Investigating the Role of Leptin Receptor (Q223R) Gene Polymorphism in Breast Cancer Development in Bangladeshi Women: 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

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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 "Investigating the Role of Leptin Receptor (Q223R) Gene Polymorphism in Breast Cancer Development in Bangladeshi Women: A Case-Control Study" submitted by J M Aousiful Islam (20346030), 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

To ensure the safety, rights, and well-being of all participants, this study was conducted in strict compliance with ethical standards. In accordance with ethical standards, the Institutional Review Board (IRB) of BRAC University (IRB No BRACUIRB120220005) was requested and granted approval. A thorough assessment of the research proposal was carried out by IRB to assure that it complied with all pertinent ethical standards and regulatory requirements. All participants were informed about the research project before starting the activity regarding this. Participants were completely aware of a wealth of information concerning the goals, methods, possible risks, and benefits of the project. The participants were ensured that they are flexible & independent enough to leave from the study at any time without incurring any further consequences.

This investigation was strict enough to follow all rules and regulations approved by the IRB. The methods and procedures were executed accordingly. These involved the secure storage of data, careful handling of biological specimens to ensure the participant anonymity. Besides, strict compliance with the protocols was followed to reduce any potential risks to the participants. The study also guaranteed that all gathered data was de-identified to ensure privacy and was only used for the purpose of this research.

Abstract

Breast cancer is well recognized as a significant cause of death, and it is well established that there is a genetic element associated with the prognosis of the disease. This study's objective is to examine the association between the LEPR Q223R polymorphism and the risk of developing in Bangladeshi women. In this study, PCR-RFLP method was carried out on 20 participants among them 10 breast cancer patients and 10 healthy control group, to examine the allele frequencies of the LEPR Q223R polymorphisms.

The results were inclusive, while variations of the frequencies of the defendant genotypes were observed, those variations were not statistically significant in terms of the association between the single nucleotide polymorphism and the risk of developing breast cancer in the selected population. These findings contribute to the scientific fields of genetics and factors related to breast cancer, which emphasizes the need for larger projects and studies to confirm these findings in this project. Gaining a more comprehensive understanding of genetic variants in various populations is necessary for the advancement of treatment approaches focused preventative measures for breast cancer.

Keywords: LEPR; Polymorphism; Breast Cancer; Genetic Risk; PCR RFLP;

Dedication

The foundation of my accomplishments is my parents' unwavering support, sacrifices, and affection. I dedicate this work to them.

To my wife, whose unwavering encouragement and forbearance have provided me with the fortitude to endure, you have been my most significant source of inspiration.

Special recognition is due to my elder brother, whose unwavering support and confidence in my capabilities have been instrumental in my accomplishments.

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List of Acronyms

IARC	International Agency for Research on Cancer
RFLP	Restriction Fragment Length Polymorphism
PCR	Polymerase Chain Reaction
EGFR	Estimated Glomerular Filtration Rate
IGF-I	Insulin-like Growth Factor I
HER2	Human Epidermal Growth Factor Receptor 2
CDK2	Cyclin-dependent Kinase 2
SPSS	Statistical Package for Social Sciences
GWAS	Genome-wide Association Studies

Chapter 1

Introduction

Breast cancer is a serious health problem that Bangladesh is now experiencing. The International Agency for Research on Cancer (IARC) reports that, over 13,000 women receive a breast cancer diagnosis each year, and over 7,000 of them lose their lives to the illness. (Nessa et al., 2018). According to GLOBOCAN, 13,028 new breast cancer cases were diagnosed in 2020, which highlights the significant pressure that the nation's healthcare system is under (Sung et al., 2021). There are around 22.5 cases of breast cancer diagnosed per 100,000 women in Bangladesh, with the majority of cases occurring in women between the ages of 15 and 44 (Ahmed et al., 2018). The particular causes of breast cancer are not completely understood, despite the fact that it is a very common disease. A number of factors, including age at menarche and menopause, dietary habits, reproductive history, oestrogen exposure, genetic predispositions, and obesity, are all potential risk factors. Obesity is especially prevalent in postmenopausal women (Opoku et al., 2023).

On a global scale, breast cancer continues to be the most prevalent form of cancer among females. On a global scale, it ranks as the fifth leading cause of cancer-related mortality and the second most common cancer type overall (Azamjah et al., 2019). Breast cancer accounts for around thirty percent of all newly diagnosed instances of cancer each year (Shang & Xu, 2022). Breast cancer frequently develops without any obvious signs, which in turn contributes to delayed diagnoses and worse outcomes. A total of 9.6 million people lost their lives to cancer in 2018, with 18.1 million new cases being recorded (Bray et al., 2018). This indicates that the worldwide cancer burden has greatly increased. A number of factors, including hormonal, lifestyle, and environmental variables, as well as genetic

predispositions, are known to be linked to the chance of developing breast cancer (Roheel et al., 2023).

Globally, women are diagnosed with breast cancer, which is perhaps the most prevalent form of cancer among women (Roheel et al., 2023). There is a lifetime chance of having breast cancer. Despite the fact that breast cancer is a rather frequent disease, the particular mechanisms contributing to its development are not fully understood (Conklin & Keely, 2012). Many other factors, including the age at which a woman begins 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 identified as potential risk drivers (Vincent, 2015). Menarche is the age at which a woman begins menstruation for the first time. It would appear that a larger body weight is also connected with the development of breast or mammary thyroid cancer (Argolo et al., 2018). It has been established that obese women who have gone through menopause are more likely to get breast cancer, according to research (García-Estévez et al., 2021). Being overweight or obese raises the risk of breast cancer through four primary mechanisms: through leptin and its receptors; through inflammation of adipose tissue that persists for an extended period; through alterations in sex hormones; and through communication through insulin and IGF-1 (Taghizadeh et al., 2017). A low-grade chronic inflammation is promoted in white adipose tissue as a result of these activities, which contribute to increasing the amount of the adipokine leptin. An energy imbalance is caused because of this, and that activates signaling pathways that produce cytokines and inflammatory mediators (Taghizadeh et al., 2017). When the leptin hormone and its receptor is taken into consideration, a quite important mechanism which links obesity to breast cancer emerges in the profile. Through its large impact on hunger and spending of energy in the human body, the hormone leptin plays a crucial role in the controlling of

body weight, it is largely produced by white adipose tissue and has a molecular weight of 16 kilodaltons (Andò et al., 2019). Leptin serves as an adjuvant to the weight-loss process in the human body; it also has therapeutic significance in the pathogenesis of immunological and hematopoietic disorders and in controlling infertility, angiogenesis (Andò et al., 2019). Including the leptin receptor, often referred to as Ob-R, the class I cytokine receptor family includes the leptin receptor, which was first found in the brain (Schaab & Kratzsch, 2015). However, it is also expressed in a variety of other organs, such as the liver, immune cells, placenta, endometrial, stomach, and breast cancer cells (Schaab & Kratzsch, 2015).

Leptin is a component of several pathways that are linked to cancer, including the escalation of the epithelial-mesenchymal transition, which makes it easier for cancer cells to move around cells and spread throughout the body (Olea-Flores et al., 2020). LEPR also interacts with growth factors such as IGF-I, EGFR, and HER2/neu, which contributes to the development of breast cancer, its progression, and its evolution into malignancy (Guo et al., 2007). Breast cancer patients with lymph node involvement, metastases, and a poor prognosis are more likely to have high leptin counts or profound expression of leptin receptors (Takada et al., 2020).

Leptin encapsulates both estrogen-dependent and estrogen-independent mechanisms in breast cancer patients. It stimulates the signaling pathways, such as STAT3 and MAPK, which increase cell growth and viability. By boosting the expression of CDK2 and cyclin D1, leptin dictates the progression of the cell cycle, while at the same time suppressing the activity of cell cycle inhibitors such as pRb (Benot-Dominguez et al., 2022). Leptin also has a different phenomenon of elevating the number of cells and changing their shape in cells of breast cancer tumors (Easterling et al., 2019).

The scientific community has been rigorously investigating the genetic differences found between the leptin hormone and its receptor genes, and trying to figure out what percentage is possibility is that the Q223R polymorphism (rs1137101) in the LEPR gene contributes to developing breast cancer (Atoum & Hamaid Alparrey, 2022). The extracellular domain of the leptin receptor was investigated extensively, which gave the finding that the Q223R polymorphism causes a glutamine-to-arginine substitution at position 223 (Atoum & Hamaid Alparrey, 2022). The leptin binding and signaling effectiveness is largely disturbed by the glutamine-to-arginine substitution. Because of the substantial connection of this polymorphism with elevated levels of leptin, which also is inclusive of leptin resistance, this contributes to the physiological processes of breast cancer and obesity (Becer et al., 2013). This is yet to be evidenced-based, and scientific research-oriented study if Leptin receptor Q223R gene has a crucial role in the etiological development of breast cancer among Bangladeshi female population. Objective: The present study was undertaken to investigate the association of Q223R polymorphism in Leptin receptor (LEPR) gene with a normal control group and in patient subjects affected by breast cancer among Bangladeshi women. By investigating this genetic variation, the research aims to grasp an understanding of the potential hit that it may have on the susceptibility to breast cancer development and the progression of the disease among the Bangladeshi female population. This study will give a detailed genetic assessment that may bring some truth out on the etiological role that the Q223R single nucleotide polymorphism plays in developing and progression of breast cancer. The specific scientific technique that was used for the genetic analysis in this project is called Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP). This study has only one main goal, which is to explore the potential connection between these genetic variants in those with the ability to develop breast cancer in the long run. Moreover, it is hoped that this project can explore what kind of impact these genetic

variations will have on the prognosis after diagnosis if their existence ruins even part or all hope barring some sort of miracle.

This study also hopes to raise the question about what genetic risk factors are linked to breast cancer in order to facilitate efforts to detect or prevent disease at an early stage. This research project aims at the establishment of personalized screening methods and preventive services tailored to Bangladeshi genomes. We will accomplish this by identifying individual genetic markers--such as the Q223R polymorphism. By offering guidance for more precise hazard sources and, at the same time, an entry into tailored medications the envisioned result of this project is improved health outcomes for women in Bangladesh.

Chapter 2

Materials and Methods

2.1 Approach Participants and Ethical Considerations

A total of 20 female participants were included in this study, with 10 being breast cancer patients and the other 10 being healthy control subjects. The study received ethical approval from the BRAC University Institutional Review Board (IRB No BRACUIRB120220005) as a B. Pharm. final year thesis project. Before subjects participated, they had to provide informed permission, proving that they fully understood the goals and procedures of the study.

2.2 Collection of Sample

In 2022, samples of breast cancer cases were collected from the National Cancer Research Institute Hospital, Mohakhali, Dhaka. 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 required to meet the following inclusion criteria: a verified diagnosis of breast cancer (for the patient group), absence of any family history of breast cancer, being 18 years of age or older, and identifying 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 Documentation of Data

A standardized questionnaire was implemented to guarantee the collection of exhaustive and accurate data, which included detailed information regarding medical history, demographic characteristics, and lifestyle behaviors.

Demographic and Medical Data

- Reports on any family history of breast cancer or any other kind of cancer are involved in the family history.
- Past medical conditions which include information about serious medical conditions for instance hormonal abnormalities, diseases of breast which are not malignant.
- Factors that must be taken into account in case of reproductive history involve age at which full-term pregnancy happened, the total number of pregnancies, the age at which menopause occurs, the age at which first menstruation begins, history of hormonal replacement therapy, lactation details.
- Any history of breast surgery or other reproductive organs.
- Individual's actual age, occupation, ethnic heritage specific factors about the residence.

Psychosocial and Lifestyle Factors

- Consistent and patterned eating schedule.
- Duration of physical activity and the kind of daily exercise performed.
- Tobacco and alcohol consumption past and present status.
- Sleep pattern and individual height and weight to calculate BMI.
- Information regarding the household's income and insurance coverage.
- Information on anxiety, stress level, social inclusion.

Confidentiality and Data Management

The gathered data was recorded consciously and kept in a secured database. Data encryption protocols were made to safeguard personal information, confirming the preservation of study participants' confidentiality according to established standards. The data was only accessible for the researchers carrying out the study, ascertaining compliance with data protection legislation and ethical standards. The entire methodology for data documenting made a solid base for analyzing and interpreting study outcomes, by ensuring the collection of dependable and reliable data.

2.4 Procedures Conducted in a Laboratory

Genomic DNA Extraction

Firstly, through venipuncture blood sample were taken out and a sterile vacutainer tube were taken with 3 ml of blood and dipotassium-ethylenediaminetetraacetic acid (EDTA)-K_2 as an anticoagulant due to prevent blood coagulation. Following manufacturer's guidelines. The FavorPrep™ Blood Genomic DNA Extraction Kit (Favorgen Biotech Corporation, Taiwan) was utilized for extraction of genomic DNA from the collected blood samples. The DNA extracted from collected and conserved blood sample was kept at a temperature of -20°C until it could be further analyzed and tested.

Analysis of PCR-RFLP

Polymerase Chain Reaction-Restriction Fragment Length Polymorphism method was used to identify the Genotyping of the Q223R polymorphism of the leptin receptor.

Amplification of the Q223R polymorphism in the DNA samples was done by following primers-

- Forward: 5'-ACCCTTTAAGCTGGGTGTCCCAAATAG-3'
- Reverse: 5'-AGCTAGCAAATATTTTGTAAGCAATT-3'

The conditions for polymerase chain reaction (PCR) amplification were mentioned below:

- First of all, the initial denaturation stage was continued for 2 minutes with the temperature 94°C.
- Then, a 40-cycle phase was started which includes denaturation at 94°C with the duration of 30 seconds. Afterwards annealing was continued at 58°C for 30 seconds.
- After annealing, extension was done at 72°C for 30 seconds.
- Lastly, the final extension was carried out at 72°C for 10 minutes.

An electrophoresis experiment was carried out on a 1% agarose gel using 135 volts for a duration of 20 minutes. The objective of this experiment is to examine the polymerase chain reaction and accomplish the successful amplification of the selected DNA fragments. Just after the polymerase chain reaction (PCR), the PCR product were digested with the help of 0.5 microliters of restriction enzyme MspI (New England Biolabs) in a reaction mixture which was designed to target the LEPR gene. The enzymatic digestion process was initiated and continued for a duration of 16 hours with 37°C. Then the resulting digests were kept on a gel made of 3% agarose. Afterwards, the electrophoresis was carried out with 75 volts for 25 minutes in order to separate the digested fragments.

The Q223R polymorphism exhibited the following band patterns-

- In case of GG homozygous genotype, a single band was observed at 416bp
- In the case of AA homozygous genotypes, two bands were found at 291bp and 125bp.
- In case of AG heterozygous genotypes, three bands were found at 426bp, 291bp and 125bp.

The genotypes breast cancer patients and healthy control groups were determined by analyzing the digestion patterns observed on the agarose gel as reported in the study conducted by Atoum & Hamaid Alparrey, 2022.

Statistical tools used for analysis of data

Statistical analysis was accomplished with SPSS version 16.0 (SPSS Inc., Chicago, IL). The Chi-squared test was utilized to compare the Q223R LEPR polymorphism patterns in people with breast cancer and people without breast cancer. Odds ratio (OR) and 95% CIs were used to evaluate the association between the two groups. Furthermore, the average leptin levels in breast cancer patients with the Q223R LEPR polymorphism were determined using a one-sample t-test, which distinguished between non-obese and obese individuals. If the p-value was less than 0.05, it was considered statistically significant.

Chapter 3

Result

Every genotype is contained within Hardy Weinberg equation Chi square = 0.855807901 with p-value = 0.5 (Table 1).

Table 1: A Hardy-Weinberg Equilibrium Analysis of the Control Group's MspI Polymorphism and Genotype.

Genotype	Observed	Expected	X^2	p-value
AA	0	0.225	0.855807901	0.5
AG	3	2.55		
GG	7	7.225		
Total	10	10		

The Chi-square (X^2) test was employed. A p-value less than 0.05 was deemed to be statistically significant.

The frequency of AA, AG and GG genotypes (0, 30, and 70; respectively) among breast cancer females are shown in Table 2. Since the AA genotype had zero counts, the odds ratio calculation was not possible for AA as a reference in a traditional sense. There was no discernible statistical difference between control group (n=10) and the breast cancer (n= 10) among the GG genotype (OR= 3.86; 95%CI= 0.33 to 45.62; p= 0.5) when compared to AG genotype. The very wide confidence interval indicates high uncertainty,

which is common with small sample sizes.

Table 2: Analysis of the Genotypes Frequency, Odds Ratio, and 95% Confidence Interval between Breast Cancer Cases and Control.

SNP/Genotype/Allele	Case (%)	Control (%)	OR (95%CI)	p-value
AA	0 (0%)	0 (0%)	Ref	0.5
AG	1 (10%)	3 (30%)	N/A	0.5
GG	9 (90%)	7 (70%)	3.86 (0.33 - 45.62)	

The reference genotype is AA, and a p-value of less than 0.05 was deemed significant on a statistical level.

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

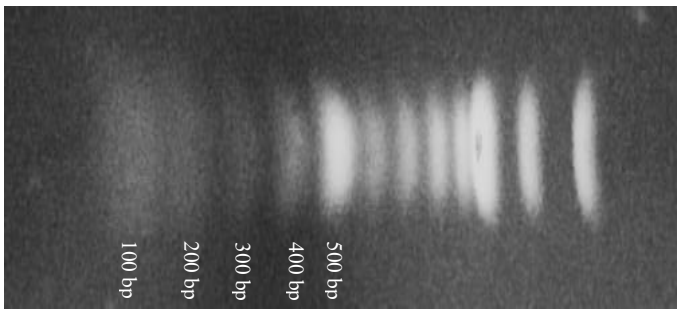

Fig 1: Photograph of gel electrophoresis of DNA Ladder (100bp)

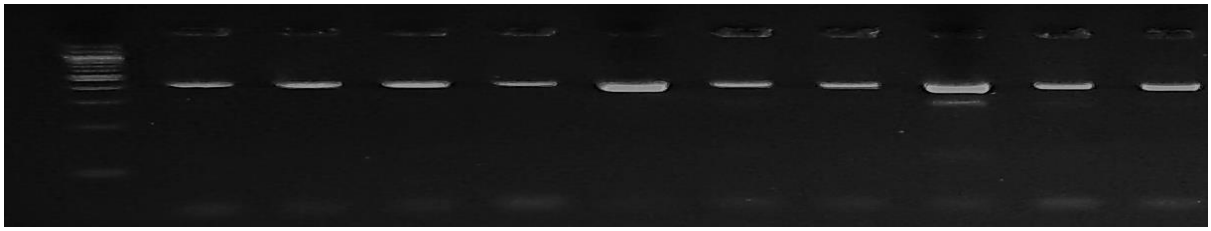

Fig 2: Photograph of gel electrophoresis of LEPR Q223R gene polymorphism detected in Breast Cancer (Case) group after restriction-digestion.

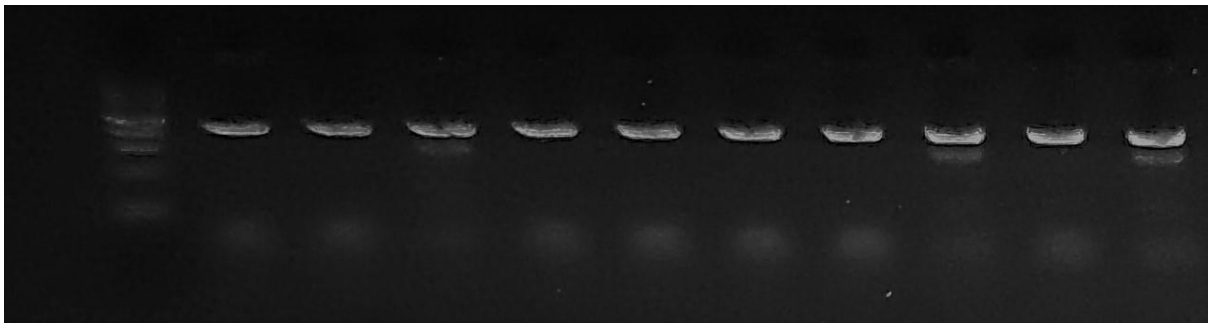

Fig 3: Photograph of gel electrophoresis of LEPR Q223R gene polymorphism detected in Healthy Volunteer (Control) group after restriction-digestion.

Chapter 4

Discussion

4.1 Key Findings Summary

Our study aimed to investigate the relationship between a Bangladeshi cohort's risk of breast cancer and the LEPR Q223R polymorphism. Several significant discoveries were identified during the investigation:

Hardy-Weinberg Equilibrium (HWE):

- The control group's genotypic distribution aligned with the Hardy-Weinberg equilibrium (Chi-square = 0.856, p-value = 0.5). This implies that the allele frequencies are stable and not subject to significant evolutionary pressures, thereby validating the control group's representativeness.

Genotype Frequencies:

- The AA, AG, and GG genotypes were detected at frequencies of 0%, 10%, and 90%, respectively, in the breast cancer cohort.
- Conversely, the control group demonstrated frequencies of 0%, 30%, and 70% for the same genotypes.

Probability Ratios and Statistical Significance:

- There was no discernible variation in the GG versus AG genotypes between the controls and patients with breast cancer that was statistically significant (OR = 3.86; 95% CI = 0.33 to 45.62; p = 0.5). The tiny sample size is likely the cause of the wide confidence interval, which suggests that there is substantial uncertainty.

4.2 Comparison with Existing Literature

Previous investigations regarding the LEPR Q223R polymorphism and breast cancer have yielded inconsistent outcomes. While some studies have reported substantial associations, others have not. Our research supports the latter, indicating that in the Bangladeshi population under study, there is no meaningful association between this polymorphism and the incidence of breast cancer. The complexity of genetic contributions to breast cancer is underscored by this inconsistency across studies, which implies that the impact of the LEPR Q223R polymorphism may differ across various populations and study designs.

In our study, the GG genotype was more prevalent in both breast cancer patients and controls than in certain other populations. This emphasizes the importance of undertaking genetic studies in a variety of populations to learn everything there is to know about how genetic variations affect breast cancer. The variations in genotype distributions that have been observed may be due to environmental factors or population-specific genetic backgrounds.

4.3 Consequences of Results

Clinical Relevance: Since our research did not find a strong association between the LEPR Q223R polymorphism and breast cancer, it is reasonable to assume that this variant is not a good indicator of breast cancer risk in Bangladeshi women. These results underscore the necessity of conducting more extensive and thorough research to definitively ascertain the function of this and other polymorphisms in breast cancer.

To develop tailored risk assessment instruments and focused preventative approaches, it is imperative to understand the genetic components of breast cancer. Our research, despite the absence of a substantial association, provides valuable information to the broader endeavour to elucidate the genetic basis of breast cancer.

Genetic Counselling and Risk Assessment: Results from the study indicate that a comprehensive approach to genetic counselling for risk of developing breast cancer should be conducted, in which multiple genetic and environmental factors should be considered, rather than relying simply on the LEPR gene's Q223R polymorphism. In order to generate more accurate and personalized risk profiles, personalized risk assessment strategies should integrate a diverse range of genetic markers.

4.4 Limitations

Size of Sample: The small sample size is a limitation of our study because it reduces statistical power and results in wide confidence intervals. Due to this limitation, the results should be viewed with caution. However, larger samples should be used in the future to verify the results obtained in this study as well as to give more reliable estimates for the association between breast cancer risk and LEPR Q223R polymorphism.

Generalizability: The implications of our findings might be limited to a certain group, namely, the people of Bangladesh because they have got distinct genetic features and ecological circumstances. It is known that breast cancer susceptibility is associated with genotypic differences and environmental exposures. More studies on other populations are required in order to confirm the results we have observed and to examine possible population-specific impacts.

Potential Biases: Despite efforts to reduce biases, like selection bias and measurement error, these factors cannot be completely removed. If the participants were not selected randomly from the general population, selection bias may have occurred. The accuracy of the genotype classification could have been affected due to mismanagement in proper measurement. The objective of future research on this topic should be to overcome these obstacles by using research methodology and design with higher benchmarks.

4.5 Prospective Research Areas

Larger Cohort Studies: In order to enhance statistical range and make more reliable estimates of breast cancer risk related to the LEPR Q223R polymorphism, it is highly suggested that larger populations are included in future research. Furthermore, larger studies would be able to allow for detailed subgroup analyses, such as looking at the reciprocity between genetic polymorphisms and environmental facets.

Functional Studies: Functional and proper studies are quite necessary for understanding the possible roles of LEPR Q223R polymorphism in the development and progression of breast cancer. They will help determine the hidden prospects, thus identifying potential personalized therapeutic solutions. Moreover, understanding the functional outcomes of genetic alterations is critical for translating these genetic findings into clinical practice for the patients.

Examination of Additional Polymorphisms:

Further studies must be done to find out other gene polymorphisms that may either interact with the LEPR Q223R polymorphism or independently increase risk of developing breast cancer. Digging up the cause of this disease from top to bottom will require different genetic factors to be examined together. Genome-wide association studies (GWAS) and next-generation sequencing methods can be used to find new genetic variations that may be connected to risk of developing breast cancer (Jurj et al., 2020).

Chapter 5

Conclusion

The purpose of this study was to understand the correlation between the LEPR Q223R polymorphism and the risk of developing breast cancer in the population of Bangladeshi women who fall in the obese category in BMI range. Despite the reasonable biological relations of LEPR Q223R increasing breast cancer risk, the results of our study did not indicate a statistically significant link in the selected population. Genomic analysis is quite a useful tool in the determination of variability among genetic connections. Genomic analysis of the obtained data from this study showed that of the total genotypes, 0% were found in the control group, 30% in the breast cancer patients, and 90% in the AG and GG groups, respectively. At 3.86 (95% CI: 0.33 to 45.62; p = 0.5), the odds ratio between the GG and AG genotypes was not significantly different, indicating that there was no statistically significant change. Moreover, in the control group, the distribution of genotypes was in Hardy-Weinberg equilibrium, which ensured the representativeness of the sample control. Our results are in concordance with other previously published studies that have also reported a lack of significant association between the LEPR Q223R polymorphism and the risk of breast cancer, while others have shown the opposite. This inconsistency across the populations and contexts suggests that the effect of the LEPR Q223R polymorphism may be variable in regard to populations and contexts, making genetic factors for breast cancer more complex. These findings should thus be interpreted with caution, considering the study limitations, including the small sample size. The broad confidence interval and loss of statistical power further underline the need for obtaining a larger, informative prospective study to confirm these findings. Moreover, the fact that different genetic and environmental background may have limited generalizability of these findings. Functional studies, larger cohort studies and the

exploration of other genetic polymorphisms are warranted to increase our understanding of the biology underlying breast cancer. Such extensive studies can reveal the biological mechanisms by which these polymorphisms may affect breast cancer risk and more accurate estimates could be obtained. It is quite necessary to understand these mechanisms to create personalized risk assessment tools and targeted prevention techniques. This study provides valuable data to the breast cancer genetics field, even though it resulted in non-significant correlations. It underscores the necessity of conducting ongoing research into the intricate interactions between genetics and breast cancer, as well as the significance of taking population-specific genetic factors into account. This emphasizes the need for continued studies on genetic backgrounds underpinning interactions between genes and breast cancer as well implication of unique population genetics. We encourage initiatives for enhancing the early detection, prevention and treatment of breast cancer as well as personalized medicine approaches to expand our capabilities that will ultimately improve patient care and relieve human suffering. Learning more about it will help us in doing so.

In summary, from the findings, there is no correlation between the LEPR Q223R polymorphism and the frequency of breast cancer in women from Bangladesh. However, it is important because it signifies that genetic research holds significance in learning the causes of this disease. It has thus been quite important to continually strive within this sphere for the betterment of treatments and in providing personalized care for patients with breast cancer throughout the world.

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