Assessing the Prevalence and Impact of Leptin Receptor (Q223R) Polymorphism in Bangladeshi Breast Cancer Patients: A Case Control Study.

By

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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

School of Pharmacy
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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

The thesis titled "Assessing the Prevalence and Impact of Leptin Receptor (Q223R) Polymorphism in Bangladeshi Breast Cancer Patients" submitted by Sheikh Shajia Islam Shithi (20346047), of Spring, 2024 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of Bachelor of Pharmacy.

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Ethics Statement

This study was conducted in accordance with rigorous ethical guidelines to safeguard the safety, rights, and well-being of all participants. The Institutional Review Board (IRB) of BRAC University has given approval of the request (IRB No BRACUIRB120220005) in accordance with ethical principles. A thorough assessment of the research proposal was done by IRB to confirm that the study was compatible with all applicable ethical standards and regulatory requirements.

Before participating in this study, each subject gave their informed consent. The purpose of the study, methods, potential risks and benefits all were informed completely to the participants. Additionally, It was assured to the participants that their involvement was completely voluntary and flexible enough to leave the project at any point without fear of consequences.

The investigation's methods and procedures used in this study strictly followed the rules and regulations that IRB has approved. This included the careful handling of biological specimens, secured storage of data, assurance of anonymity of participants and complete compliance with the protocols aimed at reducing potential risks to the participants. Moreover, the study ensured that all collected data was anonymized enough to safeguard privacy, thereby merely used to achieve the study's goals.

Abstract

Breast cancer is the leading cause of mortality among women globally, including in Bangladesh, which reports over 13,000 cases annually. This study inquired about the recurrence and impact of the LEPR (Q223R) polymorphism in patients of breast cancer from Bangladesh. The leading purpose of this research is evaluating the repetition of this genetic variation in individuals with breast cancer and healthy controls groups, looking into its probable impact on the likelihood of developing cancer. PCR-RFLP method was utilized to perform genotyping of 10 breast cancer patients and 10 healthy controls. The final outcomes showed that the GG genotype was prevalent in both groups, and no substantial association was detected between the (Q223R) polymorphism and an increased hazard of breast cancer. This study aids in understanding the genetic elements that lead to the onset of breast cancer and emphasizes the necessity for additional studies to inspect the role of the LEPR (Q223R) variant in this subset.

Keywords: LEPR; Genetic Susceptibility; LEPR Q223R Polymorphism; Breast Cancer; PCR-RFLP;

Dedication

I would like to dedicate this thesis to my beloved parents whose firm support, unwavering love and selflessness have always boosted me up to reach all of my goals. Their steadfast confidence in me has been the main catalyst in my journey.

To my husband, whose unconditional support, encouragement and patience have been the foundation of my strengths and dedication throughout this endeavor.

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List of Acronyms

IARC	International Agency for Research on Cancer
STAT3	Signal Transducer and Activator of Transcription 3
MAPK	Mitogen-Activated Protein Kinase
RFLP	Restriction Fragment Length Polymorphism
CDK2	Cyclin-dependent kinase 2
SNP	Single nucleotide polymorphisms
NCoA	Nuclear Receptor Coactivator
EDTA	Ethylenediaminetetraacetic Acid
LEPR	Leptin Receptor
OR	Odds Ratio
PCR	Polymerase Chain Reaction

Chapter 1

Introduction

Breast cancer is the most prevalent malignancy among women, ranking second globally in incidence and fifth in mortality (Lei et al., 2021). Every year almost 10% of patients are diagnosed with this (Wilkinson & Gathani, 2022). Without causing noticeable symptoms breast cancer consistently progresses. In 2018, an anticipated 9.6 million fatalities contributed to a global cancer burden of 18.1 million new cases in previous years (Hafez & Mohamed, 2022). The International Agency for Research on Cancer (IARC) indicates that annually, over 13,000 women in Bangladesh are diagnosed with breast cancer, with more than 7,000 succumbing to the illness (Howlader et al., 2020). The International Agency for Research on Cancer (IARC) reported 12,764 new cases of breast cancer in Bangladesh in 2018 (Tasdida Shamsi, 2021).

By the end of 2015, breast cancer had surpassed all other cancers in Bangladeshi women (Begum et al., 2019). 69% of women in Bangladesh have passed away as a direct result of this, which has placed a significant burden on the country's healthcare facilities (Sarker et al., 2022). Breast cancer affects 22.5 out of every 1,00,000 women in Bangladesh (Begum et al., 2019). This numbers the number of women that are affected by the disease. If we compare this cancer to others, we see that it disproportionately affects women 15–44 years old (Tasdidaa Shamsi, 2021).

Women are diagnosed with breast cancer, the most frequent type of cancer. Breast cancer is the most frequent sort of cancer and is something that one has a lifetime chance of having. Despite being a rather prevalent disease, the precise causes of breast cancer are not fully known. Numerous factors, such as the age at which a woman enters menstruation for the first time (menarche) and the age at which she enters menopause, dietary habits, reproductive history,

oestrogen consumption, and genetic predispositions, have been proposed as potential risk drivers (Sun et al., 2017). A higher body weight also seems to be linked to the development of mammary or breast tumors (Cleary et al., 2010). Researchers have asserted that women who have gone through menopause are more likely to get breast cancer if they are obese (García-Estévez et al., 2021). Four main ways that being overweight or obese is connected to getting breast cancer are through leptin and its receptors, fatty tissue inflammation that lasts for a long time, changes in sex hormones, and communication through insulin and IGF-1. These pathways contribute to an increase in the amount of the adipokine leptin that is found in white adipose tissue. Additionally, these mechanisms contribute to the promotion of low-grade chronic inflammation (Snoussi et al., 2006). This causes an imbalance of energy and sets off signalling pathways that make cytokines and inflammatory mediators (Atoum & Hamaid Alparrey, 2022).

A key role in the regulation of body weight is played by the hormone leptin, which has a molecular weight of 16 kilodaltons and is mostly produced by white adipose tissue. Leptin has an effect on the amount of food that is consumed as well as the amount of energy that is utilized (Fantuzzi, 2005). Leptin not only regulates body weight, but also has an impact on hematopoiesis, reproduction, angiogenesis, and immunological functions (Zhang & Scarpace, 2006). In a successful endeavor, Zhang and Scarpace were able to effectively clone and sequence the leptin gene, which is the human equivalent of the rat obese gene (OB). This particular gene can be found on chromosome 7q31.3 as well as the production of a messenger RNA with 4.5 kilobases in adipose tissue (Snoussi et al., 2006). The leptin receptor, a member of the class I cytokine receptor family, is crucial for regulating the physiological effects of leptin. The presence of the leptin receptor was first recognized in the brain. Further study has indicated that leptin is also present in other organs, including the liver, immune cells, placenta, endometrium, and stomach (Lei et al., 2021). Furthermore, Ob-R expression has been identified in pathological tissues, including brain and pituitary tumors, hepatocellular carcinoma, gastric

cancer cells, and breast cancer (Jardé et al., 2008). In addition, leptin is involved in the epithelial-mesenchymal transition, which facilitates the movement and dissemination of cancer cells (Krause et al., 2012). Moreover, leptin alters the functions of insulin-like growth factor-I, HER2/neu, and epidermal growth factor receptor (Ray et al., 2007). The initiation, development, and dissemination of breast cancer are all linked to the leptin-mediated signaling network. Elevated blood levels of leptin or the expression of leptin receptors are correlated with lymph node involvement, metastasis, and a poor prognosis (Atoum & Hamaid Alparrey, 2022).

Moreover, Leptin facilitates the proliferation of both estrogen-dependent and estrogen-independent breast cancer cells. Research indicates that leptin concentrations of 25–100 ng/ml activate the STAT3 (Signal Transducer and Activator of Transcription 3) and MAPK (Mitogen-Activated Protein Kinase) pathways, facilitating the proliferation of T47D breast cancer cells and non-transformed HBL100 mammary epithelial cells (Cirillo et al., 2008). Leptin does not facilitate anchorage-independent proliferation in HBL100 cells. Cell lines expressing estrogen receptors (MCF7, T47D, MDAMB361) and those lacking them (MDAMB231, SKBR3) are both encouraged to proliferate by leptin (Cirillo et al., 2008).

STAT3 interacts with nuclear receptor coactivator (NCoA) 1, recruiting transcriptional activators to gene promoters to boost cell development, according to one study (Takahashi et al., 2017). Through STAT3 and histone acetyltransferase activity, leptin also upregulates cyclin D1 and transactivates its promoter (Saxena et al., 2007). The advancement of the cell cycle stimulated by leptin correlates with elevated amounts of cyclin-dependent kinase 2 (CDK2) and cyclin D1, with the inactivation of the cell cycle inhibitor retinoblastoma protein (pRb) via excessive phosphorylation (Andò & Catalano, 2012). Leptin possesses the unique capacity to not only promote cell proliferation, but also induce cellular metamorphosis in T47D breast

cancer cells, a phenomenon that is absent in typical mammary epithelial cells (Garofalo et al., 2006).

There are a number of genes that are involved in the expression and regulation of leptin. Variations in these genes have the potential to change the levels of leptin and/or the activity of leptin at the receptor location. The LEPR polymorphism Q223R (rs1137101) is a frequent variant that is thought to be associated to a lower ability of the leptin receptor to transfer signals, which is of functional significance. This is a condition that has been linked to a number of different diseases. It has been established that the presence of homozygous variation AA is associated with higher levels of leptin in the blood circulation (Dallal et al., 2013).

The location of the LEPR gene is on chromosome Ip31 in humans. Recently there has been widespread inquisitions into the LEPR gene to unlock any variants that may have a factual impact on the development of human obesity. The LEPR gene has been exhibited to possess SNPs (single nucleotide polymorphisms), among which is a specific one called Q223R. Within the region of the leptin receptor the Q223R polymorphism is a situation which encodes the extracellular domain. This polymorphism turns into the substitution of glutamine with arginine at the place of LEPR protein. The Q223R polymorphism is connected to a decline in the efficiency of leptin to attach, resulting in the development of leptin resistance (Becer et al., 2013). The binding of leptin to its receptor is disrupted by the Q223R polymorphism. There is a substitution of a nucleotide with a G nucleotide at exon 6, more precisely at nucleotide 668 and codon 223 of the start codon. This is the effect of the substitution (Atoum & Hamaid Alparrey, 2022).

The fundamental objective of this research project, which is titled "Assessing the Prevalence and Impact of Leptin Receptor Q223R Polymorphism in Bangladeshi Breast Cancer Patients," is to establish a connection between the Q223R polymorphism in the leptin receptor (LEPR)

gene and a healthy control group that is comprised of breast cancer patients. Besides, the study is aimed at addressing the probable impact of this genetic variation on the vulnerability and advancement of breast cancer in the population of Bangladesh. This project pursues to offer widespread genetic analysis which can help to identify the causative factors for breast cancer by utilizing the Restriction Fragment Length Polymorphism (RFLP) process. Furthermore, the objective of this project is to emhance the consciousness of the reasons associated with breast cancer among women, thereby performing initial detection and prevention measures. By recognizing genetic markers such as the Q223R polymorphism, the research can provide information about personalized screening methods and preventive measures, thereby expanding elevated health outcomes of women in Bangladesh.

Chapter 2

The Methods and the Materials

2.1 Approach Participants and Ethical Considerations

There was a total of 20 female participants who took part in this investigation. Among them 10 being breast cancer patients and another 10 being healthy control groups. IRB (Institutional Review Board) of BRAC University has granted ethical approval of this study as a B. Pharm. final year thesis project. Each and every individual was provided with comprehensive information regarding the objectives and procedures of the study before they agreed to take part in it.

2.2 Collection of Sample

The National Cancer Research Institute Hospital in Mohakhali, which is located in Dhaka, was the location where samples of breast cancer cases were collected in the year 2022. All the breast cancer patients, who were confirmed through histological examination, came from different parts of Bangladesh. Demographic and lifestyle data, such as age, weight, height, and smoking status, were collected through comprehensive personal interviews conducted by two skilled nurses after all diagnostic tests were finished. The control group consisted of 10 healthy females from various regions of Bangladesh, all of whom had no previous incidence of breast cancer. All participants were mandated to fulfill the subsequent inclusion criteria: a confirmed diagnosis of breast cancer (for the patient cohort), lack of any familial history of breast cancer, a minimum age of 18 years, and identification as female. The exclusion criteria encompassed individuals who were below 18 years old, or had significant physical ailments, and were either pregnant or had other concurrent problems.

2.3 Data Documentation

A standardized questionnaire was utilized to thoroughly detail the features of both cases and controls. This encompassed comprehensive data regarding the individual's medical history, demographic characteristics, and lifestyle behaviour.

2.4 Procedures Conducted in a Laboratory

Genomic DNA Extraction

Peripheral blood samples were acquired through venepuncture and collected in sterile vacutainer tubes with 3 ml of blood and ethylenediaminetetraacetic acid (EDTA)-Na2 as an anticoagulant. The FavorPrep™ Blood Genomic DNA Extraction Kit (Favorgen Biotech Corporation, Taiwan) was utilized to extract genomic DNA from blood samples in accordance with the manufacturer's instructions. The obtained DNA was preserved at a temperature of 4°C until it could be further analyzed.

Analysis of PCR-RFLP

For the purpose of carrying out the procedure of genotyping the LEPR (Q223R) polymorphism, the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) instrument was applied. The type of primers implemented here is mentioned below-

- Sense: 5'-ACCCTTTAAGCTGGGTGTCCCAAATAG-3'
- Anti-sense: 5'-AGCTAGCAAATATTTTGTAAGCAATT-3'

The installations of polymerase chain reaction (PCR) amplification are given below-

● **Initial denaturation:** A preliminary denaturation phase commenced at 94°C for 4 minutes.

● **Protocol of thermal cycling:** Denaturation was conducted for 40 cycles at a constant temperature for 30 seconds, while annealing occurred at 58°C for 30 seconds, and extension was executed at 72°C for 30 seconds.

● **Final extension:** Final extension was achieved by maintaining a temperature of 72°C for 10 minutes.

The PCR results were examined using electrophoresis on a 1% agarose gel to confirm the successful amplification of the targeted fragments. Following the completion of the polymerase chain reaction (PCR), the samples were subjected to digestion using the MspI restriction enzyme (New England Biolabs), which specifically targets the LEPR gene. The digestion process was conducted for 16 hours, subsequently followed by electrophoresis on a 3% agarose gel to isolate the fragments.

By analyzing the following band patterns, the Q223R polymorphism was uncovered-

- The AA homozygous genotype is defined by the presence of two bands at 291 bp and 125 bp.
- The AG heterozygous genotype is defined by the presence of three bands at 416 bp, 291 bp, and 125 bp.
- The GG homozygous genotype is defined by the presence of a single band at 416 bp.

The genotypes of breast cancer patients and control participants were determined by analyzing the digestion patterns found on the agarose gel.

Statistical methods to do quantitative analysis of data

For the purpose of carrying out the statistical analysis, the computer program Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago, Illinois) was utilized. In order to compare the distribution of Q223R LEPR polymorphisms between individuals who have breast cancer and individuals who do not have breast cancer, the Chi-squared test was utilized. Odds ratios (OR) and confidence intervals (CI) with a 95% level of certainty were utilized in order to evaluate the connection between the case group and the control group.

Additionally, a one-sample t-test was utilized in order to ascertain the average levels of leptin in breast cancer patients who possessed the Q223R LEPR polymorphism. This was done in order to differentiate between persons who were obese and those who were not fat. It was determined that a p-value that was lower than 0.05 was statistically significant.

Chapter 3

Result

Every genotype falls within the range of the Hardy-Weinberg equation, which has a p-value of 1 and a Chi square value of 0.0277 (Table 1).

Table 1: In the control group, the results of the Hardy-Weinberg equilibrium test for the MspI polymorphism and its respective genotype are presented.

Genetic Constitution	Observed value	Expected value	X^2	p-value
AA	0	0.025	0.0277	1
AG	1	0.95		
GG	9	9.025		
total	10	10		

This test was a Chi square (X2) analysis. P-values that were less than 0.05 were regarded as statistically significant.

The frequencies of the AA, AG, and GG genotypes (0, 10, and 90, respectively) among females with breast cancer are presented in Table 2. The absence of counts for the AA genotype rendered the calculation of the odds ratio infeasible for AA as a reference in a conventional manner. No statistically significant difference was seen between breast cancer (n=10) and

control (n=10) for the GG genotype (OR=1.0; 95% CI=0.054 to 18.575; p=1) in comparison to the AG genotype. The extensive confidence interval signifies considerable uncertainty, a frequent occurrence with limited sample sizes.

Table 2: Comparison of Genotype Frequencies, Odds Ratios, and 95% Confidence Intervals between Breast Cancer Cases and Controls.

SNP/Allele	Case in percentage (%)	Control in percentage (%)	OR (95%CI)	p-value
AA	0 (0%)	0 (0%)	Ref	1
AG	1 (10%)	1 (10%)	N/A	1
GG	9 (90%)	9 (90%)	1.0 (0.054 - 18.575)	

AA serves as the reference genotype; a p-value of less than 0.05 was deemed statistically significant.

The p-values in the table indicate that none of these results are statistically significant at the 0.05 level.

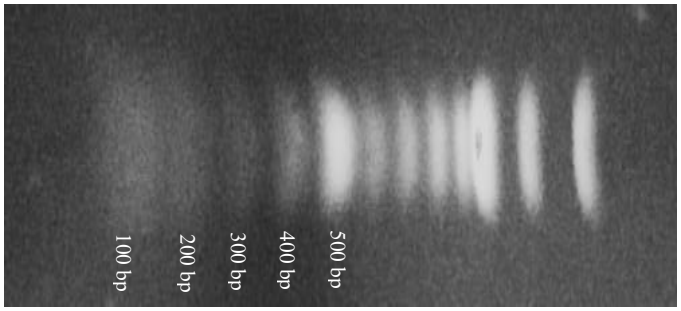

Fig 1: Image of gel electrophoresis depicting a DNA ladder (100 bp)

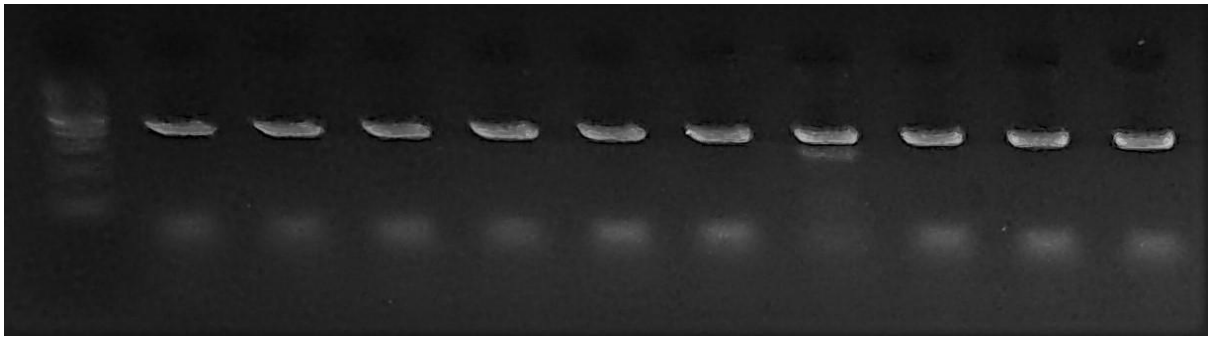

Fig 2: Image of gel electrophoresis illustrating the LEPR Q223R gene polymorphism identified in the Breast Cancer (Case) group post-digestion.

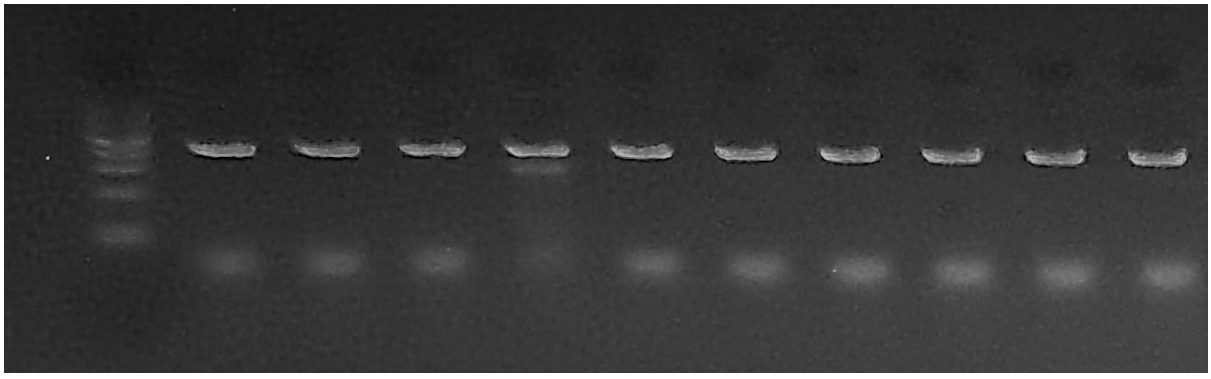

Fig 3: Image of gel electrophoresis illustrating the LEPR Q223R gene polymorphism identified in the Healthy Volunteer (Control) group post-digestion.

Chapter 4

Discussion

The objective of the research project that was given the title "Assessing the Prevalence and Impact of Leptin Receptor Q223R Polymorphism in Bangladeshi Breast Cancer Patients " was aimed to ascertain the presence or absence of the Q223R polymorphism in the leptin receptor (LEPR) gene is present among women in Bangladesh and to investigate whether or not it is associated with breast cancer. For the aim of providing dynamic information into the genetic determinants that influence breast cancer susceptibility and development in this particular cohort, the findings of this study contribute to a more comprehensive understanding of breast cancer genetics.

4.1 Prevalence of the LEPR Q223R gene polymorphism

The results that were obtained from the genotyping that was carried out in this study indicated that there was a considerable prevalence of the GG genotype in the case of breast cancer patients as well as in the healthy control group. Specifically, this particular gene was exhibited by 90% of the people in both groups. Amongst the two groups, AA genotype was notably absent but AG genotype was observed in 10% of the participants. Taking into consideration the distribution that has been presented here, it indicates that the Q223R polymorphism is not extremely prevalent in this population. The frequency of particular polymorphisms might vary greatly from one ethnic group to another, which is compatible with these findings, which are consistent with the observed genetic variance in distinct ethnic groups.

4.2 Correlation with the probability of developing breast cancer

The statistical analysis revealed no significant link between the Q223R polymorphism and the likelihood of getting breast cancer in the studied population. The odds ratio (OR) for the GG genotype relative to the AG genotype was 1.0 (95% confidence interval [CI]: 0.054 to 18.575; p=1), indicating no increased risk linked to this genetic variation. The lack of the AA genotype in both the breast cancer patients and the control group prevented a direct comparison with this reference genotype, which complicates the interpretation of the results. The broad confidence interval indicates a significant amount of uncertainty resulting from the limited sample size, emphasising the necessity for conducting larger research to validate these results.

4.3 Biological Consequences of LEPR Polymorphism

Notwithstanding this fact, the study did not demonstrate any significant correlation between the LEPR Q223R mutation and breast cancer; still, the biological implications of leptin and its receptor in cancer formation remain highly intriguing. Fat cells produce the hormone leptin which has a very important role in metabolism, energy level and controlling hunger. Additionally, many physiological processes are linked with leptin which includes haematopoiesis, reproduction, angiogenesis, and modulation of immunological response.

The binding of leptin to its receptor, known as Ob-R, which belongs to the class I cytokine receptor family, is the mechanism by which leptin is able to exercise its regulatory effect. This receptor can be found in a wide number of tissues, such as the brain, liver, immune cells, placenta, endometrium, and stomach, among others. In addition to that, it can be found in tissues that are healthy, such as breast cancer cells. One of the many signaling pathways that are engaged as a result of the interaction between leptin and its receptor is the Janus kinase/signal transducer and activator of transcription (JAK/STAT) route. Another pathway that is activated is the mitogen-activated protein kinase (MAPK) pathway (Ben-Eliezer et al.,

2007). The most significant function of these pathways includes regulation of cell growth, specialisation, and viability.

4.4 Leptin and Breast Cancer

One of the most important factors in the development of cancer is the fact that leptin is responsible for facilitating the proliferation of breast cancer. Several distinct systems, some of which are dependent on estrogen and others of which are not dependent on estrogen, are responsible for the effects that this causes. Evidence suggests that leptin has the capacity to stimulate the proliferation of breast cancer cell lines that express estrogen receptors, including those that express estrogen receptors positively (MCF7, T47D, and MDAMB361) and those that express estrogen receptors negatively (MDAMB231, SKBR3) (Dubois et al., 2014). This is accomplished by leptin through the activation of important signaling pathways, such as STAT3 and MAPK, which play a significant role in the regulation of cell growth. Furthermore, leptin has the ability to interact with other carcinogenic factors, such as insulin-like growth factor-I (IGF-I), epidermal growth factor receptor (EGFR), and HER2/neu, which results in the acceleration of the growth of tumors.

The Q223R polymorphism in the LEPR gene, located on chromosome 1p31, causes the substitution of glutamine (Q) with arginine (R) at position 223 in the receptor's extracellular domain (Vauthier et al., 2012). There is a probability that this change will further cause leptin resistance by reducing its effectiveness of receptor signaling and binding. In case of human obesity, leptin resistance is present where increased levels of leptin in blood do not show the desired physiological responses. A significant correlation exists between obesity and postmenopausal breast cancer. Leptin resistance may enhances this chance by causing long-

term inflammation, messing up metabolic homeostasis, and changing sex hormone levels (Pérez-Pérez et al., 2020).

4.5 Limitations of the Study and Suggestions for the Future

When considering the results of this study, it is important to keep in mind that it may have some limitations. One of the most significant limitations of the study is that the sample size was rather small, which has an effect on both the statistical power of the study and the extent to which the findings may be practically applied. As the existence of AA genotype was not found in the population under investigation, it was quite complicated to carry out thorough investigation into all possible genotypic variants. Additionally, this study merely focused on Q223R polymorphism and it didn't concentrate on any other polymorphisms within the LEPR gene or any other genes that are involved in leptin signaling that might be significant.

In further studies, it is consulted that the study should have the objective of adding a larger and more diversified cohort with the aim of improving the vigor and validity of the findings. It may be possible to obtain a more thorough understanding of the genetic factors that contribute to breast cancer susceptibility by expanding the scope of the genetic research to include more polymorphisms and genes that are involved in the leptin signaling pathway. Furthermore, the combination of genetic information with other factors that may be responsible for the development of breast cancer, such as environmental exposures, lifestyle variables, and hormone profiles, has the potential to provide a more comprehensive knowledge of the complex characteristics of breast cancer.

The research project, titled "Assessing the Prevalence and Impact of Leptin Receptor Q223R Polymorphism in Bangladeshi Breast Cancer Patients," has been made substantial. contributions in the field of breast cancer genetics by providing vital information. With a view to understanding their possible role in disease susceptibility and progression, this study

illuminates the significance of examining genetic variations in different populations. While the Q223R mutation did not demonstrate a substantial association with breast cancer predisposition in this particular cohort, the study emphasizes the importance of establishing such a correlation. Continuing research in this segment is necessary because of discovering genetic markers that can be utilized to develop customized screening, preventive, and treatment measures, which will consequently result in enhanced health outcomes for women in Bangladesh and globally.

Chapter 5

Conclusion

This thesis project proposed to supervise the prevalence and potential impact of the LEPR Q223R polymorphism on the susceptibility of Bangladeshi women to breast cancer. The specific genetic factors which are contributing to breast cancer in the Bangladeshi population, widely acknowledged as a major public health concern, have not yet been thoroughly examined. This study's objective is to address the lack of information by investigating the potential correlation between the Q223R mutation in the leptin receptor gene and an elevated vulnerability to breast cancer.

The results indicated that the GG genotype was prevalent in both the breast cancer patient cohort and the healthy control group. Conversely, there is no statistically significant association between the Q223R polymorphism and the likelihood of developing breast cancer. The project's results indicate that the Q223R polymorphism has no significant impact on breast cancer risk. Nonetheless, the extent to which this data can be generalized is constrained due to the absence of the AA genotype among the subjects. The limited sample size of this study is likely accountable for this outcome.

Leptin remains an important element in the research of breast cancer due to its extensive involvement in biological processes which includes angiogenesis, cell proliferation and carcinogenesis (Mertens et al., 2023). This study does not have the effect of lowering the potential significance of leptin and its receptor in the biology of breast cancer. This is the case despite the fact that the study did not have a considerable amount of data. Instead, it places an emphasis on the significance of performing a study that is more diverse, with a sample size that is both larger and more diverse, in order to investigate the association between the

polymorphisms in leptin receptor genes and the likelihood of developing breast cancer in the Bangladeshi community among the population.

When carrying out additional research, it is essential to keep in mind that the potential connection exists between genetic predispositions and environmental, lifestyle, and dietary factors. In addition, a greater number of genetic markers which ought to be included in this research project. Approaches that take into account everything could potentially result in a more comprehensive knowledge of the factors that contribute to the development of breast cancer. This eventually will trun into the development of various screening methods which are more successful, as well as therapies and preventative strategies that are personalized enough to fulfil the needs of specific Bangladeshi communities. The ultimate objective is to lessen the impact of breast cancer in Bangladesh, which will ultimately result in an improvement in the health of women. This will be accomplished once everything has been said and done.

References

Andò, S., & Catalano, S. (2012). The multifactorial role of leptin in driving the breast cancer microenvironment. *Nature Reviews Endocrinology*, *8*(5), 263-275. https://doi.org/10.1038/nrendo.2011.184

Atoum, M., & Hamaid Alparrey, A. A. A. (2022). Association of Leptin Receptor Q223R Gene Polymorphism and Breast Cancer Patients: A Case Control Study. *Asian Pacific Journal of Cancer Prevention*, *23*(1), 177-182. https://doi.org/10.31557/apjcp.2022.23.1.177

Becer, E., Mehmetçik, G., Bareke, H., & Serakıncı, N. (2013). Association of leptin receptor gene Q223R polymorphism on lipid profiles in comparison study between obese and non-obese subjects. *Gene*, *529*(1), 16-20. https://doi.org/10.1016/j.gene.2013.08.003

Begum, S., Mahmud, T., Rahman, T., Zannat, J., Khatun, F., Nahar, K., Towhida, M., Joarder, M., Harun, A., & Sharmin, F. (2019). Knowledge, attitude and practice of Bangladeshi women towards breast cancer: a cross sectional study. *Mymensingh Med J*, *28*(1), 96-104.

Ben-Eliezer, M., Phillip, M., & Gat-Yablonski, G. (2007). Leptin regulates chondrogenic differentiation in ATDC5 cell-line through JAK/STAT and MAPK pathways. *Endocrine*, *32*, 235-244.

Cirillo, D., Rachiglio, A. M., La Montagna, R., Giordano, A., & Normanno, N. (2008). Leptin signaling in breast cancer: an overview. *Journal of cellular biochemistry*, *105*(4), 956-964.

Cleary, M. P., Grossmann, M. E., & Ray, A. (2010). Effect of obesity on breast cancer development. *Veterinary pathology*, *47*(2), 202-213.

Dallal, C., Garte, S., Ragin, C., Chen, J., Lloyd, S., Modugno, F., Weissfeld, J., & Taioli, E. (2013). Plasma Leptin Levels, LEPR Q223R Polymorphism and Mammographic Breast Density: A

Cross-sectional Study. *The International Journal of Biological Markers*, *28*(2), 161-167. https://doi.org/10.5301/jbm.5000016

Dubois, V., Jardé, T., Delort, L., Billard, H., Bernard-Gallon, D., Berger, E., Geloen, A., Vasson, M.-P., & Caldefie-Chezet, F. (2014). Leptin Induces a Proliferative Response in Breast Cancer Cells but Not in Normal Breast Cells. *Nutrition and Cancer*, *66*(4), 645-655. https://doi.org/10.1080/01635581.2014.894104

Fantuzzi, G. (2005). Adipose tissue, adipokines, and inflammation. *Journal of Allergy and clinical immunology*, *115*(5), 911-919.

García-Estévez, L., Cortés, J., Pérez, S., Calvo, I., Gallegos, I., & Moreno-Bueno, G. (2021). Obesity and breast cancer: a paradoxical and controversial relationship influenced by menopausal status. *Frontiers in Oncology*, *11*, 705911.

Garofalo, C., Koda, M., Cascio, S., Sulkowska, M., Kanczuga-Koda, L., Golaszewska, J., Russo, A., Sulkowski, S., & Surmacz, E. (2006). Increased Expression of Leptin and the Leptin Receptor as a Marker of Breast Cancer Progression: Possible Role of Obesity-Related Stimuli. *Clinical Cancer Research*, *12*(5), 1447-1453. https://doi.org/10.1158/1078-0432.ccr-05-1913

Hafez, A., & Mohamed, H. (2022). Assessment of the Perception of Aesthetics and Smile Attractiveness among Dental and Health Students. *International Journal of Oncology Research*, *5*(1). https://doi.org/10.23937/2643-4563/1710037

Howlader, N. R., Rahman, M. M., Hossain, M. A., Sultana, R., Hossain, S. M., Mazid, M. A., & Rahman, M. M. (2020). Genetic polymorphisms in DNA repair genes XRCC1 and 3 are associated with increased risk of breast cancer in Bangladeshi population. *Breast Cancer Research and Treatment*, *182*(3), 739-750. https://doi.org/10.1007/s10549-020-05738-8

Jardé, T., Caldefie-Chézet, F., Damez, M., Mishellany, F., Penault-Llorca, F., Guillot, J., & Vasson, M. (2008). Leptin and leptin receptor involvement in cancer development: a study on human primary breast carcinoma. *Oncology Reports*. https://doi.org/10.3892/or.19.4.905

Krause, S., Jondeau-Cabaton, A., Dhimolea, E., Soto, A. M., Sonnenschein, C., & Maffini, M. V. (2012). Dual regulation of breast tubulogenesis using extracellular matrix composition and stromal cells. *Tissue engineering Part A*, *18*(5-6), 520-532.

Lei, S., Zheng, R., Zhang, S., Wang, S., Chen, R., Sun, K., Zeng, H., Zhou, J., & Wei, W. (2021). Global patterns of breast cancer incidence and mortality: A population-based cancer registry data analysis from 2000 to 2020. *Cancer Communications*, *41*(11), 1183-1194. https://doi.org/10.1002/cac2.12207

Mertens, E., Barrenechea-Pulache, A., Sagastume, D., Vasquez, M. S., Vandevijvere, S., & Peñalvo, J. L. (2023). Understanding the contribution of lifestyle in breast cancer risk prediction: a systematic review of models applicable to Europe. *BMC cancer*, *23*(1), 687.

Pérez-Pérez, A., Sánchez-Jiménez, F., Vilariño-García, T., & Sánchez-Margalet, V. (2020). Role of leptin in inflammation and vice versa. *International journal of molecular sciences*, *21*(16), 5887.

Ray, A., Nkhata, K. J., & Cleary, M. P. (2007). Effects of leptin on human breast cancer cell lines in relationship to estrogen receptor and HER2 status. *International journal of oncology*, *30*(6), 1499-1509.

Sarker, R., Islam, M. S., Moonajilin, M. S., Rahman, M., Gesesew, H. A., & Ward, P. R. (2022). Knowledge of breast cancer and breast self-examination practices and its barriers among university female students in Bangladesh: Findings from a cross-sectional study. *Plos one*, *17*(6), e0270417.

Saxena, N. K., Vertino, P. M., Anania, F. A., & Sharma, D. (2007). Leptin-induced growth stimulation of breast cancer cells involves recruitment of histone acetyltransferases and mediator complex to CYCLIN D1 promoter via activation of Stat3. *Journal of Biological Chemistry*, *282*(18), 13316-13325.

Shamsi, T. (2021). Burden of breast cancer in Bangladesh-current and future and financing treatment with link to willingness to pay. *International Journal of Community Medicine and Public Health*, *8*(11), 5525-5528. https://doi.org/https://dx.doi.org/10.18203/2394-6040.ijcmph20214295

Shamsi, T. (2021). Burden of breast cancer in Bangladesh-current and future and financing treatment with link to willingness to pay. *International Journal of Community Medicine and Public Health*, *8*(11), 5525. https://doi.org/10.18203/2394-6040.ijcmph20214295

Snoussi, K., Strosberg, A. D., Bouaouina, N., Ahmed, S. B., Helal, A. N., & Chouchane, L. (2006). Leptin and leptin receptor polymorphisms are associated with increased risk and poor prognosis of breast carcinoma. *BMC Cancer*, *6*(1). https://doi.org/10.1186/1471-2407-6-38

Sun, Y.-S., Zhao, Z., Yang, Z.-N., Xu, F., Lu, H.-J., Zhu, Z.-Y., Shi, W., Jiang, J., Yao, P.-P., & Zhu, H.-P. (2017). Risk factors and preventions of breast cancer. *International journal of biological sciences*, *13*(11), 1387.

Takahashi, M., Muromoto, R., Kojima, H., Takeuchi, S., Kitai, Y., Kashiwakura, J.-i., & Matsuda, T. (2017). Biochanin A enhances RORγ activity through STAT3-mediated recruitment of NCOA1. *Biochemical and biophysical research communications*, *489*(4), 503-508.

Vauthier, V., Jaillard, S., Journel, H., Dubourg, C., Jockers, R., & Dam, J. (2012). Homozygous deletion of an 80 kb region comprising part of DNAJC6 and LEPR genes on chromosome 1P31. 3 is associated with early onset obesity, mental retardation and epilepsy. *Molecular*

genetics and metabolism, *106*(3), 345-350.

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

Zhang, Y., & Scarpace, P. J. (2006). The role of leptin in leptin resistance and obesity. *Physiology & behavior*, *88*(3), 249-256.