

I would dedicate my work to my people who are nearest to my heart, whose unconditional prayers and support have enabled me to have the physical strength and mental support to complete this work. The courage from my father, the support system from my mother, the inspiration from my brother, the unconditional support & endless motivation from my best friend, Md Refat Hossain Rafi, they are the people without whom I would not be able to do this work alone.

- Nusrat Jahan (21346045)

I would like to thank both my parents, who have always loved and guided me, my supervisor, who has mentored me so much, my thesis mates, whose cooperation and support have been so invaluable, and my wife, Ariba Afree, who has been there to always lend support and encouragement during my long-term pursuit of this degree.

- Md. Atik Zawad (21346052)

It is my pleasure to dedicate this work to my research supervisor whose guidance and uncompromising support has formed a major pillar of this process; and to my beloved parents, as an expression of the limitless love, sacrifices, and their prayers; to Almighty Allah, who has blessed me with strength and patience, and wisdom; and lastly, to myself--for bearing it all and struggling till this came to pass.

- Jannate Adon Wriddhi (21146052)

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We first and foremost express our sincere gratitude to Allah the Almighty for providing us with the patience, determination, and blessings necessary to do the work successfully. It would not have been possible to do this project without his blessing.

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Moreover, we are forever grateful to all the faculty members in our department for their immeasurable effort during our whole university life to teach us with enormous care which helps us in the successful accomplishment of our graduation.

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List of Acronyms

EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
HER 2	Human Epidermal Growth Factor Receptor 2
HER 3	Human Epidermal Growth Factor Receptor 3
BP	Base Pair
MAPK	Mitogen-Activated Protein Kinase
P13K	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase
PKC	Protein Kinase C
SNP	Single Nucleotide Polymorphism

Chapter 1

Introduction

1.1 Background on Breast Cancer

Breast cancer stands worldwide as the second most common cause of cancer-related deaths among women and is one of the most prevalent cancers identified in women (*Breast Cancer*, 2025). Globally, breast cancer constitutes one of the most common and fatal tumours that affects women. It appears when cells throughout the breast tissue proliferate out of control; this typically commences in the drainage canals or lobes. Around 2.3 million women acquired a breast cancer diagnosis in 2022, while 670,000 individuals died from the disease nationwide. Each nation in the world has breast cancer, which may impact women at any stage following puberty, yet is more common in the later stages of life (World Health Organization: WHO & World Health Organization: WHO, 2024). Nonetheless, although it occurs approximately one hundred times more frequently in women than men, males are not immune to it; however, a much smaller proportion do so. According to Sun et al. (2017), the primary belief is that it is part of the reason that breast carcinoma is challenging to treat so regularly because it metastasizes, most often into distant tissues, such as the kidneys, brain, lungs, and bones. Early detection of the long-term survival and prognosis can be enhanced through the detection of the disease in its early stages.

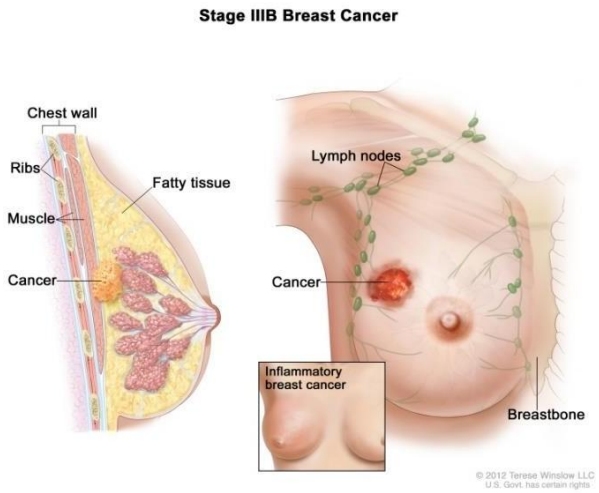

Figure 1: Visualization of Breast cancer (National Cancer Institute (US), n.d.)

At the moment when breast cancer is initially diagnosed, almost 90 percent of cases are not invasive. Therapeutic measures applicable to those patients without spreading cancer include removing the tumour surgically and preventing its recurrence (Waks & Winer, 2019). It is estimated that 30 percent of the prevalence of breast cancer could be averted due to preventable factors that include being overweight, failure to exercise, and drinking alcohol. Together with enhancing treatment procedures, the further prevention of the disorder through the implementation of the mammography test is associated with a substantial reduction in death rates caused by breast cancer (DeSantis et al., 2022).

1.2 *HER2* Expression in Breast Cancer

According to Sombal et al. (2024), *HER2*, a type of proto-oncogene often referred to as a neu-oncogene, appears on the extended arm of chromosome 17 (17q12) and comprises a structure made up of 1255 amino acid sequences and a glycoprotein found in the transmembrane (185 kDa). Approximately 20% to 30% of breast cancers are caused by polymorphisms in the *HER2* gene, and *HER2*-positive breast cancer is extremely aggressive (Sombal et al., 2024d). Increasing recurrence rates, shorter recovery times, and more serious complications are linked to *HER2* overexpression. The *HER2* receptors lack a recognizable ligand, in contrast to the remaining receptors in the EGF series. It can dimerize together with additional EGF receptors without the need for a ligand and is discovered in an active state. It is believed that the *HER2/HER3* dimer pairing is the most potent as well as tumor-promoting (Mitri et al., 2012b). Sombal et al. (2024c) state that *HER2* activates several subsequent signalling pathways that aid in controlling the development of cells and their proliferation, including mitogen-activated protein kinase (MAPK), phosphatidylinositol-4,5-bisphosphate 3-kinase (P13K), and protein kinase C (PKC). Moreover, gene expression studies have demonstrated that *HER2*-amplified breast tumours most likely correspond to one or more of the four distinct genetic subtypes of the medical condition (Perou et al., 2000).

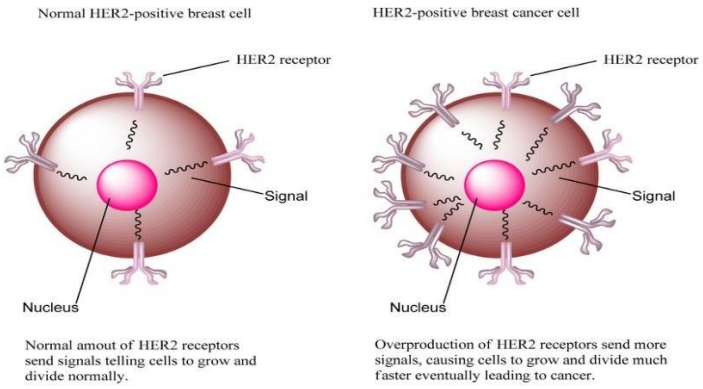

Figure 2: Normal *HER2*-positive breast cell and *HER2*-positive breast cancer cell (Okarvi & AlJammaz, 2019).

When triggered, the membrane tyrosine kinase known as the *HER2* (human epidermal growth factor receptor 2) receptor influences cell development and survival. The chromosome 17q12 contains the *HER2* oncogene. The primary manifestation of *HER2* receptor activation and a key factor in the proliferation and spread of malignancies in a particular category of breast cancer patients is *HER2* amplification.

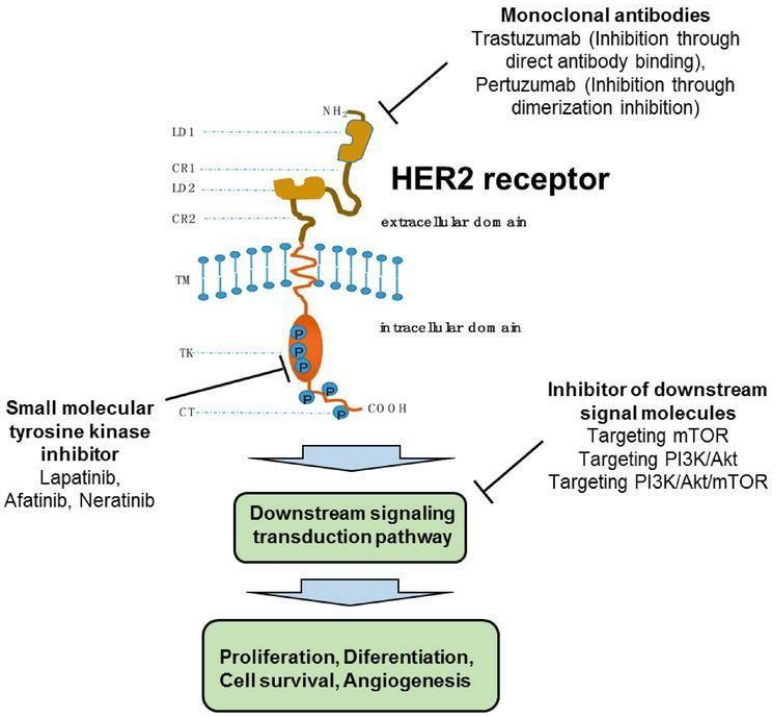

Figure 3: *HER2* molecular pathways approaches for *HER2* positive breast cancer therapy (Nguyen-Thanh et al., 2021c)

According to Nguyen-Thanh et al. (2021c), the *HER2* overexpression tumor cells may be utilized through the *HER2* cellular pathways method for *HER2*-targeted treatment options. They added that about 15–20% of breast cancers have *HER2* overexpression or amplification, which is linked to the aggressive clinical characteristics of absence cases.

1.3 SNP rs1136201 and Its Association with Breast Cancer Risk

This single-nucleotide polymorphism, referred to as SNP rs1136201, is found within the *HER2* gene. A different acronym for this polymorphism is ille655Val. Gene areas that are regulatory or essential may have single-nucleotide polymorphisms that influence the functioning of proteins. The transmembrane domain-coding area of the *HER2* locus at codon 655, which codes for both valine (Val: GTC) or isoleucine (Ile: ATC), features the *HER2*Ile655Val SNP (Nguyen-Thanh et al., 2021). Overall, it has been determined that the *HER-2* Ile655Val polymorphism is likely correlated with six main cancer categories, such as breast, thyroid, lung, ovarian, gastric, and uterine (Riaz et al., 2016). Even though *HER2* is overexpressed, Fleishman et al. (2022) demonstrated that causing instability in the production of functioning *HER2* dimers by replacing Val with Ile within that transmembrane domain results in decreased stimulation of receptors and tyrosine kinase action. Numerous writers have related the *HER2* gene's Ile655Val (rs1136201) polymorphism to the progression of breast cancer, whereas other research has not shown a link between it and an increased risk of breast cancer in females. Therefore, the overall findings are disputed and ambiguous (Sombal et al., 2024b).

Inheritance models include methods to indicate how specific genotypes relate to disease susceptibility in genetic association studies. In the additive model the effect of each successive copy of the risk allele increases risk to the disease in proportion to itself, such that heterozygotes (AG) are intermediate in risk between each homozygote (AA and GG). The predominant paradigm that is being tested is whether or not the presence of at least one risk allele (AG or GG) can elevate disease risk versus the homozygote wild-type (AA), meaning that one risk allele is enough to be vulnerable to illness. On the other hand, the recessive model deals with whether only the individuals who possess two copies of the risk allele (GG) have an increased risk in comparison with individuals who have one or no altered allele (AG + AA), and it implies that two alleles need to be changed in order to influence the disease outcome. The last is the

over dominant model that compares the effect of heterozygotes (AG) with both homozygotes (AA and GG) to determine whether the lower dose of two different alleles conveys a particular effect, either protective or deleterious, and independent of the dose. Used together, the models inform of complementary aspects of the genetic architecture of disease and assist in determining whether a variant is additively, dominantly, recessively or heterozygote-related to disease susceptibility.

1.4 Epidemiology of Breast Cancer

One of the most prevalent illnesses in the entire globe is cancer, and someone's genetic variations have a significant connection to their risk of inheriting the disease. Likewise, in numerous other developing nations, people in Bangladesh are becoming more susceptible to cancer (Babu et al., 2022). South Asian countries have been experiencing a nationwide epidemic of carcinoma of the breast, resulting in an abrupt increase in cancer incidence as well as mortality statistics. A burgeoning breast cancer crisis affects 588 million women beyond the age of 15 in these different countries (Alam et al., 2021).

Most women are victims of breast cancer. Approximately 25.2 out of 100,000 age-standardised incident rate and approximately 22.5 per 100,000 reported incidence (hospital-based) cases in Bangladesh in recent years. According to the IARC (2021, in 2018, about 12,765 women were diagnosed with breast cancer, and about 6846 women died from this disease in that same year. Another report says about 13,000 women are affected, and about 7000 deaths occur annually due to breast cancer in the women's population in Bangladesh. (Anadolu Agency, 2021).

Breast cancer has become much more prevalent throughout recent years; however, there is currently no national central cancer database that can offer exhaustive information on the entirety of the nation. Consequently, it is largely unreported what the actual rates and morbidity of breast cancer are. Nonetheless, according to GLOBOCAN, 4930 new instances of breast

cancer have been documented during this time, per the National Institute of Cancer Research and Hospital's (NICRH) 2015–2017 cancer registry records. According to GLOBOCAN in 2020, 13,028 new instances were detected, leading to an age-standardized incidence rate (ASR) of 17 per 100,000.

According to *Breast Cancer Mortality Statistics* (2025), age has a significant correlation with breast cancer mortality, with older individuals having the greatest rates. Nearly half of all fatalities in the UK between 2017 and 2019 (48%) occurred among those 75 and older. The greater incidence and poorer likelihood of survival of breast cancer in the elderly are mostly responsible for this. For both men and women, the most elevated rates are found in the 90+ age range. The difference is greatest between the ages of 35 and 39, when the age-specific death rate for females is 475 times greater than that of males (*Breast Cancer Mortality Statistics*, 2025).

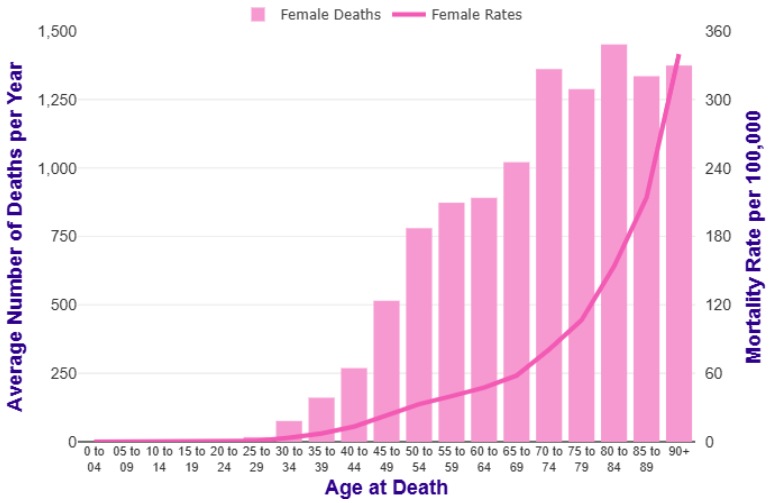

Figure 4: Breast Cancer (C50), Average Number of Deaths per Year and Age-Specific Mortality Rates per 100,000 Female Population, UK, 2017-2019 (*Breast Cancer Mortality Statistics*, 2025)

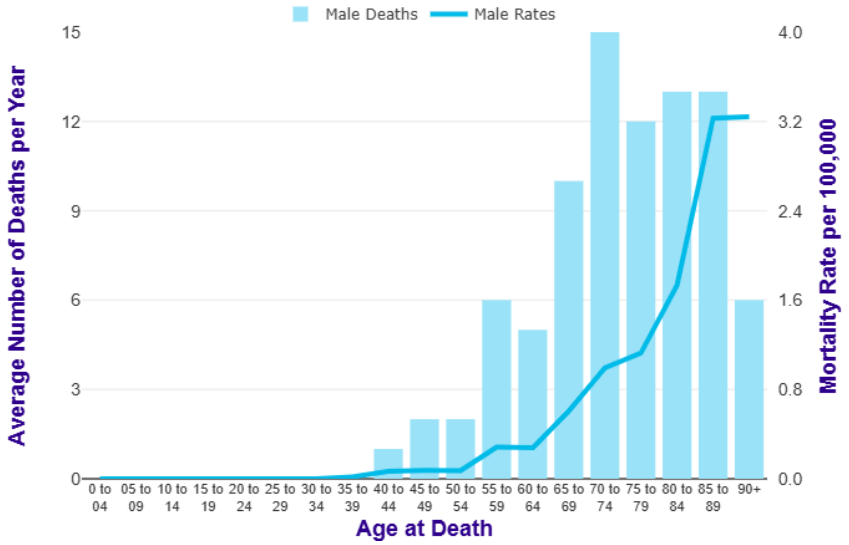

Figure 5: Breast Cancer (C50), Average Number of Deaths per Year and Age-Specific Mortality Rates per 100,000 Male Population, UK, 2017-2019 (*Breast Cancer Mortality Statistics*, 2025)

Therefore, it is imperative to examine the risk of breast cancer development in the Bangladeshi female population by examining the relationship between the *HER2* rs1136201 polymorphism and comparing healthy and cancer patients. This will allow researchers to determine whether the polymorphism is significantly present and whether it raises or reduces the risk of breast cancer using a variety of statistical analyses and a case-control design. Since ethnicity affects the risk of genetic factors, it is crucial to support this kind of research globally, including in Bangladesh.

Chapter 2

Materials & Methods

2.1 Materials

TaKaRa Taq DNA Polymerase Master Mix, restriction enzymes, and DNA ladder were obtained from TAKARA BIO INC. (Japan). Tris-borate-EDTA (TBE) buffer was purchased from Solarbio Life Sciences (China), and SeaKem® LE Agarose was sourced from Lonza (USA). Genomic DNA was extracted using a kit supplied by Favorgen (USA). Oligonucleotide primers were synthesized by Macrogen (Korea). Midori Green staining dye was obtained from Nippon Genetics (Europe).

2.2 Study Subjects

The 236 Bangladeshi women who participated in this case-control study between April 1, 2023, and June 30, 2023, comprised 124 healthy controls who had been assigned by age and gender and 112 breast cancer sufferers. People between the ages of 18 and 50 with a BMI between 18.5-24.9 kg/m² and serious neurological or physical conditions as determined by thorough laboratory, neurological, and physical examinations were not allowed to participate in the study. The study involved the random recruitment of age-matched healthy persons (controls) of Bangladeshi ethnicity and histopathologically proven breast cancer patients (cases). Every participant that was enrolled finished the trial; there were no attrition cases. There was no need for a formal power calculation because this was a pilot research. The National Cancer Research Institute Hospital in Mohakhali, Dhaka, was the source of the BC patients. For respondent evaluation, the study made use of well-equipped inpatient and outpatient settings. Based on predetermined diagnostic standards, a certified oncologist evaluated HCs and made diagnoses for BC patients. The blood specimens were analysed genetically, and clinical and demographic

information was gathered. Unbiased sampling was guaranteed by randomised selection, and bias was reduced by blinding laboratory staff to the case-control state. In Dhaka, Bangladesh, at the Rufaida BioMeds Laboratory, a genotyping analysis was performed. The authors were not given access to personally identifying information either during or after the data collection process in order to protect participant confidentiality.

2.3 DNA Extraction and Genotyping

Everyone who took part had their blood drawn into tubes that contained ethylenediaminetetraacetic acid. For a single nucleotide polymorphism (SNP) investigation, genomic DNA was extracted according to the manufacturer's instructions utilising a commercial genomic DNA extraction kit (Favorgen, USA). In short, the blood samples that were collected were lysed, and the resulting lysate was carefully poured into tubes that were bound with columns. After being cleaned with a variety of buffers, the DNA was eluted with water devoid of nuclease. The extracted DNA was kept in storage at -20°C.

1% agarose gel electrophoresis was used to evaluate the purity of the extracted DNA. Tetra-primer amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR) method was conducted for genotyping of the *HER1*-rs11543848 and *HER2*-rs1136201 polymorphisms, as previously optimized in the laboratory. Outer and inner primer sequences (forward and reverse) (Table 1) were extracted from the publication by Sombal et al. (2024) to identify homozygous and heterozygous alleles [17].

Through electrophoresis using a 100 bp DNA ladder, the amplicons were verified by amplification products on a 2% agarose gel dyed with Midori Green (Figure 1). 25% of the sample population as a whole underwent double testing to guarantee the accuracy of the findings.

Gene, SNP	Primers' sequence	Thermal condition	Fragments, BP
HER2 rs1136201	Forward outer: 5'-GAGCCAAGGCAGGTTTTAGAG-3' Reverse outer: 5'-TCTGCGCCTGGTTGGG-3' Forward inner: 5'-GCCCTCTGACGTCCATCG-3' Reverse inner: 5'-GCCAACCACCGCAGAGAT-3'	5 min of denaturation at 98°C, then 30 cycles of 98°C for 40s, 55°C for 30s, and 72°C for 60s. The final cycle had a 5 min extension at 72°C	A-allele: (195 bp) G-allele: (269 bp)

- SNP stands for single-nucleotide polymorphism
- BP stands for base pairs

Table 1: Primer Sequences, Thermal Conditions, and Amplified Products' Size

For ensuring quality of the process, random sample of 25% of the total sample population was tested for reproducibility of genotyping results, revealing 100% concordance between original and repeated genotyping.

2.4 Statistical Analysis

R software (version 4.4.1; R Foundation for Statistical Computing, Vienna, Austria; https://www.r-project.org/) was used to conduct the statistical analysis. This 'Hardy-Weinberg' principle was used to test the Hardy-Weinberg equilibrium. The connection between categorical variables across several genotype groups was examined using Pearson's Chi-square tests. Age and BMI were included as factors in multivariate logistic regression models to compute odds ratios (ORs) with 95% CIs.

Chapter 3

Result

3.1 Overview

In this chapter, the most important findings of the research that was dedicated to an investigation of the relationship between the *HER2* gene single-nucleotide polymorphism (SNP) - rs1136201 and the susceptibility of breast cancer in Bangladeshi women are outlined. The findings are categorized into three major segments, which are the demographic profiles of the research respondents, the testing of the Hardy-Weinberg and the compliance with Hardy-Weinberg equilibrium, and the evaluation of the association between *HER2* polymorphism and breast cancer with different genetic models

3.2 Demographic Characteristics

The entire study entailed 236 female participants, who were split into two groups: 112 cases of histopathologically confirmed patients with breast cancer and 124 age-matched healthy individuals who acted as controls. The average age among the cases (Table 3) was 46.9 ± 12.5 years, and that of the controls was 47.8 ± 13.1 years, with no significant difference between the two groups ($p = 0.58$). In the same vein, the mean body mass index (BMI) in the case and control groups was 25.7 ± 4.9 kg/m² and 25.2 ± 5.1 kg/m², respectively, with no significant variance ($p = 0.32$).

Occupations were divided into housewives, working women, and students. In the two groups, most of the participants were working women, and there was no statistically significant difference in the occupation of cases and controls ($p = 0.46$). The mean number of children participating was also similar between Cases (2.13 ± 0.91) and controls (2.09 ± 0.87), with a p-value of 0.45, and this difference was non-significant.

These demographic factors indicate that there was good matching between the two groups, which made it less likely that confounding variables might have come into play in this genetic association study.

Variable	Cases (n=112)	Controls (n=124)	p-value*
Age (years)	46.9 ± 12.5	47.8 ± 13.1	0.58
BMI (kg/m²)	25.7 ± 4.9	25.2 ± 5.1	0.32
Occupation			0.46
Housewife	38 (33.9%)	44 (35.5%)	
Working Women	68 (60.7%)	73 (58.9%)	
Student	6 (5.4%)	7 (5.6%)	
Number of Children	2.13 ± 0.91	2.09 ± 0.87	0.45

Table 2: Demographic Characteristics of Breast Cancer Cases and Healthy Controls

- Data are shown as mean ± SD (median) for continuous variables and % (n) for categorical variables.
- *p-values: t-test for continuous variables (age, BMI, number of children); Chi-square test for occupation.

3.3 Genotype Distribution & Hardy-Weinberg Equilibrium

To identify the genotype frequencies of *HER2* rs1136201 SNP in the case and control, the Tetra-primer ARMS-PCR approach was used and the results were visualized via agarose gel electrophoresis at a concentration of 2%. There were genotypes observed; AA (common homozygous), AG (heterozygote), and GG (Rare homozygote).

Of the 112 participants affected with breast cancer; 24 (21.43%) had the AA genotype, 84 (75.00%) had the AG genotype and 4 (3.57%) had the GG genotype. Of the 124 individuals in

the control group, 29 were AA (23.39%), 90 were AG (72.58%) and 5 were GG (4.03%).

Chi-square goodness-of-fit tests were done, separately in cases and in controls, to determine whether the genotype distribution complied with the concept of Hardy-Weinberg equilibrium (HWE). The findings referred to the two groups being in HWE with a p-value exceeding 0.05 (cases: $p = 0.5827$; controls: $p = 0.6114$). This helps in the legitimacy and reproducibility of the genotypic information (Wigginton et al., 2005).

3.4 Association Between *HER2* (rs1136201) Polymorphism & Breast Cancer

Investigating the interaction between the *HER2* rs1136201 SNP and breast cancer risk, some genetic models were used, including the additive model, dominant model, recessive model, and overdominant model. The odds ratios (ORs) with 95% confidence intervals (CIs) and corresponding p-values of each model are shown in Table 2. These genetic models also give the results graphically in the forest plot (Figure 1), which can be used as a comparison of the confidence intervals and effect sizes.

Allele	Cases (n = 112)	HWE		Controls (n = 124)	HWE		Genetic models	OR	95% CI	*P*-value
		X^2	*P*		X^2	*P*				
AA Common homozygote	(n=24; 21.43%)	0.3018	0.5827	(n=29; 23.39%)	0.2582	0.6114	Additive model 1 (AG vs. AA)	1.1278	0.608 - 2.091	0.703
							Additive model 2 (GG vs. AA)	0.9667	0.233 - 4.006	0.963
AG Heterozygote	(n=84; 75%)			(n=90; 72.58%)			Dominant model (AG+GG vs. AA)	1.1193	0.606 - 2.068	0.719
GG Rare homozygote	(n=4; 3.57%)			(n=5; 4.03%)			Recessive model (GG vs. AG+AA)	0.8815	0.231 - 3.368	0.854
							Over dominant model (AG vs. GG+AA)	1.8333	1.024 - 3.281	0.041
***p*>0.05 obeyed Hardy-Weinberg equilibrium**										

Table 3: Association between Breast Cancer with *HER2* (rs1136201) Polymorphism

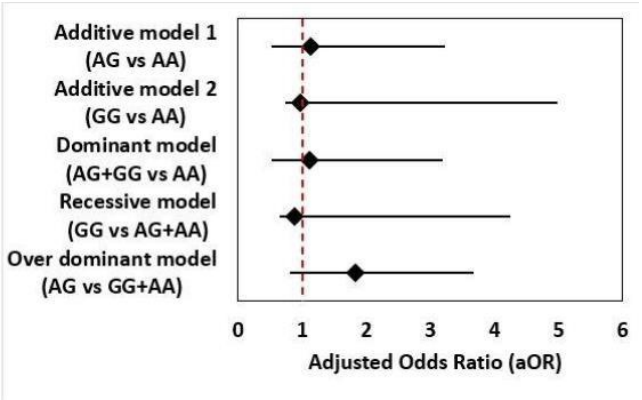

Figure 6: Forest Plot. Association between Breast Cancer with *HER2* (rs1136201) polymorphism

3.5 Genetic Inheritance Models

In the additive model, where each genotype was compared with AA genotype as a reference, both comparisons did not show statistically significant differences between rare homozygous and common homozygotes; AG versus AA: OR 1.1278 (95% CI: 0.608-2.091; p = 0.703), GG versus AA: OR 0.9667 (95% CI: 0.233-4.006; p = 0.963). In the same direction, the influential model, which synthesized AG genotypes and GG and compared it with AA, did not show a significant relation to breast cancer (OR = 1.1193; 95% CI: 0.606-2.068; p = 0.719). The recessive model (comparing GG to AG+AA) also showed no significant association with breast cancer risk (OR = 0.8815, 95% CI: 0.231-3.368; p = 0.854). The over-dominant model, however, yielded statistically significant results, with the AG genotype compressed against the AA+GG, which resulted in an OR of 1.8333 (95% CI: 1.024-3.281; p = 0.041), indicating that heterozygotes were more likely to be exposed to breast cancer. This observation represents overdominance, in which the two alleles have modified the expression or functionality of the gene to differ with the expression of either of the alleles alone, possibly through gene-gene or gene-environment interactivity unique to this population (Kardia et al., 2003).

3.6 Gel Electrophoresis Confirmation

The representative gel electrophoresis images (Figure 2) proved that the HER2 rs1136201 polymorphism has been successfully amplified. Consistent with the predicted allele-specific amplicons were the band size associated with the A-allele (195 bp) and the G-allele (269 bp).

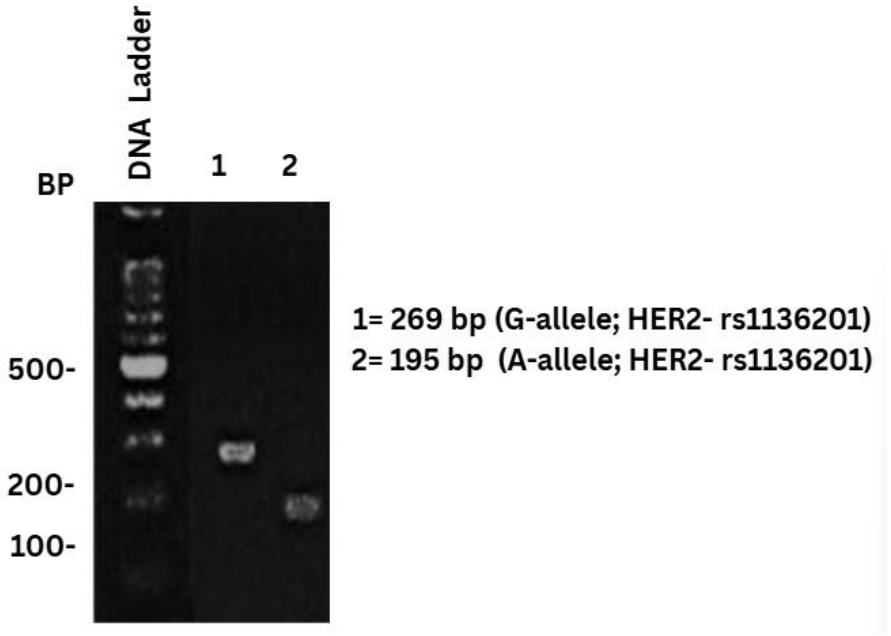

Figure 7: Representative image of agarose gel electrophoresis

The genotyping quality was also supported by the fact that all the samples produced clear bands, and the background was minimal.

3.7 Summary of Findings

HER2 rs1136201 polymorphism was not significantly related to an increase in BC risk using additive, dominant, or recessive models. Nonetheless, a significant relationship was recorded under the overdominant model, where the AG genotype was found to have a high probability of breast cancer relative to the homozygous types. There were no deviations in HWE in the control group, and the technical reliability of the genotyping process was high. Considering these results, the role of *HER2* polymorphism in the predisposition of breast cancer among Bangladeshi women can receive partial confirmation.

Chapter 4

Discussion

The research study evaluated the role of *HER2* gene polymorphism (rs1136201) in relation to breast cancer susceptibility in a Bangladeshi case-control study of the female population. The genotype was determined by Tetra-primer ARMS-PCR, and the alleles were analyzed under different models of genetics. Although results do not reveal statistically significant associations in most of the models, statistically significant results were obtained under the overdominant model, where the heterozygous AG genotype recorded a likelihood of the occurrence of breast cancer. This chapter addresses the importance of the findings, their relation to the literature, as well as the potential effects regarding biology and the shortcomings of the study, as well as recommended future studies.

4.1 Interpretation of Key Findings

The overdominant model showed that the AG genotype of *HER2* rs1136201 demonstrated a statistically significant correlation with the risk of developing breast cancer (OR = 1.8333, 95% CI = 1.024-3.281, p = 0.041), and individuals with the two-allele AG genotype had an increased risk compared to those with homozygous genotypes of AA or GG. This implies a possible heterozygote disadvantage, or overdominant effect, in which the expression of both alleles potentially interferes with normal *HER2* activity, perhaps by affecting receptor activity or expression levels.

Conversely, the additive (AG vs. AA, GG vs. AA), dominant (AG + GG vs. AA) and recessive (GG vs. AG + AA) all yielded no statistically significant results (all $p > 0.05$). These results suggest that the heterozygous genotype has an exclusive relationship, not a linear rise or fall of the risk [proportional to the number of G alleles]. This is agreeable with the fact that certain

polymorphisms do not act in additive modes i.e. through gene-gene interactions, or allele-specific regulation mechanisms (Cordell, 2009).

4.2 Comparison with Previous Studies

HER2 (alternatively known as ERBB2) is a proto-oncogene that is known to have an essential role in breast cancer development, particularly in the *HER2*-positive types of breast cancers, where it overexpresses the *HER2* receptor (Slamon et al., 1987). It is an A>G substitution leading to an amino acid change in lysine 655 by valine that resides within the transmembrane domain of the *HER2* receptor (rs1136201 or Ile655Val). It has also been postulated that this structural alteration may influence the stability and dimerization of receptors, which may influence downstream signaling pathways that result in cell proliferation and survival (Xie et al., 2000).

Analysis of various groups of people has given conflicting results as far as the relationship between rs1136201 and breast cancer is concerned. To illustrate, a meta-analysis carried out by Li et al. (2016) revealed that the G allele (Val) was linked to a slight increase in the risk of breast cancer and especially among the Asian community. However, additional research studies like the one done on Sombal et al. (2024) in a South Asian cohort found no significant correlation in any of the genetic models, indicating the lack of significance of the results of this study in their additive, dominant, and recessive models.

The overdominance effect that was observed in this work correlates with a limited number of population-specific results. To illustrate the point, Saeed et al. (2017) noticed that the heterozygous AG genotype in Pakistani cohorts was much more prevalent in individuals with breast cancer, indicating the presence of a potential regional or ethnic factor that plays an influential role in genetic susceptibility. The possibility of observing a context-dependent

effect of the heterozygous genotype is feasible and may be caused by environmental factors, way of life, or interaction with some other genetic variants that are common in the Bangladesh population.

4.3 Biological Implications

The overdominant effect of association in this study demonstrates that there might have been an allelic interaction effect in the *HER2* receptor region. The Ile655Val mutation is in a nonpolar area that is essential to dimerization in the receptor. The heterozygosity resulting from this locus can have a consequence on conformational dynamics of the *HER2* protein, leading to a possible effect on the efficiency of signal transduction. It has been previously reported that this structural difference could influence the membrane anchoring and internalization of the receptor (Wang et al., 2011). Hence, the AG genotype could possibly be a biologically unstable intermediate form, augmenting the susceptibility of cells to oncogenic transformation.

Moreover, traditionally, the between-SNP association of rs1136201 does not imply a functional effect of this polymorphism, but it could interact with other polymorphisms or upstream regulatory sequences. As *HER2* signaling is broadly characterized by an intricate crosstalk with pathways such as PI3K/AKT and MAPK, the presence of the AG genotype could alter associated networks compared to homozygous alleles and cause aberrant proliferation of cells in some situations (Arteaga et al., 2011).

4.4 Strengths of the Study

The current research is supported by a few strengths. First, this case-control design using age-matched healthy controls was a way of reducing confounding effects. Second, the T-ARMS-PCR approach facilitated a reliable and affordable rapid genotyping procedure that was confirmed through gel electrophoresis, as well as repetition of all the tests on a fourth of the

samples, which were in full concordance. The Hardy-Weinberg equilibrium analysis established that the control group had the natural distribution of alleles, minimising the probability of selection or genotyping bias.

4.5 Limitations

The study has certain shortcomings despite its strong points that the researcher has to consider. First, the smallish sample size (112 cases and 124 controls) could have contributed to the lack of statistical power, particularly in the case of identifying associations among the homozygous types (GG). The sample would have to be enlarged to enhance the degree to which OR is reliable and may indicate relationships that could not have been statistically significant in this study.

Second, the research excluded the analysis of other SNPs of the *HER2* gene. Since the genetics of breast cancer may be complicated, it is probable that other polymorphic molecules in *HER2* or related genes can be involved as part of the risk. Also, the genetic risk interacted with environmental and lifestyle influences (e.g., diet, hormonal status, family history) that were not controlled or analysed, which might have affected the outcomes.

Lastly, functional assays were not employed to determine whether the SNP influences *HER2* expression (biological effect) or activity. Although the genotyping data give a statistical association, at this stage, there is no ability to make deductions about the mechanism, since the genotyping data cannot be used as molecular evidence.

4.6 Implication & Future Direction

This result, that there is a paramount connection between AG genotype and breast cancer under the overdominant model, augments the expanding data of genetic vulnerability among South

Asian groups of people. The finding should be explored using replication in bigger studies and in multi-centers in Bangladesh and other surrounding countries.

A multi-gene approach, taking into account other SNPs in some pathways related to oncogenesis (e.g., *EGFR, BRCA1/2, TP53*) to reflect the polygenic nature of breast cancer risk, is also a question of future research. Also, gene-environment interaction analysis might be used to add further understanding of the origin of the genetic predisposition as contributed to by the alterable lifestyle choices.

At the molecular level, functional studies including profiling of gene expression, protein modelling, and in vitro assessment should be carried out on how the AG genotype affects the dynamics of the *HER2* receptor and signalling. These studies may form the basis of more individualized preventative risk assessment and intervention in the female population of Bangladesh.

Chapter 5

Conclusion

The study investigated the correlation between the *HER2* gene polymorphism (rs1136201) and breast cancer predisposition among Bangladeshi women. The study used Tetra-primer ARMS-PCR genotyping and statistical analysis to test the genotype distribution in 112 breast cancer patients and 124 healthy controls. The results showed that the risk of breast cancer does not depend on the *HER2* rs1136201 polymorphism. However, a significant correlation was found in the overdominant model, where subjects with the AG genotype had an increased risk of breast cancer development compared to those with either the AA or GG genotypes. This suggests that the interaction between the A and G alleles may play a role in the functionality of the *HER2* receptor, which plays a role in oncogenesis. The robustness of the overdominant effect was confirmed by the Hardy-Weinberg equilibrium among the control group. The study highlights the potential of the *HER2* rs1136201 polymorphism, particularly the heterozygous AG, in breast cancer susceptibility in female Bangladeshis. The findings underscore the need for population-specific studies to identify genetic markers for early detection procedures and personalized treatment packages. Future studies could involve larger and more varied cohorts and include further functional tests.

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