"STUDY ON EXPRESSION OF BRCA1 AND BRCA2 GENES IN CARCINOMA PROSTATE AND ITS CORRELATION WITH HISTOPATHOLOGY"

By

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DOCTOR OF MEDICINE

IN

PATHOLOGY

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LIST OF ABBREVIATIONS

Abbreviation	Full Form
ADME/T	Absorption, Distribution, Metabolism, Excretion, and Toxicity
ANOVA	Analysis of Variance
AR	Androgen Receptor
BPH	Benign Prostatic Hyperplasia
BRCA1	Breast Cancer 1
BRCA2	Breast Cancer 2
cfDNA	Cell-Free DNA
CT	Cycle Threshold
cDNA	Complementary DNA
DRE	Digital Rectal Examination
ELISA	Enzyme-Linked Immunosorbent Assay
EMT	Epithelial-to-Mesenchymal Transition
FFPE	Formalin-Fixed Paraffin-Embedded
GAPDH	Glyceraldehyde 3-Phosphate Dehydrogenase
hK1	Human Kallikrein 1
hK2	Human Kallikrein 2
hK3	Human Kallikrein 3 (Prostate-Specific Antigen)
hK4	Human Kallikrein 4
HRR	Homologous Recombination Repair
ISUP	International Society of Urological Pathology
mCRPC	Metastatic Castration-Resistant Prostate Cancer
mRNA	Messenger Ribonucleic Acid
NCBI	National Center for Biotechnology Information
NTC	No Template Control
PCa	Prostate Cancer
PDB	Protein Data Bank
PPI	Protein-Protein Interaction
PSA	Prostate-Specific Antigen
qPCR	Quantitative Polymerase Chain Reaction

RIN	RNA Integrity Number
RNA	Ribonucleic Acid
SIR	Standardized Incidence Ratio
SWISS-ADME	A web tool for predicting Absorption, Distribution, Metabolism, Excretion, and Toxicity properties
TRUS	Transrectal Ultrasound
ΔCT	Delta Cycle Threshold
ΔΔCT	Delta-Delta Cycle Threshold

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ABSTRACT

Introduction:

Prostate cancer is one of the most prevalent cancers in men worldwide, with increasing incidence noted particularly in regions like India. Tumor suppressor genes such as BRCA1 and BRCA2, which are essential for genomic stability, have been implicated in prostate cancer progression. Elevated PSA levels and higher Gleason scores further correlate with aggressive tumor phenotypes. In addition, computational methods, including molecular docking, offer insights into protein–protein interactions that may aid in identifying novel therapeutic targets.

Objectives:

1. To assess mRNA expression of BRCA1 and BRCA2 genes in carcinoma prostate and its correlation with PSA levels and Gleason score.
2. To study *in silico* analysis of BRCA 1 and BRCA 2 in relation to prostate specific antigen (PSA) like human kallikreins (hK1, hK2, hK3 and hK4).

Methods:

A prospective observational study was conducted involving prostate tissue samples from patients diagnosed with carcinoma prostate and Benign Prostatic Hyperplasia (BPH). RNA was extracted from formalin-fixed, paraffin-embedded (FFPE) samples and reverse transcribed to cDNA. Quantitative PCR (qPCR) was employed to quantify BRCA1 and BRCA2 expression, normalized to GAPDH using the ΔΔCT method. Statistical analyses, including Spearman's rank correlation and ANOVA, were used to correlate gene expression with PSA levels and Gleason grades. Additionally, molecular docking simulations were performed using AutoDock 4.2 and visualized with Discovery Studio to evaluate the binding affinities between BRCA proteins and kallikrein peptides.

Results:

The qPCR analysis revealed a statistically significant under expression of both BRCA1 and BRCA2 in carcinoma prostate samples compared to BPH controls. A significant negative correlation was observed between PSA levels and BRCA2 expression, while BRCA1 showed a less pronounced relationship. Moreover, a progressive decline in mRNA expression of both genes was noted with increasing Gleason grades, suggesting an association with tumor aggressiveness. Molecular docking studies demonstrated favorable binding interactions between BRCA proteins and kallikreins, highlighting potential avenues for targeted therapeutic interventions.

Conclusion:

The findings underscore the clinical relevance of BRCA1 and BRCA2 as molecular markers in prostate cancer, where their underexpression is linked with higher PSA levels and more aggressive Gleason grades. The molecular docking results further suggest that interactions between BRCA proteins and PSA like kallikreins could be used to predict expression of BRCA1 and BRCA2 along with development of targeted therapies, supporting the integration of genetic and computational approaches in prostate cancer management.

Keywords:

Prostate Cancer, BRCA1, BRCA2, PSA, Gleason Grade, qPCR, Molecular Docking, In Silico Analysis, Kallikreins, Tumor Suppressors

“STUDY ON EXPRESSION OF BRCA1 AND BRCA2 GENES IN CARCINOMA PROSTATE AND ITS CORRELATION WITH HISTOPATHOLOGY”

1. INTRODUCTION

1.1 Overview of Prostate Cancer

With over 1.4 million new cases identified each year and 375,000 deaths in 2022, prostate cancer (PCa) is the second most prevalent cancer in males (Figure 1). By 2030, it is anticipated that the global burden of PCa will have increased significantly, resulting in roughly 1.7 million new diagnoses and 499,000 fatalities[1]. The aging population, changes in lifestyle, and advancements in diagnostic techniques are all responsible for the increase [2].

India used to have lower prostate cancer rates than Western countries. However, current trends show that the number of men in India's rural and urban areas receiving a prostate cancer diagnosis is steadily increasing [3]. This rise can be attributed to increased urbanization and shifts in dietary and lifestyle habits. Improvements in healthcare facilities, diagnostic tools, and fair access have resulted in the disease being identified earlier and more often[4–6]. Programs for screening, like the “PSA (Prostate-Specific Antigen)” test, are aiding in early identification of diseases, resulting in improved survival rates in both India and Western countries[7,8].

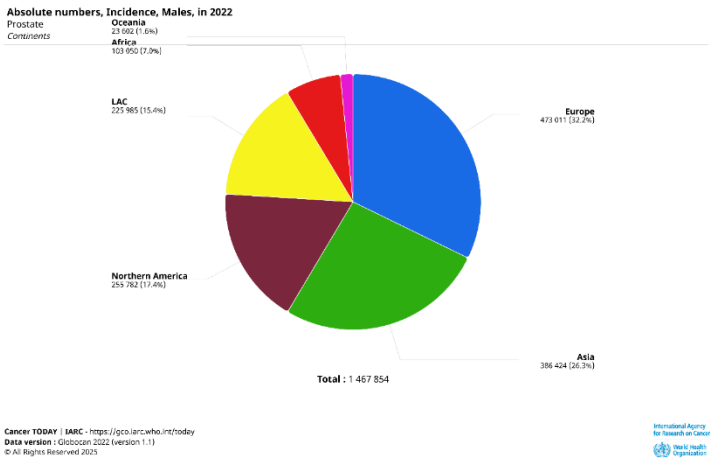


Figure 1: Continent-wise distribution of incidence of prostate carcinoma (Source: "GLOBOCAN 2022, International Agency in Research on Cancer, World Health Organization")

The prostate comprises epithelium and stroma, which are maintained in equilibrium to ensure optimal prostate function[9]. Perturbation of this equilibrium, resulting from aberrant proliferation of stromal cells, may lead to prostatic disorders that include "BPH (Benign Prostatic Hyperplasia)"[10]. Prostate cancer development, as well as progression, are greatly impacted by the interplay between epithelial and stromal components, which is essential for maintaining normal prostate homeostasis[11]. Fibroblasts linked to cancer in prostate tumors release more growth factors as well as cytokines, that promote the growth as well as metastasis of cancer cells[12].

Because PSA screening is more affordable than transrectal ultrasound (TRUS) and is better at detecting prostate malignancies than either digital rectal examination (DRE) or TRUS, it is a commonly utilized method for prostate cancer detection[13,14]. In prostate cancer, increased serum PSA levels are caused by altered vascular structure rather than a notable increase in prostate size [15].

The most often employed grading scheme is the "Gleason system". This technique relies on examining the glandular structure's appearance under a microscope at low magnification. The most prevalent cancer pattern in the sample is given a primary grade by pathologists, while the next most frequent pattern is given a secondary grade. These are grouped together to determine the "Gleason score". Gleason score is further grouped into Gleason Grade group which ranges from 1 to 5[16].

1.2 Role of BRCA1 and BRCA2 in Cancer Biology

The genes BRCA1 and BRCA2 function as tumor suppressors and produce proteins essential for homologous recombination, a repair mechanism [17]. BRCA1 functions as a detector for Double-Stranded Breaks (DSBs), aiding in assembly of repair proteins at the site of damage [18]. In order to prevent cells with DNA damage from completing the cell cycle, it also interacts with a number of other proteins involved in checkpoint activation. By loading the RAD51 recombinase onto single-stranded DNA at the break site, BRCA2 on the other hand, plays a critical part in the repair process[19–21].

The repair pathway is compromised when BRCA1 and BRCA2 are lost or mutated, resulting in the buildup of genetic errors [22]. Because cells with BRCA1 or BRCA2 gene mutations cannot effectively repair "DSBs (Double-Strand Breaks)" by homologous recombination, they must rely on more error-prone repair mechanisms like "Non-Homologous End Joining (NHEJ)" [23]. This increases the likelihood of genetic rearrangements, mutations, and chromosomal abnormalities—all of which are related to the development of cancer [24].

Impact of Mutations

Mutations in the BRCA1 and BRCA2 genes impair DNA repair processes, resulting in higher mutation accumulation and genomic instability[25]. This defective repair process promotes tumorigenesis, with BRCA-mutated cells adopting a hypermutated state that drives cancer progression [26]. In PCa, BRCA2 mutations are particularly related to aggressive clinical features[27].

Relevance in Prostate Cancer

The clinical importance of BRCA1 and BRCA2 with regard to PCa has been confirmed by numerous investigations[28]. Prostate carcinoma patients bearing these mutations generally have a worse prognosis and reduced survival compared to non-mutated instances[28].

By illuminating the functions of BRCA1 and BRCA2 in maintaining genomic integrity and their consequences when they are altered, this study seeks to establish a connection between molecular mechanisms and clinical outcomes in prostate cancer. This will lead to better and improved ways of treating and managing the disease[29].

1.3 Computational Approaches in Cancer Research

Molecular Docking

A computational method called molecular docking predicts how a ligand, or tiny molecule, will interact with a target protein[30]. By discovering possible treatment alternatives based on their binding affinity and interaction with particular proteins, it makes significant contributions to drug discovery and development[31]. It offers insights into how molecules interact with proteins by modeling their fit within a protein's active site, shedding light on both protein-protein and protein-ligand interactions [32].

In drug discovery, docking algorithms explore three-dimensional structures of proteins to identify binding sites and evaluate the stability of complexes formed with ligands[33]. Scoring functions are used to rank docking candidates based on their binding energies, helping researchers prioritize compounds for further experimental validation[34]. This method greatly cuts down on the time and expenses linked to conventional drug screening procedures[35].

It is a useful technique for comprehending the function of particular changes in genes and how they interact with biological pathways in the setting of prostate cancer[36]. It can also aid in pinpointing new therapeutic targets. For instance, examining how BRCA proteins interact with certain ligands can guide the creation of inhibitors that specifically address cancers with BRCA mutations[37]. It can also assist in forecasting the effectiveness of current medications, like Poly (ADP-ribose) polymerase (PARP) inhibitors, by simulating their interaction with proteins that have BRCA mutations[33]. These models can also be instrumental in identifying other small

molecules that can upregulate BRCA expression or modulate pathways associated with DNA repair[32,35,38–42]. Molecular docking models are much needed in the current cancer research for the development of precision medicine approaches[43].

2. AIMS AND OBJECTIVES

Aims: Aim of the dissertation is to evaluate BRCA1 and BRCA2 gene expression in carcinoma prostate in relation to Gleason score by *in silico* and molecular pathology approach.

Objectives:

1. To assess mRNA expression of BRCA1 and BRCA2 genes in carcinoma prostate and its correlation with PSA levels and Gleason score.
2. To study *in silico* analysis of BRCA 1 and BRCA 2 in relation to a prostate-specific antigen (PSA) like human kallikreins (hK1, hK2,hK3, and hK4).

3. REVIEW OF LITERATURE

3.1 Anatomy and Physiology of the Prostate

The prostate gland is essential to the male reproductive system. It surrounds the urethra and is situated right below the bladder (Figure 2). The prostate gland, shaped like a walnut, is made up of epithelial and stromal components that work in unison to support its normal function. The primary role of the prostate is to generate seminal fluid, an essential constituent of semen that facilitates and sustains sperm [44].

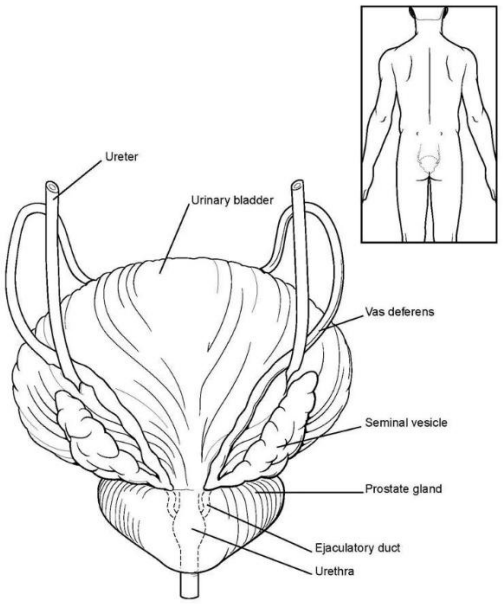


Figure 2: Location of Prostate Gland (WebMD)

Anatomically, the gland is divided into the anterior fibromuscular stroma, transition zone, core zone, and peripheral zone. Most PCa originate in peripheral zone, making it a vital area for clinical assessments (Figure 3). In contrast, BPH is usually related to the transition zone [44].

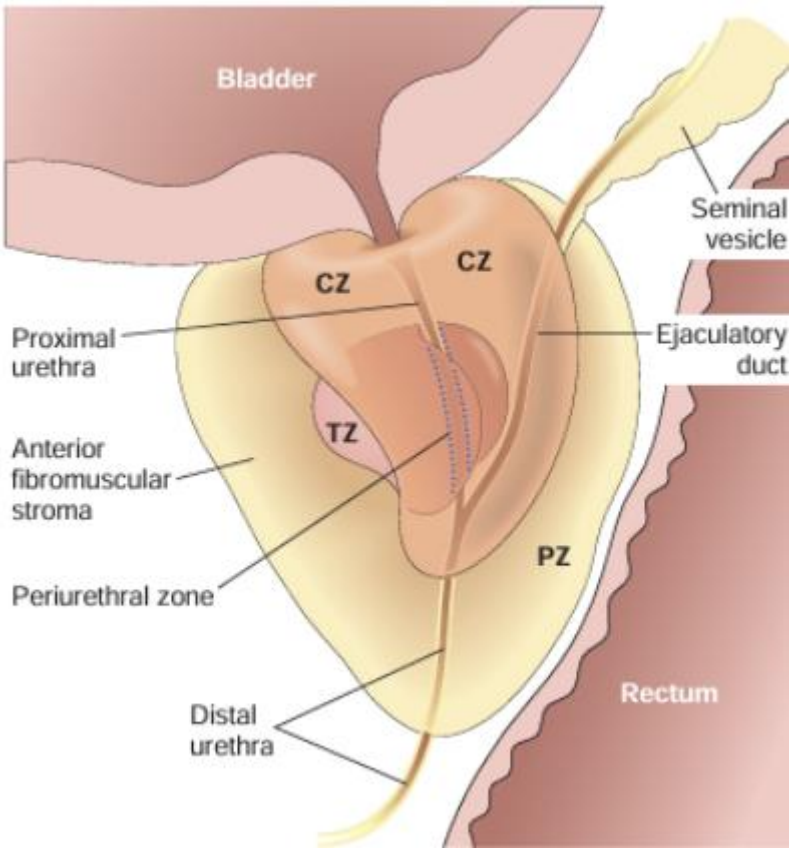


Figure 3: Lobes of Prostate Gland (Kumar V, Abbas AK, Aster JC. Robbins & Cotran pathologic basis of disease. 10th ed. Philadelphia, PA: Elsevier Saunders; 2021.)

3.2 Pathophysiology of Prostate Cancer

A complex relationship of genetic, molecular, and environmental variables leads to prostate cancer (PCa). Both acquired somatic changes, and inherited genetic abnormalities are indicative of its pathophysiology, which disrupts normal cellular development and proliferation (Figure 4)[45].

Cellular and Molecular Mechanisms in Prostate Carcinogenesis

Genetic and epigenetic changes that disrupt the balance between cell division and programmed cell death cause PCa to develop. Prostate cell survival and proliferation depend on the "AR (Androgen Receptor)" signaling system. In cases of PCa, mutations in AR genes or an increase

in AR signaling enable tumor cells to continue growing even in environments with low androgen levels, thereby facilitating the progression of the disease[46].

Genomic instability, which is typified by mutations or deletions in DNA repair genes like BRCA1 and BRCA2 as well as tumor suppressor genes like PTEN and TP53, causes DNA damage to accumulate and promote carcinogenesis. BRCA1 and BRCA2 hold particular importance because their absence disrupts homologous recombination, a vital DNA repair mechanism, which in turn elevates the likelihood of cancer-causing mutations[45,47].

By deactivating tumor suppressor genes and activating oncogenes, epigenetic changes like DNA methylation and histone acetylation contribute significantly to the progression of illness. It helps prostate cancer progress from an androgen-dependent stage to a more difficult and aggressive castration-resistant stage[48].

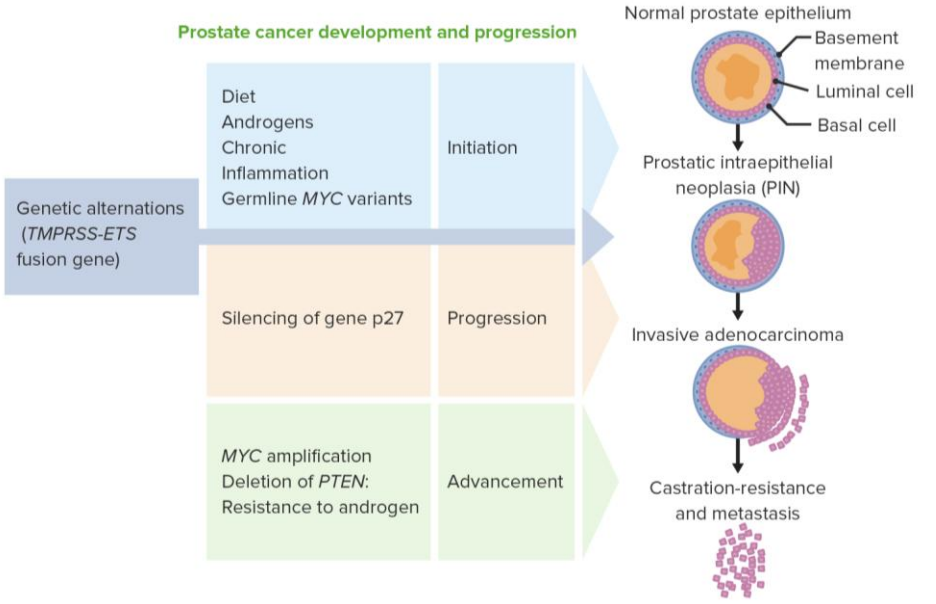


Figure 4: Pathogenesis of Prostate Carcinoma (LecturIo)

Role of Cancer-Associated Fibroblasts, Cytokines, and Growth Factors

The progression of PCa is significantly influenced by the "TME (Tumor Microenvironment)". A major component of the TME, "CAFs (Cancer-Associated Fibroblasts)" actively promote

tumor growth and cancer metastasis. They release a range of growth factors, cytokines, and proteases which alters the extracellular matrix, establish a pro-tumorigenic setting, and aid in invasion of cancer cells[47–49].

Cytokines and Growth Factors:

- **Transforming Growth Factor-beta (TGF-β):** Initially serving as a tumor suppressor, it later facilitates invasion and metastasis during the advanced stages of cancer [49].
- **Fibroblast Growth Factor (FGF):** Stimulates angiogenesis and supports the proliferation of cancer cells [47].
- **Interleukin-6 (IL-6):** Enhances AR signaling and promotes tumor growth in PCa by acting as a growth factor and pro-inflammatory cytokine[45].
- **Vascular Endothelial Growth Factor (VEGF):** Induces angiogenesis, which provides the tumor with the nutrition and oxygen it needs to keep growing [49].

The crosstalk between cancer cells and stromal components of the TME, mediated by these factors, plays a pivotal role in driving metastasis. By releasing matrix metalloproteinases (MMPs), which degrade the extracellular matrix, stromal cells enable cancer cells to infiltrate surrounding tissues and spread to other locations[48].

Metastasis in Prostate Cancer

Prostate cancer cells exhibit a distinct metastatic pattern, with a strong predilection for bones, followed by lymph nodes, lungs, and liver. Cancer spreads to bones more easily when malignant cells interact with the bone's microenvironment. Tumor-released substances such as parathyroid hormone-related protein (PTHrP) increase osteoclast activity, which breaks down bone and creates an environment that is conducive to growth of cancer cells [50].

3.3 Diagnostic Tools in Prostate Cancer

Prostate-Specific Antigen (PSA):

PSA is a glycoprotein enzyme produced by prostate epithelial cells, primarily secreted into seminal fluid. Disruption of glandular architecture in prostate cancer allows PSA to enter the bloodstream, leading to elevated serum levels. PSA testing is commonly employed for the early identification of PCa, tracking the progression of disease, and evaluating the efficacy of treatments. Elevated PSA levels are often the first indication of prostate abnormalities, making it a cornerstone in prostate cancer screening and management[8].

3.4 Gleason Grading System

One well-known histological method for evaluating the structural patterns of PCa is the "Gleason grading system". This technique, that was first presented by Dr. Donald Gleason in the 1960s, evaluates the level of glandular differentiation in prostate cancers and offers vital prognostic data [51].

Explanation of Grading and Groups

The Gleason grading system evaluates a tumor by assigning a score based on the two most common histological patterns it exhibits. It rates each pattern on a scale of 1 to 5 (Figure 5), with lower values indicating well-differentiated glands and higher scores indicating aggressive, poorly differentiated tumors with little glandular structure [51,52].

- **Primary Grade:** Attributed to the most common pattern found in the tumor.
- **Secondary Grade:** Attributed to the second most common pattern.
- **Gleason Score:** The total of the elementary and secondary grades, which range from two to ten.

For clinical purposes, the Gleason scores are grouped into Gleason Grade Groups:

- **“Grade Group 1 (≤6):** Well-differentiated, low-risk cancer.”
- **“Grade Group 2 (3+4=7):** Intermediate risk with predominant well-formed glands”.
- **“Grade Group 3 (4+3=7):** Intermediate risk but with a higher proportion of poorly formed glands”.
- **“Grade Group 4 (8):** High risk, poorly differentiated or cribriform architecture”.
- **“Grade Group 5 (9-10):** High risk, undifferentiated or comedonecrosis patterns”.

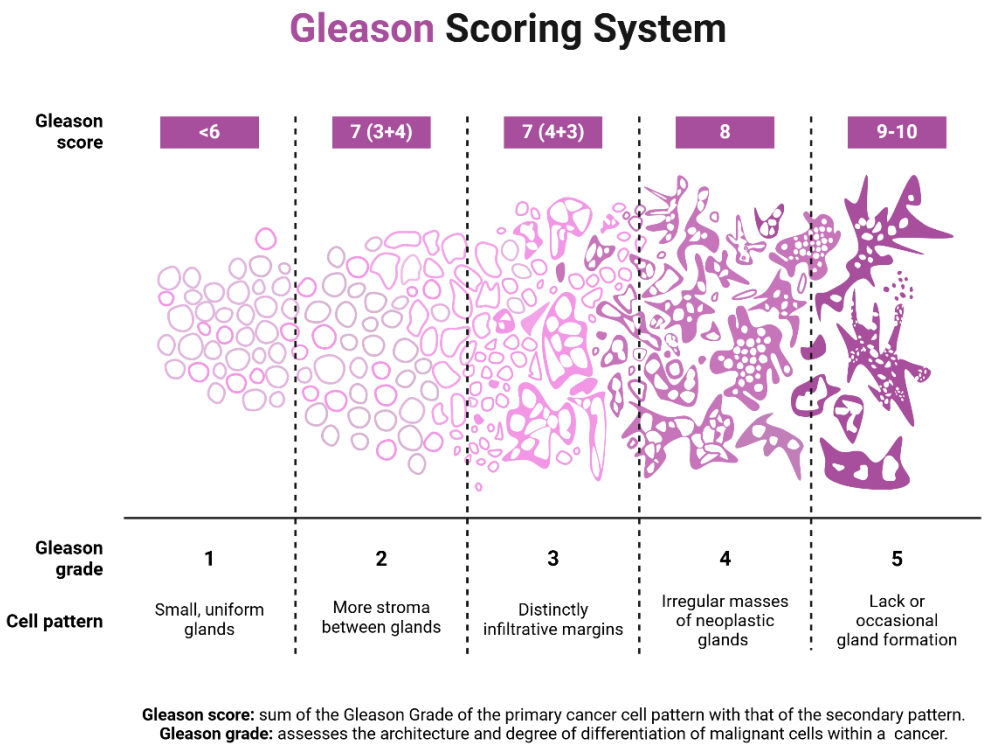


Figure 5: Gleason Grade Group pattern (BioRender)

The Gleason score is essential for risk stratification, guiding treatment decisions, and predicting patient outcomes. A more aggressive type of disease, a higher risk of metastasis, and lower survival rates are all associated with elevated scores. [53].

The 2015 Revision of the Gleason Grading System

In 2015, "International Society of Urological Pathology (ISUP)" introduced significant revisions to the "Gleason system" to improve its accuracy and clinical relevance:

- Introduction of the Grade Group System to simplify communication with clinicians.
- Recognition of cribriform and intraductal carcinoma as adverse prognostic patterns.
- Improved stratification of Gleason 7 tumors into Grade Groups 2 (3+4) and 3 (4+3) to reflect differences in clinical behavior.

The revised system enhances the prognostic utility of the Gleason grading system, aligning pathological findings more closely with clinical outcomes [16,51,52].

3.5 PSA and its Correlation with BRCA1/BRCA2

Studies Correlating PSA Levels with BRCA1/BRCA2 Mutations

Recent research indicates that PSA levels might have specific associations with BRCA1 and BRCA2 mutations in PCa. More advanced stages of the illness and greater "Gleason scores" are common in those with these BRCA1/BRCA2 mutations. Interestingly, some research has suggested that BRCA2-mutated prostate cancers may not always exhibit proportionally elevated PSA levels despite aggressive disease progression. According to a study by Han et al., patients with BRCA2 mutations exhibit lower PSA levels when their disease is advanced, which may cause a delay in detection. Conversely, BRCA1 mutation carriers typically show PSA levels more consistent with disease burden, highlighting differences in how these genetic alterations influence PSA expression[54,55].

The correlation between PSA levels and BRCA mutations further supports the need for genetic testing in prostate cancer patients, particularly in high-risk or early-onset cases. Integrating PSA levels with BRCA mutation status can enhance diagnostic accuracy and risk stratification.

Limitations of PSA in Predicting Aggressive Forms of Prostate Cancer

While PSA continues to be a fundamental tool in PCa screening, its shortcomings in accurately identifying aggressive types of disease are well-recognized. PSA levels alone do not reliably distinguish between indolent and aggressive tumors, leading to potential overdiagnosis and overtreatment. In BRCA-mutated prostate cancers, this limitation is particularly pronounced, as some aggressive cases may present normal or only mildly elevated PSA levels [56].

3.6 Role of BRCA1 and BRCA2 in Cancer

BRCA1/2 expression and high Gleason scores, which signify poorer differentiation and more aggressive tumor characteristics, were shown to be significantly correlated in a research performed in Uganda [57]. BRCA1/2 mutations are uncommon in the general population, yet they occur more frequently within certain ethnic groups, such as Ashkenazi Jews and Nordic populations[58]. Metastatic cases show a significantly higher mutation frequency in prostate carcinoma[59].

Clinical Implications of BRCA Mutations

BRCA mutations are predictive markers for therapeutic responses, particularly in targeted treatments. Individuals with BRCA mutations show heightened responsiveness to “Poly ADP Ribose Polymerase (PARP)” inhibitors that involve olaparib and rucaparib. By specifically targeting cancer cells lacking efficient homologous recombination repair mechanisms, these inhibitors apply the concept of synthetic lethality [60].

The prognostic implications of BRCA mutations extend to survival outcomes. Men with BRCA2 mutations exhibit reduced metastasis-free and cancer-specific survival, emphasizing the need for genetic testing in high-risk populations. Additionally, BRCA1/2 mutation carriers

often experience rapid disease progression, reinforcing their role as critical markers for aggressive disease[1].

Recent advancements in the understanding of BRCA-deficient metastatic prostate cancer (mCRPC) have highlighted the pivotal role of BRCA mutations, particularly BRCA2, as significant prognostic biomarkers. Research utilizing sophisticated cell-free DNA (cfDNA) tests found that individuals with BRCA2 mutations experienced greatly decreased "PFS (Progression-Free Survival)" and "OS (Overall Survival)" in contrast to those without BRCA mutations. Furthermore, BRCA-deficient tumors displayed a distinct genomic phenotype, with a greater incidence of actionable mutations in pathways that include AR and PI3K, highlighting potential for co-targeting strategies in future therapeutic approaches. These findings affirm the critical need for genomic profiling in optimizing treatment for BRCA-associated prostate cancer and advancing precision oncology [61].

3.7 Gleason Grading System

Studies Highlighting the Association Between Gleason Score and BRCA1/BRCA2 Expression

In research by Agalliu et al., 1,251 control individuals and 979 patients with prostate cancer were all Ashkenazi Jews. This case-control study focused on the correlation between three different founder mutations ("BRCA1-185delAG", "BRCA1-5382insC", and "BRCA2-6174delT") and the clinical characteristics and risk of prostate cancer. The findings showed that individuals with the "BRCA2 mutation" had a 3.2-time increased risk of high-grade prostate cancer, defined as a Gleason score of 7 or above (OR 3.18, 95% CI 1.52-6.66) [62].

Mateo et al. analyzed 470 PCa specimens from patients who acquired deadly "mCRPC (metastatic Castration-Resistant Prostate Cancer)" after not receiving any previous treatment. Among these, 61 patients had samples from both their primary tumors and metastatic lesions,

allowing for comparative study. BRCA2 mutations were found in 7% (33 patients) of the cases, which were linked to increased genomic instability and more aggressive disease characteristics. Additionally, the study demonstrated the therapeutic importance of BRCA mutations since patients with these mutations showed increased susceptibility to PARP inhibitors, which are now crucial for the treatment of BRCA-mutated malignancies [63].

Clinical genetics clinics in the UK and Ireland recruited 447 male carriers of the BRCA2 mutation and 376 carriers of the BRCA1 mutation for prospective cohort research by Nyberg et al. The participants had been monitored over a median period of 5.3 years for the BRCA2 group and 5.9 years for the BRCA1 group. 26 people with the BRCA2 mutation were found to have PCa during the follow-up, suggesting a 4.45-fold higher risk (SIR 4.45, 95% CI 2.99–6.61) than the general population. The fact that 65% of BRCA2-related tumors had a "Gleason score" of 7 or higher indicated an address to more aggressive illness. However, BRCA2 carriers under 65 had a 3.99-fold higher risk (95% CI 1.88–8.49), and those with a family history of prostate cancer had a 7.31-fold higher risk (95% CI 3.40–15.7) [64].

Amsi et al. used tissue samples that had been stored to analyze 188 males from Uganda who had been diagnosed with PCa between January 2005 and December 2014. The immunohistochemical assessment showed BRCA1 expression in 26.1% of the cases (49 out of 188) and BRCA2 expression in 22.9% (43 out of 188), with both proteins being co-expressed in only 7.4% (14 out of 188) of the tumors. BRCA1/2 expression was shown to be statistically significantly related to higher Gleason scores (P=0.013 for BRCA1; P = 0.041 for BRCA2), and these proteins were more frequently expressed in tumors that were poorly differentiated than those that were well or moderately differentiated [57].

Lack of BRCA was observed to be significantly related to lower progression-free survival (PFS) and higher Gleason scores in a recent investigation by Fettke et al. that looked at 13

patients with mCRPC. This research highlighted the prognostic importance of BRCA1 and BRCA2 under-expressions or mutations and their impact on predicting clinical outcomes [61].

3.8 Molecular Docking in Cancer Research

Overview of Molecular Docking

The computer technique known as molecular docking, which predicts the preferred orientation of a tiny molecule (ligand) when it is bound to a target protein (receptor), facilitates understanding of molecular interactions that are crucial to drug discovery and development. AutoDock and Discovery Studio are important tools used in molecular docking. AutoDock, developed by Scripps Research, is widely used for protein-ligand docking and has been instrumental in various drug discovery projects. Discovery Studio provides a comprehensive suite for molecular modeling and simulation, enabling visualization and analysis of docking results[32,35].

3.9 Evidence from Global and Indian Studies

Studies conducted worldwide have shown a clear correlation between a greater risk of PCa and mutations in BRCA1 and BRCA2 genes. An extensive investigation featured in Nature charted numerous mutations within the BRCA2 gene, pinpointing specific variants that elevate the risk of numerous cancers, that include PCa [65,66].

There is little information available in India regarding the frequency and consequences of BRCA mutations in patients with PCa. The Indian Council of Medical Research (ICMR) acknowledges that “Inherited mutations of the BRCA1 or BRCA2 genes are linked with familial breast and ovarian cancers and may also be associated with prostate cancer” [67].

PCa is becoming more common in India, and additional investigation is needed to determine the significance of BRCA mutations in Indian males. However, specific studies quantifying this risk in the Indian male population are scarce [68].

4. Materials and Methods

4.1. Study Design

4.1.2 Study Type

This is a prospective observational study designed to evaluate the mRNA expression levels of BRCA1 and BRCA2 in patients diagnosed with prostate carcinoma (PCa) and benign prostatic hyperplasia (BPH). Serum PSA level was analyzed in all cases of carcinoma prostate. Gleason Scoring was applied to slides stained with H&E in every case of prostate carcinoma, and "RT-qPCR (Real-Time quantitative Polymerase Chain Reaction)" was applied on paraffin-embedded tissue samples for all instances of both BPH and prostate carcinoma.

4.1.3 Study Period: The study was conducted from 1st April 2023 to 31st December 2024.

4.1.4 Sample Size Determination

The study's sample size was chosen to provide sufficient power for detecting statistically significant variations in BRCA1 and BRCA2 mRNA expression levels between patients with BPH and those with PCa. A 1:4 ratio between the BPH and PCa groups was anticipated based on the study design. To achieve a statistical power of 80% and a significance level of 5% (two-tailed), a minimum of 43 patients in the PCa group and 10 patients in the BPH group, for a total sample size of 48 patients [57]. The following formula was used to determine the sample size:

$$\boldsymbol{N = 2\left[\frac{\left(Z_{\propto} + z_{\beta}\right) * S}{d}\right]^{2}}$$

Z- Level of significance=95%

Z--power of the study=80%

d=clinically significant difference between two parameters

S = Common standard deviation.

4.2 Patient Recruitment

4.2.1 Inclusion Criteria

- Histopathologically diagnosed cases of carcinoma prostate and benign prostatic hyperplasia.

4.2.2 Exclusion Criteria

- Samples with poor RNA quality (“RNA Integrity Number, RIN <6”), as assessed by an Agilent Bioanalyzer.

4.2.3 Consent and Confidentiality

Participants were provided with detailed information about the study's objectives, procedures, potential benefits, and hazards before enrollment. Each participant provided written informed consent, adhering to institutional and ethical standards.

4.2.4 Ethical Clearance

The Institutional Ethics Committee -BLDE (Deemed to be University) BLDE(DU)/IEC/935/2023-24 granted ethical approval for this investigation.

4.3 Histopathology

4.3.1 Sample Preparation

All prostate biopsy specimens involved in the investigation were preserved in 10% buffered formalin and processed using routine histopathological techniques. The tissue specimens were encased in paraffin blocks, and thin sections were created using a microtome. Hematoxylin and Eosin (H&E) staining was then applied to these sections to help with histomorphologic diagnosis.

4.3.2 Gleason Score Assessment

For prostate carcinoma cases, the “Gleason scoring system” was utilized to grade and classify tumors based on glandular growth patterns. This grading system assigns tumors into 5 distinct “Gleason Grade Groups”:

- “Grade Group 1: Gleason score ≤6”
- “Grade Group 2: Gleason score 3 + 4”
- “Grade Group 3: Gleason score 4 + 3”
- “Grade Group 4: Gleason scores 4 + 4, 3 + 5, or 5 + 3”
- “Grade Group 5: Gleason scores 4 + 5, 5 + 4, or 5 + 5”

The “Gleason Grade Group system” provided a standardized method to evaluate the histopathological aggressiveness of prostate carcinoma and enabled correlations with molecular findings from BRCA1 and BRCA2 expression analyses.

4.4 RNA Extraction

4.4.1 Sample Collection and Storage

All patients with a diagnosis of BP) or PCa had tissue samples taken using FFPE (Formalin-Fixed Paraffin-Embedded), which were kept at room temperature until processing. After RNA extraction, the isolated RNA was stabilized and stored in RNase-free tubes (Eppendorf, Germany) at -80°C to preserve integrity for downstream analyses.

4.4.2 Sectioning and Deparaffinization

Tissue blocks that had been fixed in paraffin and stored in formalin were cut with a microtome into sections that were 8–10µm thick in order to extract RNA. The tissue sections were then transferred into RNase-free microcentrifuge tubes (Eppendorf, Germany) to ensure contamination-free processing. The paraffin was removed from the tissue sections using the following deparaffinization protocol:

1. Xylene Washes: Two washes with 1 mL of xylene for 5 minutes each.
2. Graded Ethanol Series: Tissue rehydration was performed using ethanol solutions of decreasing concentrations:
    - 100% ethanol for 5min.
    - 95% ethanol for 5 minutes.
    - 70% ethanol for 5 minutes.

After deparaffinization and rehydration, the tissue sections were air-dried briefly to remove residual ethanol before proceeding with RNA extraction.

4.4.3 Isolation Protocol

In accordance with the manufacturer's guidelines, total RNA was extracted from deparaffinized tissue sections using the "RNeasy FFPE Kit (Qiagen, Germany)." The key steps in the isolation process were as follows:

1. Proteinase K Digestion: Tissue sections were digested with Proteinase K to break down cross-linked proteins and release RNA from the fixed tissue matrix.
2. RNA Purification: RNA was isolated using a spin-column method, which involved several washing steps to remove contaminants like proteins, DNA, and other impurities.
3. Elution: The RNA that had been purified was dissolved in RNase-free water to ensure it was suitable for subsequent applications.

4.4.4 Quality and Quantity Assessment

The following techniques were employed to evaluate the extracted RNA's quality and quantity:

1. Spectrophotometry:
    - Utilizing a "NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, USA)", the concentration and purity of RNA had been determined.
    - At 260/280nm, the absorbance ratio was measured, with values in the range of 1.8–2.0 considered indicative of pure RNA with minimal protein contamination.
2. Integrity Check:
    - With the help of the "RNA 6000 Nano Kit and the Agilent 2100 Bioanalyzer (Agilent Technologies, USA)", RNA integrity was assessed.

- In order to ensure high-quality RNA for reliable gene expression analysis, samples with an RIN > 6 had been evaluated as appropriate for downstream applications.
- 3 samples showed RNA integrity < 6 which were excluded from the study.

4.5 cDNA Synthesis

4.5.1 Reverse Transcription Setup

cDNA synthesis was performed in accordance with the manufacturer's regulations with the "PrimeScript RT Reagent Kit (Takara Bio Inc., Japan)". The reaction was set up to convert high-quality RNA into cDNA for downstream quantitative PCR (qPCR) analysis. The reaction components for a total volume of 20 µL included:

- RNA Template: 1 µg of extracted RNA.
- 5 Nos. PrimeScript Buffer: 4 µL.
- dNTP Mix: 1 µL (final concentration 10mM).
- Random Primers: 1µL.
- PrimeScript Reverse Transcriptase: 1 µL.
- RNase-Free Water: Added to adjust the final volume to 20 µL.

4.5.2 Reaction Conditions

The reverse transcription procedure had been conducted in a "Bio-Rad T100 Thermal Cycler (Bio-Rad, USA)" as per the manufacturer's specifications.

1. Initial Priming: Incubate at 37°C for 15min to enable random primers to bind to the RNA template.
2. Reverse Transcription: PrimeScript Reverse Transcriptase is used to synthesize cDNA, and it is incubated at 42°C for 30min.
3. Enzyme Inactivation: For five minutes, the reverse transcription reaction is stopped, and the enzyme becomes inactive by heating it to 85°C.
4. Final Storage: The synthesized cDNA was immediately cooled on ice and stored at -20°C for downstream applications.

The reaction volume was set to 20µL, ensuring efficient conversion of RNA to high-quality cDNA for quantitative PCR (qPCR) analysis.

4.6 Primer Design and Validation

4.6.1 Primer Design

The "NCBI Primer-BLAST tool" was utilized to generate primers for the target genes BRCA1, BRCA2, and the housekeeping gene GAPDH to ensure efficiency and specificity. The following criteria were applied during primer design to optimize their performance in quantitative PCR (qPCR):

- Targeted amplicons were designed to be 70–200 base pairs in length, suitable for qPCR and degraded RNA from FFPE samples.
- The primers were designed with a Guanine-Cytosine content of 40–60% to ensure optimal binding and amplification.

- Both forward as well as reverse primers were selected to have similar melting temperature (Tm) values, ideally between 58°C and 60°C, to maintain uniform annealing conditions.
- Primer sequences were screened to minimize the formation of secondary structures such as dimers or self-annealing regions.
- The primer sequences were cross-checked against the NCBI nucleotide database to ensure specificity for the target genes and to avoid off-target amplification.

4.6.2 Primer Sequences

The following primer sequences were designed and validated for quantitative PCR (qPCR) analysis of BRCA1, BRCA2, and the housekeeping gene GAPDH:

1. BRCA1:
    - Forward: 5'-CAGAGGACAATGGCTTCCATG-3'
    - Reverse: 5'-AATTGGGCAGATGTGTGAGGCACCTG-3'
2. BRCA2:
    - Forward: 5'-CCAAGTGGTCCACCCCAAC-3'
    - Reverse: 5'-ACTGTACTTCAGGGCCGTACACTGCTCAAA-3'
3. GAPDH (Housekeeping Gene):
    - Forward: 5'-GAAGGTGAAGGTCGGAGTCAAC-3'
    - Reverse: 5'-CAGAGTTAAAAGCAGCCCTGGT-3'

These primers had been designed to produce amplicons of approximately 70–200 base pairs in length. Primer specificity was confirmed using NCBI Primer-BLAST tool.

4.6.3 Validation

The following validation steps were conducted to guarantee the precision and effectiveness of the primers that were designed:

1. Test PCR and Gel Electrophoresis
    - Using the designed primers, a PCR test was conducted, and the results had been analyzed through agarose gel electrophoresis. A 1.5% agarose gel stained with ethidium bromide (Sigma-Aldrich, USA) was utilized to evaluate the amplified results. For each target gene (BRCA1, BRCA2, and GAPDH), the appearance of a single band of the expected size confirmed the primers' specificity and the amplification process' accuracy.
2. Melt Curve Analysis:
    - A melt curve analysis was conducted after every qPCR experiment to verify the amplified product's specificity. The temperature gradually increased from 60°C to 95°C in tiny increments, such as 0.5°C per second, to produce this curve. The melt curve showed a single, clear peak, indicating that there were no primer-dimers or nonspecific products present and that the target gene had been amplified specifically.

4.7 Quantitative PCR (qPCR) Analysis

4.7.1 Master Mix Preparation

qPCR had been performed utilizing the “TB Green Premix Ex Taq II (Takara Bio Inc., Japan)”, a master mix based on SYBR Green that is fine-tuned for exceptional sensitivity and specificity. The reaction mixture for each specific gene target (BRCA1, BRCA2, and GAPDH) was assembled as follows:

- “2× TB Green Premix Ex Taq II: 10µL”
- “Forward Primer (10 µM): 0.5µL”
- “Reverse Primer (10 µM): 0.5µL”
- “Template cDNA: 1µL (equivalent to ~25 ng)”
- “Nuclease-Free Water: 8µL”

Total Reaction Volume: 20µL per well

This reaction setup was prepared in a 96-well plate format. Negative controls (no template control, NTC) were included to detect any false positive amplification. For reproducibility, all the reactions were performed in triplicate.

4.7.2 Thermal Cycling Parameters

The “Bio-Rad CFX96 Real-Time PCR System (Bio-Rad, USA)” was employed to perform quantitative PCR (qPCR) under the following thermal cycling parameters:

1. Initial Denaturation:

- Ten minutes at 95°C will activate the enzyme and ensure that the cDNA template is completely denaturated.

2. Amplification Cycles (40 cycles):
    - Denaturation: DNA strands are separated at 95°C for 15sec.
    - Annealing/Extension: 30 seconds at 60°C to enable primer binding and target gene amplification.
3. Melt Curve Analysis:
    - Performed to verify the qPCR products' specificity at the end of the amplification cycles.
    - Fluorescence was observed when the temperature increased by 0.5°C increments from 60°C to 95°C.
    - Specific amplification without primer-dimers or nonspecific products was verified by a single, distinct peak in the melt curve.

These conditions were optimized to ensure accurate and reliable quantification of gene expression for BRCA1, BRCA2, and the housekeeping gene GAPDH.

Agarose Gel Electrophoresis:

- Selected qPCR results were performed on agarose gel (1.5%) stained with ethidium bromide (“Sigma-Aldrich, USA”) in order to confirm the anticipated amplicon size.
- A “UV transilluminator (Bio-Rad Gel Doc Imaging System)” was employed for observing the gel.

- For each target gene (BRCA1, BRCA2, and GAPDH), a single band of the anticipated size provided additional evidence of the amplification's selectivity.

4.8 Data Analysis

Normalization

The housekeeping gene GAPDH was utilized as the internal control to standardize the qPCR results in order to ensure precise gene expression quantification.

- Calculation of ΔCT:

  The "cycle threshold (CT)" values for the target genes, BRCA1 and BRCA2, were adjusted relative to the CT value of reference gene, GAPDH, by applying the following formula:

$$\Delta CT = CT_{Target} - CT_{GAPDH}$$

This step adjusts for variations in sample input and ensures that the expression levels of the target genes are accurately quantified relative to the reference gene. The normalized values were used for subsequent analysis, including relative expression calculations.

Relative Quantification

To evaluate the relative expression levels of BRCA1 and BRCA2, the following steps were taken:

1. Calculation of ΔΔCT:

The normalized ΔCT values for the experimental samples (prostate carcinoma group) were compared to the ΔCT values of the control samples (benign prostatic hyperplasia group) using the formula:

$$\Delta\Delta CT = \Delta CT_{\{sample\}} - \Delta CT_{\{control\}}$$

2. Calculation of Fold Change:

   The 2^(-ΔΔCT) approach had been employed to evalaute the relative fold change in gene expression. This approach offers a quantitative assessment of the variation in expression levels between the two groups:

$$Fold\ Change = 2^{\{-\Delta\Delta CT\}}$$

3. Calculation of Log2 Fold Change:

   To present the data on a logarithmic scale, the fold change was converted to its base-2 logarithm:

$$Log2\ Fold\ Change = log2(Fold\ Change)$$

A log2 fold change that is positive signifies that the target gene is upregulated in experimental group in contrast to the control group. Conversely, a negative log2 fold change signifies that the target gene is downregulated in the experimental group. This method guaranteed precise relative measurement of mRNA expression levels for the target genes BRCA1 and BRCA2.

Statistical Analysis

Statistical analyses had been conducted by employing **Jamovi** (Version 2.6) and **R** (Version 4.4), ensuring the use of modern and reliable statistical software. The following tests and analyses were performed:

- **Normality Testing**: The “Shapiro-Wilk test” had been employed to determine whether data followed a normal distribution.
- **Group Comparisons**:
  - **Mann Whitney U Test**: To compare means between prostate carcinoma (PCa) and benign prostatic hyperplasia (BPH) groups.
  - **ANOVA**: To compare means across multiple groups.
- **Correlation Analysis**: The relationships between PSA levels and gene expression levels (as expressed as fold change) were examined using the Spearman correlation coefficient.

“Mean ± standard deviation (SD)” was employed to report the outcomes. A p-value <0.05 was considered statistically significant.

4.9 Validation and Reproducibility

Technical Replicates

To guarantee that the outcomes are both reliable and reproducible, the following steps were implemented:

- qPCR Replicates: Each sample was run in triplicate during qPCR to minimize variability and ensure consistent amplification.
- cDNA Synthesis Replicates: The reverse transcription process was repeated for selected RNA samples to confirm the consistency of cDNA synthesis and eliminate technical bias.

Controls

Appropriate controls were included in all qPCR runs to validate the accuracy of the reactions:

- **No-Template Control (NTC)**: Reactions were performed without cDNA templates to detect potential contamination in reagents or reaction setups.
- **Positive Control**: Known reference RNA was included to verify the efficiency of the qPCR process and serve as a benchmark for successful amplification.

4.10 In Silico Analysis

Retrieval of Target Protein Structures

- **Protein Source**:

  To ensure high-quality, experimentally validated molecular data for the docking experiments, the target proteins' X-ray crystallographic structures were acquired from the “Protein Data Bank (PDB)”.

  - **BRCA1**: PDB ID 3FA2
  - **BRCA2**: PDB ID 1IYJ

- **Human Kallikreins**:

  The structures of human kallikreins (hK1, hK2, hK3, and hK4) which are the fundamental proteins found in Prostate-Specific Antigen (PSA), were obtained from the PDB with the following respective IDs:

  - **hK1**: PDB ID 1SPJ

- **hK2**: PDB ID 5HEX
- **hK3**: PDB ID 3HM8
- **hK4**: PDB ID 2BDG

These protein structures were used as templates for molecular docking and protein-protein interaction (PPI) studies, forming the basis for analyzing interactions between BRCA1/BRCA2 and kallikrein proteins.

Protein Preparation

- The protein structures were prepared for molecular docking using Discovery Studio (BIOVIA, Dassault Systèmes)
- **Steps in Preparation**:
    1. To consider hydrogen bonding interactions during docking, polar hydrogen atoms were incorporated into the protein structures.
    2. Non-essential components such as water molecules, ions, and other small molecules present in the co-crystallized structures were removed.
    3. The processed protein structures were subjected to energy minimization using the Swiss PDB Viewer to stabilize the conformation and eliminate steric clashes or strain.
- **Active Site Identification**:
    - The active site of each protein was identified using the PDBsum database, supported by relevant literature.

- Active site residues were defined based on co-crystallized ligand structures and experimental evidence, ensuring accurate targeting during molecular docking studies.

Retrieval of Selected Peptide Sequences

- The selected peptides for human kallikreins (hK1, hK2, hK3, and hK4) had been obtained from the Brookhaven PDB.
- The binding sites for these peptides were confirmed by analyzing protein-ligand associations using experimental evidence and structural data from the PDB. This approach ensured precise localization of active sites, facilitating accurate docking simulations with the target proteins (BRCA1 and BRCA2).

Docking Studies

- Molecular docking simulations were conducted using **Autodock 4.2**.
- **Docking Approach**:
    1. A genetic algorithm was applied to explore the docking space, allowing for flexible conformations of the human kallikreins (hK1, hK2, hK3, and hK4) and target proteins (BRCA1 and BRCA2).
    2. Both receptor and ligand structures were kept flexible to generate multiple conformations and identify optimal binding interactions.

- The docking simulations were performed on an Intel® Core™ i9 Dell Laptop equipped with a 16GB NVIDIA RTX 4060 GPU and 32GB RAM, running on Windows 11 operating system.

Visualization and Analysis

- All docking results had been analyzed and visualized with the help of "Discovery Studio (BIOVIA, Dassault Systèmes)". This tool enabled a detailed examination of the interactions between the target proteins (BRCA1 and BRCA2) and the human kallikreins (hK1, hK2, hK3, and hK4).
- **Output Analysis**:
    1. **Protein-Protein Interactions (PPIs)**:
        - The interactions between BRCA1/BRCA2 and kallikreins were evaluated to identify key contact points, hydrogen bonding, hydrophobic interactions, and other binding characteristics.
    2. **Ranking of Results**:
        - The docking poses were ranked based on key parameters:
            - **Binding Energy**: Lower binding energy values indicated stronger binding interactions.
            - **Inhibition Constant (Ki)**: The calculated inhibition constant was used to assess the strength and potential efficacy of the interaction.
    3. **Identification of Bioactive Molecules**:

- The docking results were analyzed to pinpoint the most suitable protein for each target.

5. Results

5.1 Overview

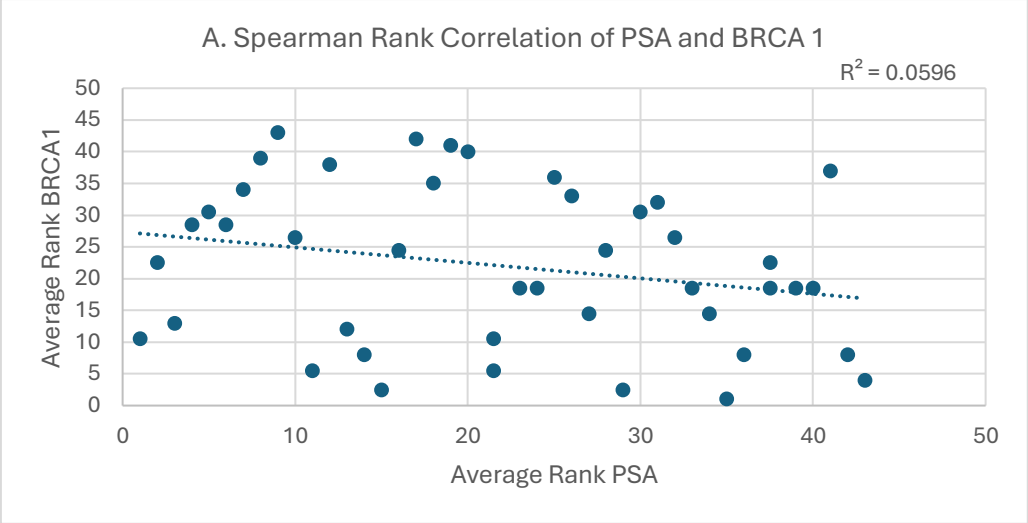
The study evaluates the relative expression of BRCA1 and BRCA2 with respect to GAPDH (Housekeeping Gene), their correlation with PSA levels, and their variation across different Gleason grades which aids in providing insights into prostate carcinoma progression and prognosis.

5.2 Results and Analysis

5.2.1 Correlation between PSA and BRCA

Table 1: Spearman's Rank Correlation Matrix Illustrating the Relationship Between PSA Levels, BRCA1 and BRCA2 Expression, and Gleason Grade

		PSA Level (ng/µL)
CT (BRCA1)	**Spearman's rho**	-0.244
	p-value	0.057
CT (BRCA2)	**Spearman's rho**	-0.291*
	p-value	0.029*
Gleason Grade	**Spearman's rho**	0.072
	p-value	0.677



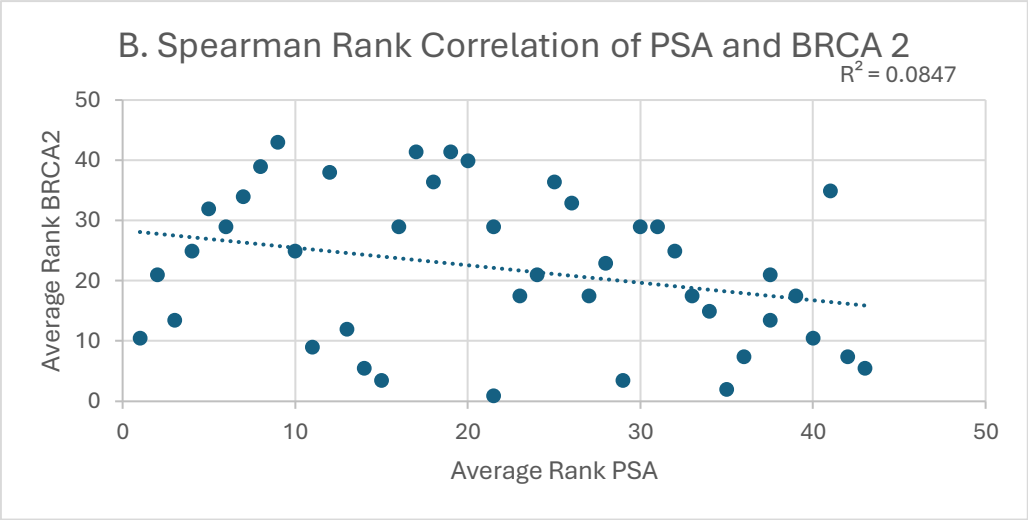


Figure 6: A) Spearman Rank Correlation of PSA and BRCA1 B) Spearman Rank Correlation of PSA and BRCA2.

Spearman's rank correlation was used to examine the relationship among PSA levels, BRCA1 and BRCA2 expression, and Gleason grade (Table 1). PSA levels and CT (BRCA1) showed a slight negative connection that was not statistically significant (Spearman's rho=-0.244, p = 0.057). Higher PSA levels are linked to reduced BRCA2 expression, according to a substantial weak negative correlation between PSA levels and CT (BRCA2) (Spearman's rho = -0.291, p = 0.029). No significant correlations had been noted between Gleason grade and the expression of BRCA1 or BRCA2 ($p > 0.05$). Scatterplots illustrating these correlations reveal a negative

relationship between PSA levels and BRCA2 expression (Figure 6B), whereas no distinct pattern is observed for BRCA1 (Figure 6A). These findings suggest a connection between prostate cancer's downregulated BRCA2 and PSA levels.

5.2.1 BPH Group

Table 2: Descriptives of BPH Group (Cycle Thresholds of BRCA1 and BRCA2 to determine mRNA expression)

	CT (GAPDH)	**CT (BRCA1)**	**CT (BRCA2)**	**ΔCT (BRCA1)**	**ΔCT (BRCA2)**
N	10	10	10	10	10
Mean	18.9	24.7	25.3	5.76	6.36
Standard deviation	0.306	0.294	0.302	0.217	0.241

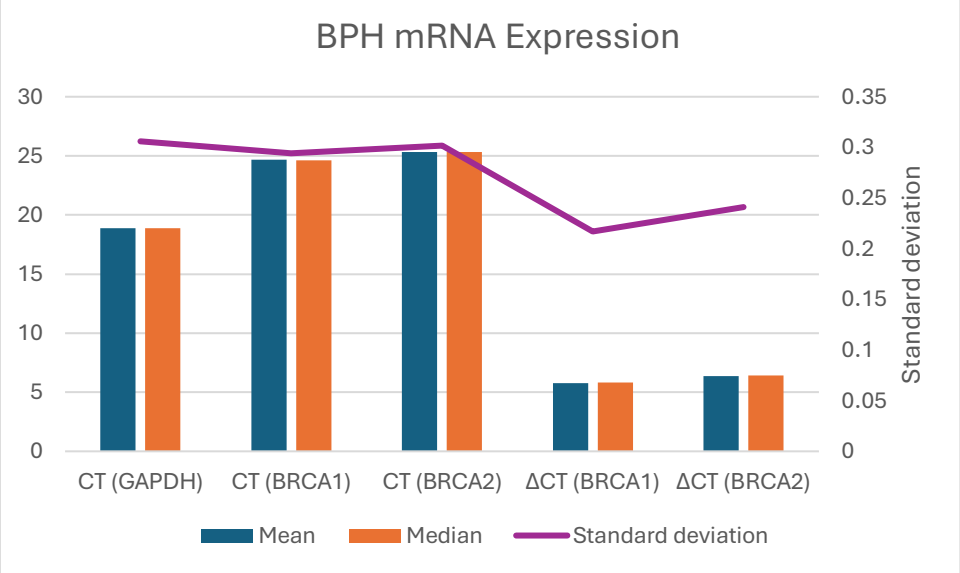


Figure 7: Barchart depicting mean mRNA Expression of BRCA1 and BRCA2 in BPH Group.

Table 2 summarizes the analysis of BRCA1 and BRCA2 mRNA expression in the BPH group (N = 10). GAPDH's cycle threshold (CT) value ranged from 18.5 to 19.5, with an average of 18.9 (±0.306). The mean CT values for BRCA1 and BRCA2 were 24.7 (±0.294) and 25.3 (±0.302), respectively. The ranges for BRCA1 and BRCA2 were 24.3 to 25.2 and 24.9 to 25.8, respectively, indicating consistent measurements across the samples.

A bar chart depicting the mean mRNA expression (Mean ± SD) of BRCA1 and BRCA2 in the BPH group is included to visually represent these findings (Figure 7).

5.2.2 Carcinoma Prostate Group

BRCA1 mRNA Expression

Table 3: mRNA Expression of BRCA1 in Carcinoma Prostate

	CT (GAPDH)	**CT (BRCA1)**	**ΔCT (BRCA1)**	**ΔΔCT (BRCA1)**	**Fold Change (BRCA1)**	**Log2 Fold Change (BRCA1)**
N	43	43	43	43	43	43
Mean	21.4	28.2	6.78	1.02	0.527	-1.02
Standard deviation	0.643	1.03	0.561	0.561	0.176	0.561

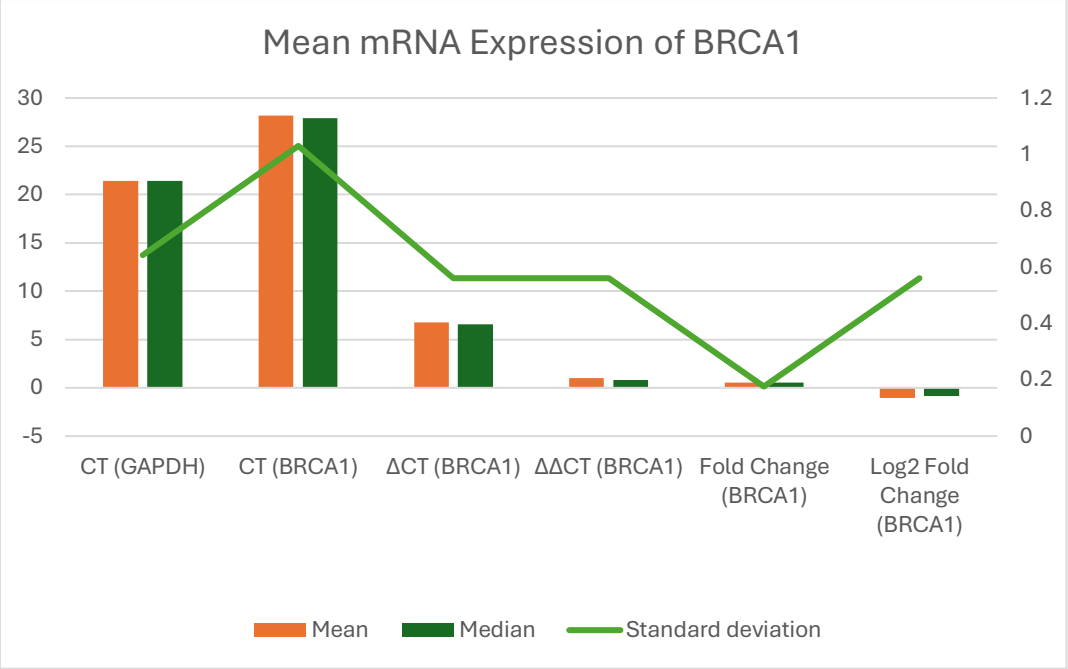


Figure 8: Mean mRNA Expression and Fold Changes of BRCA1 in Carcinoma Prostate patients.

The mRNA expression of BRCA1 was analyzed in the carcinoma prostate group (N = 43) and is summarized in Table 3. The average CT value for GAPDH was 21.4 (±0.643), with values ranging from 20.1 to 22.5. In contrast, the mean CT value for BRCA1 was 28.2 (±1.03), with a range between 26.7 and 30.4. The ΔCT (BRCA1), which shows how much BRCA1 is expressed in comparison to GAPDH, ranged from 6.0 to 7.9 and averaged 6.78 (±0.561).

The ΔΔCT (BRCA1), indicating relative expression compared to the control (BPH), had a mean of 1.02 (±0.561), with a range of 0.24 to 2.14. The fold change for BRCA1 expression was 0.527 (±0.176), with a corresponding log2 fold change of -1.02 (±0 .561), highlighting the underexpression of BRCA1 in carcinoma prostate samples compared to BPH.

A bar chart depicting the mean mRNA expression (Mean ± SD) of BRCA1 in carcinoma prostate is included to visually highlight the high CT values and low fold changes (Figure 8)

BRCA2 mRNA Expression

Table 4: mRNA Expression of BRCA2 in Carcinoma Prostate

	CT (GAPDH)	CT (BRCA2)	ΔCT (BRCA2)	ΔΔCT (BRCA2)	Fold Change (BRCA2)	Log2 Fold Change (BRCA2)
N	43	43	43	43	43	43
Mean	21.4	28.9	7.53	1.17	0.481	-1.17
Standard deviation	0.643	1.07	0.594	0.594	0.181	0.594

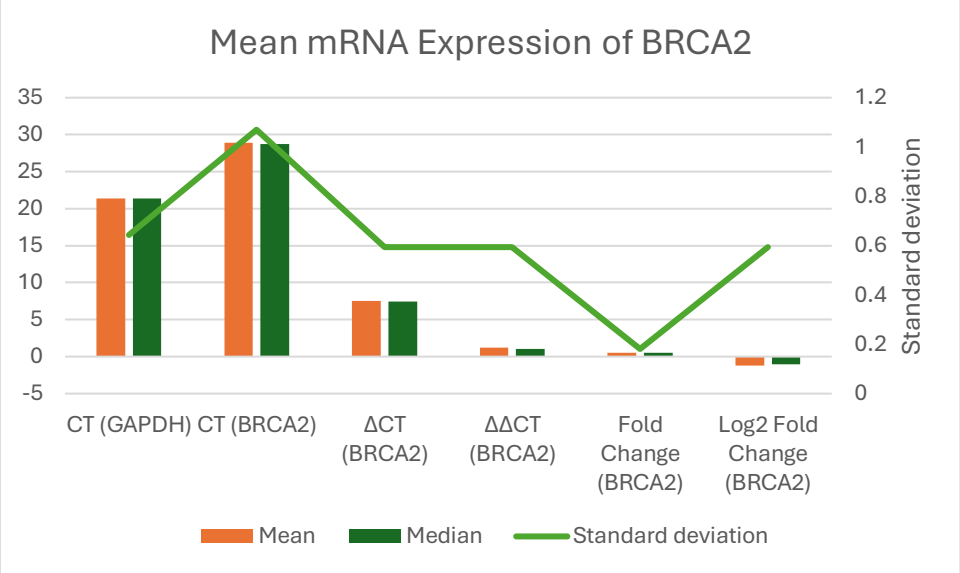


Figure 9: Mean mRNA Expression and Fold Changes of BRCA2 in Carcinoma Prostate patients

Table 4 provides a summary of the analysis of BRCA2 mRNA expression in the group with prostate carcinoma (N = 43). The mean CT value for BRCA2 was 28.9 (±1.07), ranging from 26.9 to 31.2. The relative expression of BRCA2 to GAPDH, represented by ΔCT (BRCA2), had a mean value of 7.53 (±0.594) with a range of 6.5 to 8.7.

The ΔΔCT (BRCA2) had a mean of 1.17 (±0.594), with a range of 0.14 to 2.34. The fold change for BRCA2 expression was 0.481 (±0.181), with a corresponding log2 fold change of -1.17 (±0.594). These results reflect trends similar to BRCA1, with significantly higher CT values and lower fold changes indicating marked under expression of BRCA2 in carcinoma prostate samples.

A bar chart depicting the mean mRNA expression (Mean ± SD) of BRCA2 in carcinoma prostate is included to visually demonstrate the consistent under expression trends observed in carcinoma prostate samples (Figure 9).

Both BRCA1 and BRCA2 demonstrate significantly higher CT values and lower fold changes in carcinoma prostate samples, indicating their substantial under expression compared to BPH.

5.3 Comparison Between BPH and Carcinoma Prostate

The Mann-Whitney U test was used to compare the BRCA1 and BRCA2 mRNA expression between the BPH and cancer prostate groups because of abnormalities.

For BRCA1, the carcinoma prostate group exhibited significantly higher CT values ($p < .001$), indicating reduced expression compared to the BPH group. Similarly, the ΔCT values for BRCA1 were significantly higher in the carcinoma prostate group ($p < .001$). For BRCA2, a similar pattern was observed. The group with prostate carcinoma showed notably higher CT values ($p < .001$) and ΔCT values ($p < .001$) when compared to the BPH group. This suggests a decreased relative expression of BRCA1 and BRCA2 in prostate carcinoma samples.

5.4 mRNA Expression with Respect to Gleason Grade

Table 5: mRNA Expression of BRCA1 with respect to Gleason Grade

	Gleason Grade	CT (GAPDH)	CT (BRCA1)	Fold Change (BRCA1)	Log2 Fold Change (BRCA1)
Mean	**1**	20.9	27.4	0.599	-0.74
	2	21.4	27.8	0.655	-0.622
	3	21.2	27.8	0.573	-0.84
	4	21.5	28.3	0.504	-1.06
	5	21.8	29.3	0.325	-1.73
Standard deviation	**1**	NA	NA	NA	NA
	2	0.33	0.31	0.0866	0.183
	3	0.592	0.77	0.136	0.338
	4	0.618	0.852	0.169	0.492
	5	0.852	1.24	0.148	0.549

The mRNA expression of BRCA1 showed a progressive decline across Gleason grades 1 to 5 in carcinoma prostate samples (n = 1, 11, 15, 6, and 10, respectively; Table 5). While the CT values for GAPDH remained consistent across grades, the CT values for BRCA1 increased

from 27.4 in grade 1 (n = 1) to 29.3 in grade 5 (n = 10), reflecting reduced expression. The fold change decreased from 0.599 in grade 1 to 0.325 in grade 5, and the log2 fold change became progressively more negative, ranging from -0.74 to -1.73. These findings show an inverse correlation between Gleason grade and BRCA1 expression, with higher tumor grades exhibiting significant under-expression of BRCA1, as visualized in the log2 fold change graph (Figure 10).

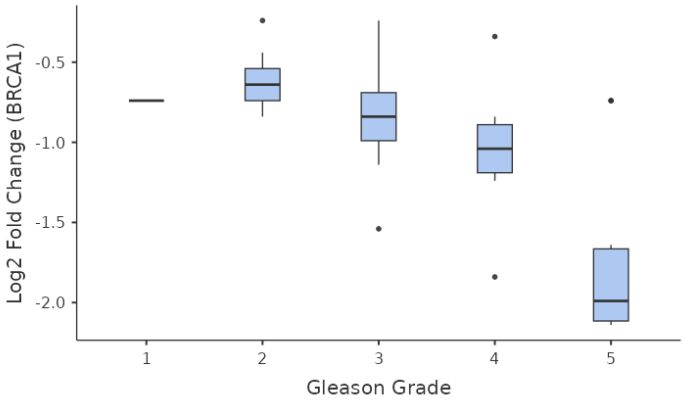


Figure 10: Log2 Fold change of BRCA1 in carcinoma prostate with respect to Gleason grade.

5.4.2 BRCA2

Table 6: mRNA Expression of BRCA2 with respect to Gleason Grade

	Gleason Grade	CT (GAPDH)	CT (BRCA2)	Fold Change (BRCA2)	Log2 Fold Change (BRCA2)
Mean	**1**	20.9	28.2	0.521	-0.94
	2	21.4	28.5	0.586	-0.795
	3	21.2	28.5	0.513	-1
	4	21.5	29	0.502	-1.11
	5	21.8	30.1	0.301	-1.88
Standard deviation	**1**	NaN	NaN	NaN	NaN
	2	0.33	0.301	0.124	0.27
	3	0.592	0.754	0.131	0.331
	4	0.618	1.16	0.227	0.615
	5	0.852	1.29	0.167	0.629

The mRNA expression of BRCA2 also demonstrated a progressive decline from Gleason's grade 1 to 5 in carcinoma prostate samples (n = 1, 11, 15, 6, and 10, respectively), mirroring the trend observed for BRCA1 (Table 6). The CT values for GAPDH showed minimal variability, ranging from 20.9 in grade 1 to 21.8 in grade 5, while the CT values for BRCA2 increased from 28.2 in grade 1 to 30.1 in grade 5, indicating reduced expression relative to GAPDH. The fold change values decreased from 0.521 in grade 1 to 0.301 in grade 5, and the corresponding log2 fold change values became progressively more negative, ranging from -0.94 in grade 1 to -1.88 in grade 5 (Figure 11).

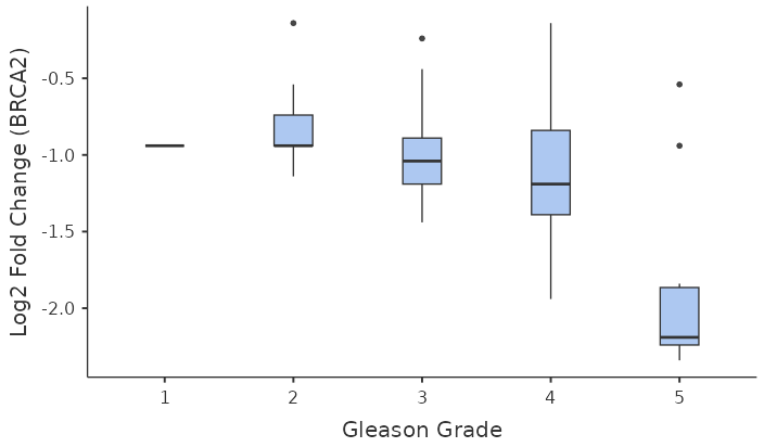


Figure 11: Log2Fold change of BRCA2 in Carcinoma Prostate with respect to Gleason Grade

5.5 Statistical Analysis of Gleason Grade

5.5.1. BRCA1

Table 7: Comparison of Log2Fold Change of mRNA Expression of BRCA1 with respect to Gleason Grade

ANOVA – Log2 Fold Change (BRCA1)	**Sum of Squares**	**p**
Gleason Grade	5.86	**<.001**
Post Hoc Comparisons - Gleason Grade	Gleason Grade	p_{tukey}
1	2	1
	3	0.993
	4	0.743
	5	0.095
2	3	0.528
	4	0.04
	5	**<.001**
3	4	0.38

	5	**<.001**
4	5	0.074

The relationship between Gleason grade and the log2 fold change of BRCA1 was analyzed using one-way ANOVA, which revealed a statistically significant effect ($F = 12.6$, $p < .001$) (Table 7). “Tukey’s HSD post-hoc analysis” further identified significant pairwise differences between Gleason grade 5 and lower grades. Specifically, grade 5 tumors exhibited significantly lower BRCA1 expression compared to grades 2 (mean difference = 0.9836, $p < .001$) and 3 (mean difference = 0.772, $p < .001$). Furthermore, grade 2 tumors showed higher expression than grade 4 (mean difference = 0.5103, $p = 0.04$). These findings highlight the progressive under expression of BRCA1 with increasing Gleason grade.

5.5.2 BRCA2

Table 8: Comparison of Log2Fold Change of mRNA Expression of BRCA2 with respect to Gleason Grade

ANOVA – Log2 Fold Change (BRCA2)	**Sum of Squares**	**p**
Gleason Grade	5.76	**<.001**
Post Hoc Comparisons - Gleason Grade	**Gleason Grade**	$\mathbf{p_{tukey}}$
1	**2**	1
	3	0.975
	4	0.658
	5	0.065
2	**3**	0.303
	4	0.022
	5	**<.001**
3	**4**	0.413
	5	**<.001**
4	**5**	0.065

A similar analysis was performed for the log2 fold change of BRCA2, and a one-way ANOVA revealed that Gleason grade had a significant impact ($F = 13.7$, $p < .001$). Significant differences between grade 5 and the other grades were found by the subsequent Tukey's HSD post-hoc test; grade 5 tumors had considerably lower BRCA2 expression than grades 2 (mean difference = 0.9877, $p < .001$) and 3 (mean difference = 0.735, $p < .001$). Furthermore, grade 2 tumors demonstrated higher expression levels than grade 4 (mean difference = 0.5277, $p = 0.022$) (Table 8). These results reinforce the consistent under expression of BRCA2 with increasing Gleason grade. These analyses confirm a strong relationship between increasing Gleason grade

and the progressive under expression of both BRCA1 and BRCA2, suggesting their potential as molecular markers of prostate cancer aggressiveness.

5.6 *In Silico* Study

Table 9: The PDB format of the crystal structures of the target proteins.

S.No	Protein Name	PDB ID	Resolution	Molecular weight (kDa)
01	BRCA1	3FA2	2.20 A	50.48
02	BRCA2	1IYJ	3.40A	200.02

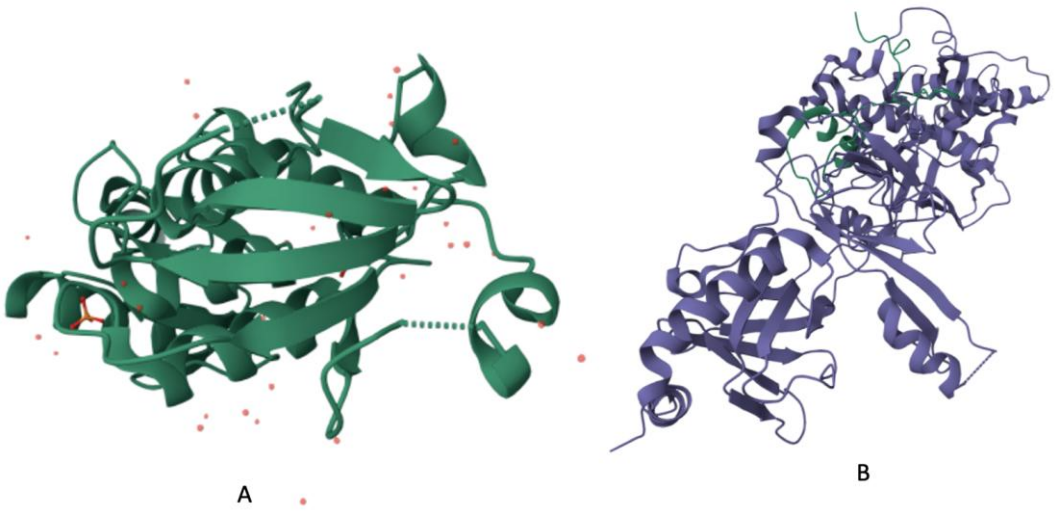


Figure 12: X-ray crystallographic structures of BRCA 1 and BRCA2 proteins retrieved from PDB

Table 9 presents the PDB ID and structural details of the target proteins, BRCA1 and BRCA2. The X-ray crystallographic structure of BRCA1, identified by PDB ID 3FA2, comprises 3439 amino acids, has a molecular weight of 50.48kDa, and is resolved at 2.20 Å. Meanwhile, the

structure of BRCA2, with PDB ID 1IYJ, includes 10092 amino acids, a molecular weight of 200.02 kDa, and is resolved at 3.40 Å, as depicted in Figure 12. These structures had been collected from the PDB.

Table 10: PDB format of the crystal structure of the peptide

S.No	Protein Name	PDB ID	Resolution	Molecular weight kDa
01	HK1	1SPJ	1.70 A	26.19
02	HK2	5HEX	2.73A	208.4
03	HK3	3HM8	2.80 A	195.23
04	HK4	2BDG	1.95 A	48.48

Table 10 presents the PDB IDs for prostate-specific antigen proteins, namely hK1, hK2, hK3, and hK4. The X-ray crystallographic structures of these proteins are as follows: hK1 (PDB ID 1SPJ) comprises 2211 amino acid residues, has a molecular weight of 26.79kDa, and is resolved at 1.70 Å. In contrast, hK2 (PDB ID 5HEX) is resolved at 2.73 Å, has 13830 amino acid residues, and has a molecular weight of 208.4kDa. hK3 (PDB ID 3HM8) is resolved at 2.80 Å, has a molecular weight of 195.23 kDa, and has 12633 amino acid residues. hK4 (PDB ID 2BDG) has a molecular weight of 48.48kDa, is resolved at 1.95 Å, and consists of 3658 amino acid residues.

Docking Studies:

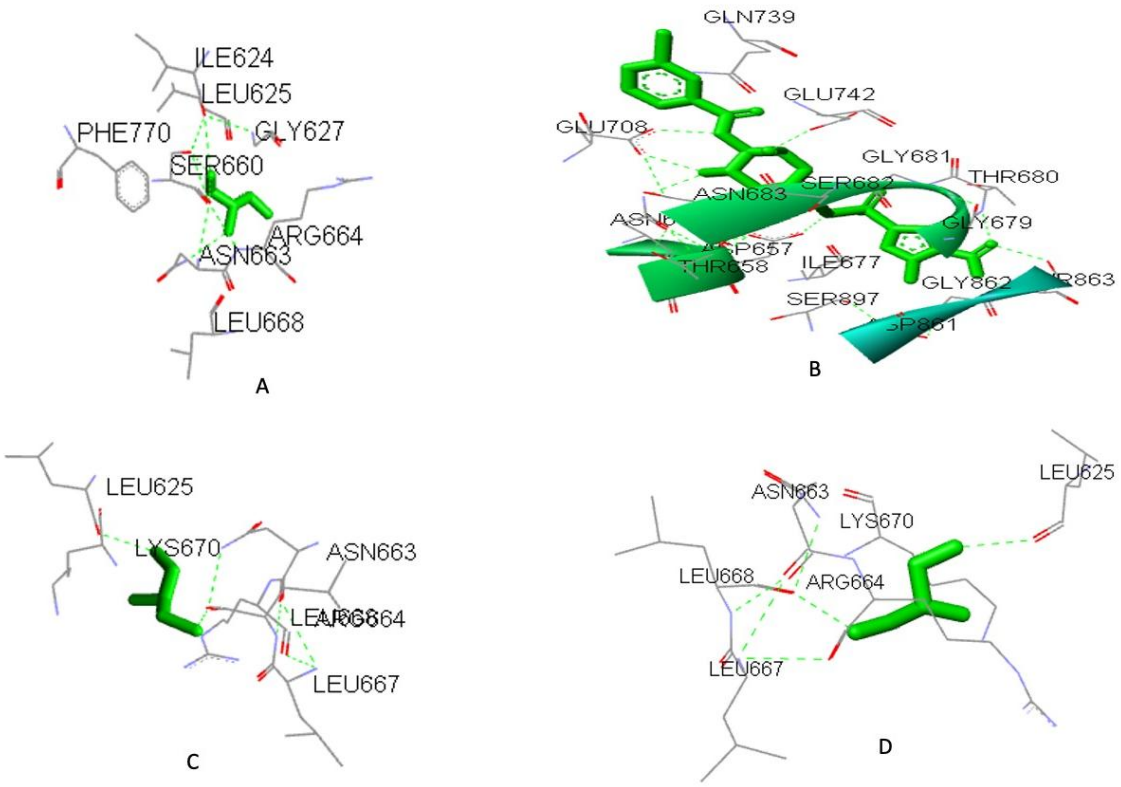


Figure 13: Molecular Docking Analysis of BRCA1 with hK Peptides

Panel A–D depicts the molecular docking interactions between BRCA1 and different hK peptides. The docked ligand (hK peptide) is shown in green, while the receptor protein (BRCA1) is represented with its secondary structure elements in blue (β-sheets) and gray (loops and helices). Hydrogen bond interactions are illustrated with green dashed lines. The red regions represent oxygen atoms, while blue indicates nitrogen atoms involved in the interaction.

A) Interaction of BRCA1 with hK1: *The ligand binds within the active site, forming hydrogen bonds with key residues including Ser660, Asn663, and Arg664.*

B) Interaction of BRCA1 with hK2: *The binding pocket accommodates the hK2 peptide, showing extensive hydrogen bonding with Asn683, Ser668, and Glu708, stabilizing the interaction.*

C) Interaction of BRCA1 with hK3: *The binding configuration demonstrates key hydrogen bond formation with Lys670, Asn663, and Arg664, suggesting moderate affinity compared to hK2.*

D) Interaction of BRCA1 with hK4: *Hydrogen bonding and hydrophobic interactions with Leu625, Arg664, and Asn663 indicate a stable interaction within the BRCA1 binding pocket.*

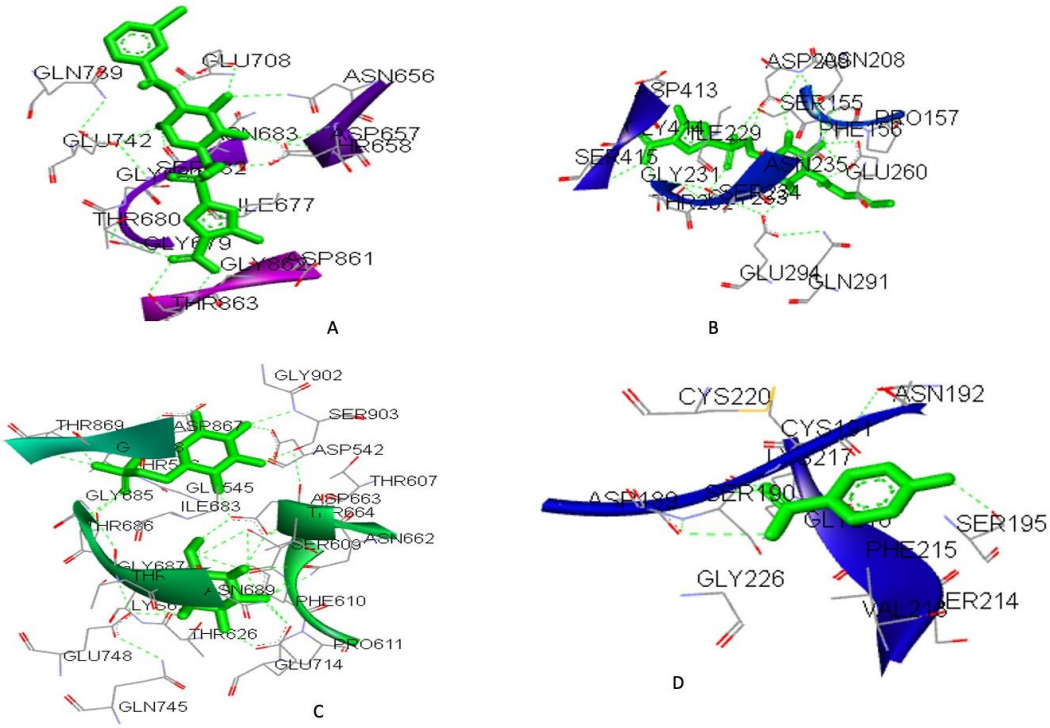


Figure 14: Molecular Docking Interactions of BRCA2 with hK Peptides

This figure illustrates the molecular docking interactions between BRCA2 and various hK peptides, highlighting key binding residues and interaction networks. The docked hK peptides (ligands) are represented in green, while the BRCA2 protein structure is shown in secondary

structural elements (β-sheets in blue, α-helices in purple/green, loops in gray). Green dashed lines represent hydrogen bonds, and the important interacting residues are identified with labels. Oxygen atoms are marked in red, while nitrogen atoms are in blue.

A) Interaction of BRCA2 with hK1:

The ligand binds within the active pocket of BRCA2, forming hydrogen bonds with Asn656, Asn683, Glu708, Glu742, and Gly681, contributing to a stable interaction network.

B) Interaction of BRCA2 with hK2:

The docking analysis shows multiple hydrogen bonds between hK2 and BRCA2, with key interactions involving Asp208, Asn208, Glu260, and Gly231, suggesting moderate affinity and stability.

C) Interaction of BRCA2 with hK3:

The hK3 peptide is accommodated within a deep groove of BRCA2, engaging in extensive hydrogen bonding with Asp542, Ser903, Thr869, and Gly865, indicating strong interaction and potential binding stability.

D) Interaction of BRCA2 with hK4:

The docking results demonstrate hydrogen bonding between Cys220, Cys211, Phe215, and Asn192, stabilizing the ligand within the BRCA2 pocket. The β-sheet region (blue) provides structural rigidity, enhancing binding affinity.

Table 11: Docking Score of Protein-Protein Interaction between BRCA1/2 and hKs

S.No	Protein-Protein Interaction	Docking Score Kcal/M	Confidential Score	Interacting residues
	BRCA1			
01	HK1	-2.80	0.9320	ILE625, GLY627, SER660, ASN663, ARG664
02	**HK2**	**-2.85**	**0.9381**	**GLU708, ASN683, ASN684, SER682, GLU742, GLY679**
03	HK3	-2.51	0.8840	LYS670, LEU667, LEU625
04	HK4	-2.47	0.9320	ASN663, LEU625, LEU668, ARG664, LEU667
	BRCA2			
05	HK1	-1.98	0.9325	GLU708, ASN683, ASN684, SER682, GLU742, GLY679
06	HK2	-2.6	0.9145	GLU708, ASN683, ASN684, SER682, GLU742, GLY679
07	HK3	-2.59	0.9005	ASN663, LEU625, LEU668, ARG664, LEU667
08	**HK4**	**-2.9**	**0.9439**	**ILE625, GLY627, SER660, ASN663, ARG664**

Molecular docking identifies the optimal binding configuration and forecasts minimal conformational energy. The intrinsic scoring function was employed to determine the most favorable complex among the protein-peptide complexes. Utilizing a genetic algorithm, enhanced precision docking was conducted with the prepared BRCA1 and BRCA2 proteins alongside PSA proteins - hK1, hK2, hK3, and hK4, as part of Protein-Protein Interaction (PPI). The server predicted that all hK peptides exhibited good activity, among all **hK2 exhibited best affinity with the BRCA1** with a docking score of **-2.85 Kcal/M and hK4 with BRCA2** with a docking score of **-2.9 Kcal/M** [Table 11 and Figure 13,14].

Screening of ADME/T properties by SWISS-ADMET online tool.

Lipinski's Rule of Five is a widely employed guideline in drug discovery based on small molecules. It helps to predict whether a compound is likely to be well metabolized in physiological system [69].

First, the molecular weight should be 500 Daltons or less, as compounds exceeding this threshold tend to have poor absorption and diffusion across biological membranes. Second, the partition coefficient (LogP) should be 5 or lower, since higher values indicate excessive lipophilicity, which can reduce solubility and bioavailability. Third, the compound should have no more than five hydrogen bond donors, such as hydroxyl (-OH) and amine (-NH) groups, as an excess of these can hinder penetration through the cell membrane. Lastly, since too many oxygen or nitrogen atoms that can form hydrogen bonds will also decrease membrane permeability, there shouldn't be more than 10 hydrogen bond acceptors. In this study, the "ADME/T (Absorption, Distribution, Metabolism, Excretion, and Toxicity)" properties of docked protein-peptide complexes had been assessed by employing the SWISS-ADME online

tool (Table 12). The analysis confirmed that all selected drug-like molecules adhered to Lipinski's Rule of Five, suggesting favourable physicochemical properties.

Table 12: ADME/T analysis of all docked complexes analyzed with Lipinski rule

Sl. No.	**Protein-Protein Interaction**	**Lipinski Rule**	
		BRCA-1	BRCA-2
01	HK1	Yes	Yes
02	HK2	Yes	Yes
03	HK3	Yes	Yes
04	HK4	Yes	Yes

6. Discussion

Prostate carcinoma is the second most cancer among men worldwide and third most common in India that ranges from slow growing to very aggressive forms[70]. It develops due to a mix of genetic changes, hormonal factors, and environmental influences, making it a complex disease to understand and treat. In India, the burden of prostate cancer is steadily rising, partly due to an aging population and changing lifestyles [11]. However, there is a notable gap in research in understanding the molecular mechanism, specifically the expression of BRCA1 and BRCA2 and its correlation with PSA and Gleason score, especially in Indian context.

The current study demonstrated a statistically significant under expression of both the BRCA1 and BRCA2 genes in prostate carcinoma samples compared to BPH controls. This under expression gradually decreased as the Gleason grade increased, indicating increased tumor aggressiveness. Further, *In silico* analysis by molecular docking showed significant interactions between BRCA proteins and kallikreins, which may be possibly be utilized for targeted therapy.

While the association of BRCA1 with PSA levels was less simple and statistically inconsequential, this investigation found a statistically significant negative correlation between PSA levels and BRCA2 expression. This suggests that lower BRCA2 expressions may be associated with elevated PSA levels, potentially influencing tumor biology and PSA dynamics. These outcomes are in line with other research demonstrating that BRCA2 mutations are linked to higher PSA levels at diagnosis and a higher risk of aggressive illness [59,71].

Walker et al. (2014) conducted a study that reinforces this link, indicating that individuals with BRCA mutations were more prone to having intermediate- or high-risk PCa (88% compared to 36%) than those with only a family history of disease. While BRCA1 carriers did not show

an evident rise in PSA levels or recurrence risk, those with the BRCA2 mutation had elevated PSA levels at the time of diagnosis and were at a higher risk of disease recurrence or metastasis (50%) [71]. This is consistent with our findings, where the PSA-BRCA1 correlation was minimal.

Given these observations, tailoring PSA screening strategies for BRCA mutation carriers—particularly BRCA2—may enhance early detection and risk stratification. Walker et al. (2014) also proposed lowering the PSA cutoff from the conventional >4.0 µg/L to >3.0 µg/L to improve the detection of clinically significant PCa in BRCA1/2 carriers[71].

Although the exact biological mechanism behind the observed association between elevated PSA and BRCA2 under expression is yet unknown, it may involve abnormalities in DNA repair pathways that cause genomic instability and an improved tumorigenic environment that encourages the generation of PSA[72]. According to some theories, BRCA1 may co-regulate the androgen receptor (AR), and its absence may alter androgen signaling, which could have an impact on PSA production[73]. Recent studies also suggest that BRCA mutations may contribute to pro-inflammatory tumor microenvironments, indirectly affecting PSA secretion patterns[74].

Our research further reveals a notable inverse relationship between BRCA1/2 expression and Gleason grade, supporting their potential function as tumor suppressors in the advancement of PCa. A progressive reduction in BRCA1/2 mRNA expression was observed with increasing Gleason grade, suggesting that the loss of BRCA function may contribute to the transition from localized to high-risk, aggressive disease. This pattern is consistent with the well-established connection between BRCA2 mutations and a worse prognosis for PCa, which has been shown in multiple cohort studies and meta-analyses [28,62,75].

BRCA2-mutated tumors are more likely to exhibit high Gleason scores, extraprostatic extension, and nodal metastases, according to multiple studies. BRCA2 mutations were

identified as significantly correlated with an elevated risk of PCa with a Gleason score of 7 or above in a prospective cohort study conducted by Castro et al. (2013). This finding indicates that the loss of BRCA2 not only heightens the risk of tumor development but also contributes to a more aggressive form of disease [75]. Further correlating BRCA2 malfunction to adverse clinical outcomes, a meta-analysis by Valsecchi et al. (2023) found that BRCA2 mutations were more common in cases of metastatic prostate cancer [28].

Although BRCA1 has been implicated in PCa risk, its function in disease progression is less well understood. Agalliu et al. (2012) reported that while BRCA2 mutation carriers exhibited a strong association with high Gleason grades, only specific BRCA1 founder mutations (such as BRCA1-185delAG) showed a similar correlation [62].

These results add to the increasing amount of data highlighting the necessity of taking BRCA1/2 status into consideration when using risk stratification models for prostate cancer[66]. Incorporating BRCA1/2 expression analysis into clinical decision-making may aid in refining prognosis, directing treatment decisions, and locating patients who might profit from PARP drugs and other targeted therapies[76].

Our study's findings of BRCA1/2 downregulation in carcinoma prostate samples, particularly in high Gleason-grade tumors, are compared with existing literature to contextualize their significance in tumor progression and clinical management in *Table 14*

Table 14: Comparison of BRCA1/2 expression findings in prostate cancer from various studies

Citation	Study Population & Methodology	Key Findings
Present Study	10 BPH & 43 carcinoma prostate cases; qPCR-based analysis	Significant underexpression of BRCA1/2 in carcinoma prostate compared to BPH; Decline in BRCA1/2 expression with increasing Gleason grade. Significant negative correlation between PSA levels and BRCA2 expression. *In silico* analyses revealed a significant interaction between human kallikreins and BRCA protein.
Agalliu et al., 2009 [62]	979 prostate cancer cases and 1,251 controls (Ashkenazi Jewish men); case-control study analyzing BRCA1/2 founder mutations	BRCA2 mutation carriers had a 3.2-fold increased risk (OR 3.18, 95% CI 1.52-6.66) of high-grade prostate cancer (Gleason ≥7)
Nyberg et al., 2020 [64]	447 BRCA2 and 376 BRCA1 mutation carriers followed for a median of 5.3 and 5.9 years, respectively.	"BRCA2 carriers had a 4.45-fold increased prostate cancer risk (SIR 4.45, 95% CI 2.99–6.61); 65% of BRCA2-associated tumors had a Gleason score ≥7; BRCA2 carriers under 65 years had a 3.99-fold increased risk"

Mateo et al., 2015 [63]	470 treatment-naïve prostate cancer biopsies, with 61 patients having matched primary and metastatic samples	7% of cases harbored BRCA2 mutations, associated with higher genomic instability and aggressive disease; BRCA-mutated tumors were highly sensitive to PARP inhibitors
Amsi et al., 2020 [57]	188 prostate cancer cases (Ugandan men); immunohistochemistry analysis of BRCA1/2 expression	BRCA1 expression in 26.1% and BRCA2 in 22.9% of cases; significant association with higher Gleason scores (P = 0.013 for BRCA1, P = 0.041 for BRCA2)
Fettke et al., 2023 [61]	13 patients with metastatic castration-resistant prostate cancer (mCRPC); cfDNA analysis	The absence of BRCA was significantly associated with shorter progression-free survival (PFS) and higher Gleason scores.
Han et al., 2022 [55]	Patients with advanced prostate cancer harboring BRCA2 mutations	Some BRCA2-mutated cases show disproportionately low PSA levels relative to tumor burden.

Present data contribute to the growing literature on BRCA2 as a prognostic and predictive biomarker. Fettke et al. (2023) confirm that BRCA2 deficiency correlates with poorer survival outcomes and increased resistance to conventional treatments [61,77]. This strengthens the theory that BRCA2 loss could function as a prediction signal for targeted therapy as well as a prognostic biomarker.

Homologous recombination repair (HRR), a critical mechanism that repairs double-strand DNA breaks and maintains genomic integrity, depends on BRCA1 and BRCA2[78]. The loss or diminished expression of these genes results in the buildup of unrepaired DNA damage, promoting genomic instability. This instability, in turn, hastens tumor development and raises the chances of acquiring mutations that lead to aggressive phenotypes [28].

Studies have demonstrated that BRCA-deficient prostate cancers exhibit increased chromosomal aberrations, tumor growth, metastasis, as well as resistance to treatment are all influenced by loss of heterozygosity and copy number changes [63]. This mutational burden may allow tumors to evade apoptotic pathways, adopt a more aggressive phenotype, and demonstrate resistance to DNA-damaging agents that include platinum-based chemotherapies and radiotherapy [61].

Furthermore, the "EMT (Epithelial-To-Mesenchymal Transition)", a critical stage in the progression of cancer, may be facilitated by decreased BRCA expression. Cancer cells can become more mobile and invasive by EMT, which involves the decrease of epithelial markers like E-cadherin and the growth of mesenchymal proteins like vimentin [75]. In prostate cancer, BRCA2 downregulation has been linked to EMT induction, allowing tumor cells to disseminate more efficiently and establish secondary metastatic sites, particularly in the bone, which is a common site of prostate cancer metastasis [64].

A further notable consequence of BRCA downregulation is the emergence of resistance to standard treatments. Tumors with BRCA mutations or diminished BRCA expression frequently exhibit either inherent or developed resistance to "ADT (Androgen Deprivation Therapy)" and chemotherapy based on taxanes [73]. When BRCA activity is lost, DNA repair is disrupted and alternative DNA repair mechanisms, like "NHEJ (Non-Homologous End Joining)", which is prone to errors and adds to further genomic changes, are selectively activated [57]. Because of their versatility, BRCA-deficient tumors often respond less well to conventional prostate cancer treatments, necessitating the use of alternative therapeutic modalities such as PARP inhibitors [79].

The therapeutic relevance of BRCA underexpression has been demonstrated in clinical trials evaluating PARP inhibitors include olaparib and rucaparib, that selectively target tumors with homologous recombination deficiency (TRITON2 Study, 2020) [80]. Our research indicates that the gradual reduction of BRCA expression in high-grade prostate cancer implies that BRCA status could serve as a crucial biomarker for both risk assessment and the development of tailored treatment plans. Individuals with tumors lacking BRCA may gain from early intervention using targeted therapies or combined treatment approaches that take advantage of their impaired DNA repair capabilities [77].

Further, the results from our *insilico* study reveal the amino acid residues of hKs that BRCA1/2 proteins bind and interact with. In this respect, hK2-BRCA1 showed a significant association mainly at the residue position such as GLU708, ASN683, ASN684, SER682, GLU742, and GLY 679. Also, the interaction between hK4 and BRCA2 at defined residue positions, ILE625, GLY627, SER660, ASN663, and ARG664, indicated higher binding activity. This shows that PSA antigens particularly hK2 and hK4 are highly sensitive to BRCA1 and BRCA2 protein

expression. Hence, PSA may also be considered as a good predictor to understand possible protein expressions of BRCA1 and BRCA2.

Moreover, the findings are promising for these "Protein-Protein interactions" to be useful in the context of diagnostic biomarkers. The values of PSA and hK2 are already used in the clinic to detect and monitor the prognosis of PCa[81]. The results here indicate that the characteristics of BRCA1/2-kallikrein interactions have great potential to improve the efficacy and specificity of these markers by increasing their sensitivity levels.

Due to their involvement in numerous physiological activities, human kallikreins can be considered a good profiling target associated with prostate cancer. Human kallikreins can be profiled using a range of methods, including "ELISA (Enzyme-Linked Immunosorbent Assays)", mass spectrometry, and immunohistochemistry. These methods can quantify and identify kallikreins in biological samples, enabling the necessary information regarding expression levels and distribution in prostate tissue.

The conventional screening technique for PC mainly relies on the measurement of serum PSA levels. Nevertheless, PSA screening is constrained by its limitations, such as the occurrence of false positives and overdiagnosis. The accuracy and sensitivity of prostate cancer detection could be increased by incorporating kallikrein profiling into the screening procedure, particularly when it relates to BRCA1/2 protein expression. For example, evaluating the concentrations of hK2 alongside PSA could enhance the ability to distinguish between malignant and benign prostatic diseases with more efficiency [38,82].

Future Directions

While our research significantly advances the understanding of expression of BRCA1/2 and its link to aggressive prostate cancer, there are still numerous opportunities for further investigation. Although the connection between BRCA1 and BRCA2 mutations and the risk and progression of PCa is well documented, there is a lack of studies specifically exploring their relationship with PSA levels. Our findings contribute novel insights into this emerging field; however, large-scale, longitudinal studies are required to confirm such observations and to clarify the clinical utility of PSA as a biomarker in BRCA-mutated tumors, particularly to ascertain whether these tumors exhibit distinct PSA kinetics. In-depth mechanistic investigations are also warranted to elucidate the regulatory networks—encompassing epigenetic modifications, microRNA-mediated regulation, and ubiquitin-proteasome degradation—that drive BRCA underexpression. Furthermore, future research should aim to integrate these molecular markers into clinical decision-making frameworks, ultimately developing personalized screening strategies and tailored therapeutic approaches for genetically high-risk populations. Such efforts will be crucial for refining risk stratification and enhancing treatment efficacy in prostate cancer.

7. Summary

This is a prospective observational study conducted from 1st April 2023 to 31st December 2024, including 43 PCa and 10 benign prostatic hyperplasia (BPH) patients, selected based on histopathological diagnosis, excluding samples with poor RNA quality (RIN<6). Quantitative real-time PCR was performed to measure BRCA1 and BRCA2 expression, normalized against GAPDH, and correlated with Gleason score and PSA levels. Protein-protein interactions between BRCA proteins and human kallikreins found in prostate-specific antigens were analyzed with an *in silico* approach by molecular docking.

This study identified a statistically significant downregulation of BRCA1 and BRCA2 mRNA expression in prostate carcinoma (PCa) relative to benign prostatic hyperplasia (BPH), with expression inversely correlating with increasing Gleason grades, suggesting a potential role in tumor progression and aggressiveness. Furthermore, a noteworthy negative relationship between BRCA2 expression and PSA levels was noted, suggesting that decreased BRCA2 expression may change PSA dynamics and impact clinical detection methods. *In silico* molecular docking analyses further elucidated specific amino acid residues mediating interactions between BRCA proteins and human kallikreins (BRCA1 with hK2 at residues GLU708, ASN683, SER682; BRCA2 with hK4 at residues ILE625 and GLY627), providing mechanistic insights into their potential regulatory role in genomic stability. The study further suggests the use of kallikrein profiling as a possible diagnostic tool for prostate carcinoma and underscores the clinical relevance of BRCA expression profiling in prostate cancer diagnostics and suggests promising targets for therapeutic intervention.

8. Conclusion:

This study reveals the critical role of BRCA1 and BRCA2 in PCa pathogenesis, demonstrating that their underexpression correlates with elevated PSA levels and higher Gleason grades, signifying a more aggressive disease. Moreover, our *in silico* docking analyses reveal specific interactions between BRCA proteins and kallikreins, providing novel insights into potential therapeutic targets. These findings collectively enhance our molecular comprehension of prostate cancer and underscore the potential of incorporating BRCA status into tailored diagnostic and therapeutic approaches.

9. Limitations:

This study was statistically powered for primary comparisons, the overall sample size remains relatively small and may not capture the heterogeneity present in larger, more diverse populations. The *in silico* docking analyses, although insightful, are predictive and require further experimental validation by ELISA method to confirm the biological relevance of the identified interactions.

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ANNEXURE I

Institutional Ethical Clearance Certificate

Azadi Ka Amrit Mahotsav

BLDE
(DEEMED TO BE UNIVERSITY)
Declared as Deemed to be University u/s 3 of UGC Act, 1956
Accredited with 'A' Grade by NAAC (Cycle-2)
The Constituent College
SHRI B. M. PATIL MEDICAL COLLEGE, HOSPITAL & RESEARCH CENTRE, VIJAYAPURA

BLDE (DU)/IEC/ 935/2023-24 10/4/2023

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on **Saturday, 18th March, 2023 at 11.30 a.m. in the CAL Laboratory, Dept. of Pharmacology,** scrutinized the Synopsis/ Research Projects of Post Graduate Student / Under Graduate Student /Faculty members of this University /Ph.D. Student College from ethical clearance point of view. After scrutiny, the following original/ corrected and revised version synopsis of the thesis/ research projects has been accorded ethical clearance.

TITLE: "STUDY ON EXPRESSION OF BRCA1 & BRCA2 GENES IN CARCINOMA PROSTATE& ITS CORRELATION WITH HISTO PATHOLOGY".

NAME OF THE STUDENT/PRINCIPAL INVESTIGATOR: DR. SAYANDEEP KUSALKANTI DAS.

NAME OF THE GUIDE: DR.SAVITRI M.NERUNE , ASSOCIATE PROFESSOR
DEPT. OF PATHOLOGY.

Dr. Santoshkumar Jeevangi
Chairperson
IEC, BLDE (DU),
VIJAYAPURA
Chairman,
Institutional Ethical Committee,
BLDE (Deemed to be University)
Vijayapura

Dr.Akram A. Naikwadi
Member Secretary
IEC, BLDE (DU),
VIJAYAPURA
MEMBER SECRETARY
Institutional Ethics Committee
BLDE (Deemed to be University)
Vijayapura-586103. Karnataka

Following documents were placed before Ethical Committee for Scrutinization.

- Copy of Synopsis/Research Projects
- Copy of inform consent form
- Any other relevant document

Smt. Bangaramma Sajjan Campus, B. M. Patil Road (Sholapur Road), Vijayapura - 586103, Karnataka, India.
BLDE (DU): Phone: +918352-262770, Fax: +918352-263303, Website: www.bldedu.ac.in, E-mail:office@bldedu.ac.in
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ANNEXURE II

B.L.D.E(DEEMED TO UNIVERSITY) SHRI B.M.PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTER, VIJAYAPURA-586103

INFORMED CONSENT FOR PARTICIPATION IN DISSERTATION/RESEARCH

I, the undersigned,_____, S/O D/O W/O_____, aged _____years,ordinarily resident of_____do hereby state/declare that Dr. Sayandeep K. Das of Shri. B.M. Patil Medical College Hospital has examined me thoroughly on _____ and it has been explained to me in my language that I am suffering from a disease (condition). Further Doctor informed me that he/she is conducting a dissertation/research titled, "**STUDY ON EXPRESSION OF BRCA1 AND BRCA2 GENES IN CARCINOMA PROSTATE AND ITS CORRELATION WITH HISTOPATHOLOGY"** under the guidance of Dr. Savitri M. Nerune requesting my participation in the study.

Further Doctor has informed me that my participation in this study will help in the evaluation of the results of the study which is a useful reference for the treatment of other similar cases soon, and also I maybe benefited in getting relieved from suffering or cure of the disease I am suffering.

The Doctor has also informed me that information given by me, observations made/ photographs/ video graphs taken upon me by the investigator will be kept secret and not assessed by a person other than me or my legal hirer except for academic purposes. The Doctor did inform me that though my participation is purely voluntary, based on the information given by me, I can ask for any clarification during treatment/study related to diagnosis, the procedure of treatment, the result of treatment, or prognosis. At the same timeI have been informed that I can withdraw from my participation in this study at any time if I want or the investigator can terminate me from the study at any time from the study but not theprocedure of treatment and follow-up unless I request to be discharged. After understanding the nature of the dissertation or research, diagnosis made, and mode of treatment,I the undersigned Shri/Smt.under my fully conscious state of mind agree to participate in the said research/dissertation.

Signature of the Patient

Signature of the Doctor

Witness

1)

2)

B.L.D.E (DEEMED TO BE UNIVERSITY)

ಶ್ರೀ ಬಿ.ಎಂ. ಪಾಟೀಲ್ ಮೆಡಿಕಲ್ ಕಾಲೇಜು, ಆಸ್ಪತ್ರೆ ಮತ್ತು ಸಂಶೋಧನಾ ಕೇಂದ್ರ, ವಿಜಯಪುರ- 586103

ಪ್ರಬಂಧ/ಸಂಶೋಧನೆಯಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಮಾಹಿತಿ ಪಡೆದ ಸಮ್ಮತಿ

ನಾನು, ಕೆಳಗಿನವರು_____ ಸಹಿಯಿಟ್ಟವರು, ಮಗ/ಮಗಳು/ಪತ್ನಿಯ _____ ವಯಸ್ಸು _____ವರ್ಷಗಳು, ಸಾಮಾನ್ಯವಾಗಿ ನಿವಾಸಿಸುವ ಸ್ಥಳದ ಹೆಸರು_____, ಇಲ್ಲಿ ಹೇಳಿದ್ದೇನೆ/ಘೋಷಿಸುತ್ತೇನೆ ಡಾಕ್ಟರ್ ಹೆಸರು Dr. Sayandeep K. Das ಅವರು ಆಸ್ಪತ್ರೆ ಹೆಸರು Shri B.M. Patil Medical College ಅವರು ನನ್ನನ್ನು ಪೂರ್ಣವಾಗಿ ಪರೀಕ್ಷಿಸಿದರು ದಿನಾಂಕದಲ್ಲಿ_____ ಸ್ಥಳ ಹೆಸರು_____ ಮತ್ತು ನನಗೆ ನನ್ನ ಭಾಷೆಯಲ್ಲಿ ವಿವರಿಸಲಾಗಿದೆ ನಾನು ಒಂದು ರೋಗ (ಸ್ಥಿತಿ) ಅನುಭವಿಸುತ್ತಿದ್ದೇನೆ. ಮುಂದುವರಿದು ಡಾಕ್ಟರ್ ನನಗೆ ತಿಳಿಸಿದ್ದಾರೆ ಅವರು ಒಂದು ಪದ್ಧತಿ/ಸಂಶೋಧನೆ ನಡೆಸುತ್ತಿದ್ದಾರೆ ಶೀರ್ಷಿಕೆಯುಳ್ಳ **STUDY ON EXPRESSION OF BRCA1 AND BRCA2 GENES IN CARCINOMA PROSTATE AND ITS CORRELATION WITH HISTOPATHOLOGY** ಡಾಕ್ಟರ್ Dr. Savitri M. Nerune ಮಾರ್ಗದರ್ಶನದಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ಕೇಳಿದ್ದಾರೆ ಅಧ್ಯಯನದಲ್ಲಿ.

ಡಾಕ್ಟರ್ ನನಗೆ ಇದನ್ನು ಕೂಡಾ ತಿಳಿಸಿದ್ದಾರೆ ಈ ಕ್ರಮದ ನಡುವಲ್ಲಿ ಪ್ರತಿಕೂಲ ಫಲಿತಾಂಶಗಳನ್ನು ಎದುರಿಸಬಹುದು. ಮೇಲೆ ಹೇಳಿದ ಪ್ರಕಟಣೆಗಳಲ್ಲಿ, ಅಧಿಕಾಂಶವು ಚಿಕಿತ್ಸಿಸಬಹುದಾದರೂ ಅದನ್ನು ನಿರೀಕ್ಷಿಸಲಾಗುತ್ತಿಲ್ಲ ಆದ್ದರಿಂದ ನನ್ನ ಸ್ಥಿತಿಯ ಹಿರಿದಾಗುವ ಅವಕಾಶವಿದೆ ಮತ್ತು ಅಪರೂಪದ ಸಂದರ್ಭಗಳಲ್ಲಿ ಅದು ಮರಣಕಾರಕವಾಗಿ ಪರಿಣಮಿಸಬಹುದು ಹೊಂದಿದ ರೋಗನಿರ್ಧಾರ ಮತ್ತು ಯಥಾಶಕ್ತಿ ಚಿಕಿತ್ಸೆ ಮಾಡಲು ಹೊಂದಿದರೂ, ಮುಂದುವರಿದು ಡಾಕ್ಟರ್ ನನಗೆ ತಿಳಿಸಿದ್ದಾರೆ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ಈ ಅಧ್ಯಯನದ ಫಲಿತಾಂಶಗಳ ಮೌಲ್ಯಮಾಪನದಲ್ಲಿ ಸಹಾಯಕವಾಗುತ್ತದೆ ಇತರ ಸಮಾನ ಪ್ರಕರಣಗಳ ಚಿಕಿತ್ಸೆಗೆ ಉಪಯುಕ್ತ ಉಲ್ಲೇಖವಾಗಿದೆ, ಮತ್ತು ನಾನು ಅನುಭವಿಸುವ ರೋಗದಿಂದ ವಿಮುಕ್ತಿ ಅಥವಾ ಗುಣಮುಖಗೊಳ್ಳುವಲ್ಲಿ ನನಗೆ ಪ್ರಯೋಜನವಾಗಬಹುದು.

ಡಾಕ್ಟರ್ ನನಗೆ ಇದನ್ನು ಕೂಡಾ ತಿಳಿಸಿದ್ದಾರೆ ನನ್ನಿಂದ ನೀಡಿದ ಮಾಹಿತಿ, ಮಾಡಿದ ಪರಿಶೀಲನೆಗಳು / ಫೋಟೋಗ್ರಾಫ್‌ಗಳು / ವೀಡಿಯೋ ಗ್ರಾಫ್‌ಗಳು ನನ್ನ ಮೇಲೆ ತೆಗೆದುಕೊಳ್ಳಲಾಗುವ ಅನ್ವೇಷಕರು ರಹಸ್ಯವಾಗಿ ಇಡುವರು ಮತ್ತು ನಾನು ಅಥವಾ ನನಗೆ ಕಾನೂನು ದೃಷ್ಟಿಯಲ್ಲಿ ಸಂಬಂಧಿತರನ್ನು ಹೊರತುಪಡಿಸಿ ಇತರ ವ್ಯಕ್ತಿಯಿಂದ ಮೌಲ್ಯಮಾಪನ ಮಾಡಲಾಗುವುದಿಲ್ಲ. ಡಾಕ್ಟರ್ ನನಗೆ ತಿಳಿಸಿದ್ದಾರೆ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆ ಶುದ್ಧವಾಗಿ ಸ್ವೇಚ್ಛಾಯಿತ, ನನ್ನಿಂದ ನೀಡಿದ ಮಾಹಿತಿಯ ಆಧಾರದ ಮೇಲೆ, ಚಿಕಿತ್ಸೆ / ಅಧ್ಯಯನದ ಸಂಬಂಧದಲ್ಲಿ ರೋಗನಿರ್ಧಾರ, ಚಿಕಿತ್ಸೆಯ ವಿಧಾನ, ಚಿಕಿತ್ಸೆಯ ಫಲಿತಾಂಶ ಅಥವ ಭವಿಷ್ಯದ ಪ್ರವೃತ್ತಿಗಳು ಬಗ್ಗೆ ಯಾವುದೇ ಸ್ಪಷ್ಟತೆ ಕೇಳಬಹುದು. ಅದೇ ಸಮಯದಲ್ಲಿ ನನಗೆ ತಿಳಿಸಲಾಗಿದೆ ನಾನು ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಈ ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಯನ್ನು ನಿಲ್ಲಿಸಬಹುದು ನಾನು ಬಯಸಿದರೆ ಅಥವಾ ಅನ್ವೇಷಕರು ಅಧ್ಯಯನದಿಂದ ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ನನ್ನನ್ನು ನಿಲ್ಲಿಸಬಹುದು. ಪ್ರಬಂಧ ಅಥವಾ ಸಂಶೋಧನೆಯ ಸ್ವಭಾವ, ಮಾಡಿದ ರೋಗನಿರ್ಧಾರ ಮತ್ತು ಚಿಕಿತ್ಸೆಯ ವಿಧಾನವನ್ನು ಅರ್ಥಮಾಡಿಕೊಂಡು, ನಾನು ಕೆಳಗಿನ ಶ್ರೀ / ಶ್ರೀಮತಿ_____ ನನ್ನ ಪೂರ್ಣವಾದ ಪ್ರಜ್ಞೆಯ ಸ್ಥಿತಿಯಲ್ಲಿ ಹೇಳಿದ ಸಂಶೋಧನೆ / ಪ್ರಬಂಧದಲ್ಲಿ ಪಾಲ್ಗೊಳ್ಳಲು ಒಪ್ಪುತ್ತೇನೆ.

ರೋಗಿಯ ಸಹಿ ಡಾಕ್ಟರನ ಸಹಿ

ಸಾಕ್ಷಿಗಳು

1)

2)

ANNEXURE III

PROFORMA

- **Name OP/IP No.:**
- **Age :**
- **Sex :** **D.O.A :**
- **Religion :** **D.O.D :**
- **Occupation :**
- **Residence :**
- **Presenting Complaints :**
- **History:**
- **Personal history :**
- **Family history :**
- **Treatment history :**
- **Examination finding :**
- **Radiological finding :**
- **PSA level :**
- **Vitals : PR:** **RR:**
  BP: **TEMPERATURE:**
- **Weight :**
- **Specimen :**
- **HPR Findings:**
- **Gross** -
  Specimen size :
  Appearance :
  Associated findings :
- **Microscopy** -

HISTOLOGICAL DEFINITION OF NEW GLEASON GRADING SYSTEM 2015

- **GRADE GROUP 1**
- **GRADE GROUP 2**
- **GRADE GROUP 3**
- **GRADE GROUP 4**
- **GRADE GROUP 5**

- **mRNA expression of BRCA1 and BRCA2 –** ____ folds
- **Molecular Docking Score -** ____ KJ/mol

KEY TO MASTER CHART

1. **BPH:** Benign Prostatic Hyperplasia.
2. **TURP:** Trans Urethral Resection of Prostate.
3. **PSA Level:** Prostate-Specific Antigen level measured in the sample (ng/µL)
4. **HPR Report:** Histopathology Diagnosis
5. **RNA Conc. (ng/µL):** Concentration of Ribo Nucleic Acid in the sample, measured in nanograms per microliter.
6. **RIN:** RNA Integrity Number, indicating the quality and degradation level of the RNA.
7. **CT (GAPDH):** Cycle threshold (CT) value for the GAPDH gene (Housekeeping Gene)
8. **CT (BRCA1):** Cycle threshold value for the BRCA1 gene.
9. **CT (BRCA2):** Cycle threshold value for the BRCA2 gene.
10. **ΔCT (BRCA1):** Delta CT for BRCA1; calculated as the difference between the CT value of BRCA1 and the CT value of the reference gene (GAPDH).
11. **ΔCT (BRCA2):** Delta CT for BRCA2; calculated as the difference between the CT value of BRCA2 and the CT value of the reference gene (GAPDH).
12. **ΔΔCT (BRCA1):** Delta Delta CT for BRCA1; a comparative measure (ΔCT of the Carcinoma Prostate mean ΔCT of a Benign Prostatic Hyperplasia) used to calculate relative gene expression differences.
13. **ΔΔCT (BRCA2):** Delta Delta CT for BRCA2; calculated similarly to ΔΔCT (BRCA1).
14. **Fold Change (BRCA1):** The relative expression level of BRCA1, typically derived from the 2^(-ΔΔCT) calculation. Indicates up- or down-regulation relative to a calibrator.
15. **Fold Change (BRCA2):** The relative expression level of BRCA2 calculated in a similar manner.
16. **Log2 Fold Change (BRCA1):** The logarithm (base 2) of the fold change for BRCA1. This transformation is often used to normalize data and simplify interpretation of up- or down-regulation.
17. **Log2 Fold Change (BRCA2):** The logarithm (base 2) of the fold change for BRCA2.

MASTER CHART
BPH GROUP

Sample ID	Sample Group	Age	Tissue Sent	PSA Level	HPR Report	RNA Conc. (ng/µL)	RIN	CT (GAPDH)	CT (BRCA1)	CT (BRCA2)	ΔCT (BRCA1)	ΔCT (BRCA2)
1814/24	BPH	71	TURP		Adenomyomatous Hyperplasia of Prostate	90.6	7.2	18.8	25	25.6	6.2	6.8
1857/24	BPH	66	TURP		Adenomyomatous Hyperplasia of Prostate	82.3	6.8	19.2	24.5	25	5.3	5.8
1781/24	BPH	75	TURP		Adenomyomatous Hyperplasia of Prostate	82.5	7.8	19	24.8	25.4	5.8	6.4
1785/24	BPH	60	TURP		Adenomyomatous Hyperplasia of Prostate	85.2	8	18.7	24.5	25.1	5.8	6.4
1627/24	BPH	62	TURP		Adenomyomatous Hyperplasia of Prostate	83.9	7.9	18.9	24.6	25.3	5.7	6.4
1632/24	BPH	57	TURP		Adenomyomatous Hyperplasia of Prostate	86.1	8.1	18.6	24.4	25	5.8	6.4
1550/24	BPH	65	TURP		Adenomyomatous Hyperplasia of Prostate	81.7	7.7	19.2	25	25.6	5.8	6.4
1525/24	BPH	87	TURP		Adenomyomatous Hyperplasia of Prostate	78.3	7.4	19.5	25.2	25.8	5.7	6.3
1527/24	BPH	65	TURP		Adenomyomatous Hyperplasia of Prostate	82	7.8	19	24.7	25.3	5.7	6.3
1491/24	BPH	50	TURP		Adenomyomatous Hyperplasia of Prostate	87.5	8.3	18.5	24.3	24.9	5.8	6.4

CARCINOMA PROSTATE GROUP

Sample ID	Age	Tissue Sent	PSA Level (ng/µL)	Gleason Grade	Gleason Score	HPR Report	Perinerual Invasion	RIN	CT (GAPDH)	CT (BRCA 1)	CT (BRCA 2)	ΔCT (BRCA 1)	ΔCT (BRCA 2)	ΔΔCT (BRCA 1)	ΔΔCT (BRCA 2)	Fold Change (BRCA1)	Fold Change (BRCA2)	Log 2 Fold Change (BRCA 1)	Log 2 Fold Change (BRCA 2)
6755/22	75	TURP Chips	58.9	3	4+3	Adenocarcinoma Prostate	Not identified	6.5	21	27.7	28.6	6.7	7.6	0.94	1.24	0.52123288	0.42337266	-0.94	-1.24
6778/22	60	TURP Chips	32.5	2	3+4	Adenocarcinoma Prostate	Not identified	7.1	20.8	27.2	28.1	6.4	7.3	0.64	0.94	0.64171295	0.52123288	-0.64	-0.94
7314/22	75	TURP Chips	68.5	4	4+4	Adenocarcinoma Prostate	Identified	6.8	21.2	27.8	28.7	6.6	7.5	0.84	1.14	0.55864357	0.45375958	-0.84	-1.14
7059/22	79	TURP Chips	58.7	4	4+4	Adenocarcinoma Prostate	Not identified	6.2	21.7	28.5	29.3	6.8	7.6	1.04	1.24	0.48632747	0.42337266	-1.04	-1.24
5816/22	80	TURP Chips	22.8	4	4+4	Adenocarcinoma Prostate	Identified	6.1	21.9	28.9	29.7	7	7.8	1.24	1.44	0.42337266	0.36856673	-1.24	-1.44

6480/22	70	TURP Chips	50.3	5	5+4	Adenocarcinoma Prostate	Not identified	6	22.1	29.5	30.3	7.4	8.2	1.64	1.84	0.32085647	0.27932178	-1.64	-1.84
6133/22	80	TURP Chips	19.2	2	3+4	Adenocarcinoma Prostate	Not identified	6.6	21.3	27.6	28.4	6.3	7.1	0.54	0.74	0.68777091	0.59873935	-0.54	-0.74
4554/22	65	TURP Chips	68.2	2	3+4	Adenocarcinoma Prostate	Not identified	6.7	21.1	27.3	28	6.2	6.9	0.44	0.54	0.73713461	0.68777091	-0.44	-0.54
2314/22	76	TURP Chips	22.4	3	4+3	Adenocarcinoma Prostate	Not identified	6.5	21.6	28.2	29	6.6	7.4	0.84	1.04	0.55864357	0.48632747	-0.84	-1.04
2416/22	50	TURP Chips	32.6	5	4+5	Adenocarcinoma Prostate	Identified	6	22.4	29.9	30.7	7.5	8.3	1.74	1.94	0.29936968	0.26061644	-1.74	-1.94
2294/22	65	TURP Chips	63.8	3	4+3	Adenocarcinoma Prostate	Not identified	6.5	21.4	28.1	28.9	6.7	7.5	0.94	1.14	0.52123288	0.45375958	-0.94	-1.14
3540/22	80	TURP Chips	51.2	5	5+4	Adenocarcinoma Prostate	Not identified	6	22.3	30.2	31	7.9	8.7	2.14	2.34	0.22687979	0.19751033	-2.14	-2.34
4661/22	83	TURP Chips	60.5	3	4+3	Adenocarcinoma Prostate	Not identified	6.3	21.5	28.3	29	6.8	7.5	1.04	1.14	0.48632747	0.45375958	-1.04	-1.14

6632/22	82	TURP Chips	29.8	5	5+4	Adenocarcinoma Prostate	Identified	6.1	22.5	30.4	31.2	7.9	8.7	2.14	2.34	0.226879 79	0.197510 33	-2.14	-2.34
6240/22	67	TURP Chips	23.4	5	5+4	Adenocarcinoma Prostate	Not identified	6.2	22.2	30	30.8	7.8	8.6	2.04	2.24	0.243163 74	0.211686 33	-2.04	-2.24
4792/22	65	TURP Chips	64.5	2	3+4	Adenocarcinoma Prostate	Not identified	6.5	21.3	27.8	28.6	6.5	7.3	0.74	0.94	0.598739 35	0.521232 88	-0.74	-0.94
305/22	79	TURP Chips	50.2	5	4+5	Adenocarcinoma Prostate	Not identified	6.1	22.4	30.3	31	7.9	8.6	2.14	2.24	0.226879 79	0.211686 33	-2.14	-2.24
3292/22	66	TURP Chips	52.7	5	4+5	Adenocarcinoma Prostate	Identified	6.2	22.3	30.1	30.9	7.8	8.6	2.04	2.24	0.243163 74	0.211686 33	-2.04	-2.24
706/22	60	TURP Chips	65.8	2	3+4	Adenocarcinoma Prostate	Not identified	6.7	21.2	27.7	28.5	6.5	7.3	0.74	0.94	0.598739 35	0.521232 88	-0.74	-0.94
4841/22	70	TURP Chips	10.3	1	3+3	Adenocarcinoma Prostate	Not identified	6.8	20.9	27.4	28.2	6.5	7.3	0.74	0.94	0.598739 35	0.521232 88	-0.74	-0.94
4296/23	76	TURP Chips	12.5	2	3+4	Adenocarcinoma Prostate	Not identified	6.6	21.4	27.9	28.7	6.5	7.3	0.74	0.94	0.598739 35	0.521232 88	-0.74	-0.94

2994/23	70	Core Biopsy	58.2	4	3+5	Adenocarcinoma Prostate	Identified	6.3	22	29.6	30.3	7.6	8.3	1.84	1.94	0.27932178	0.2606144	-1.84	-1.94
2562/23	74	TURP Chips	30.4	2	3+4	Adenocarcinoma Prostate	Not identified	6.5	21.8	28.1	28.9	6.3	7.1	0.54	0.74	0.68777091	0.598739 35	-0.54	-0.74
2893/23	75	Resection	59.7	2	3+4	Adenocarcinoma Prostate	Not identified	6.4	21.7	28	28.8	6.3	7.1	0.54	0.74	0.68777091	0.598739 35	-0.54	-0.74
2034/23	75	TURP Chips	20.8	2	3+4	Adenocarcinoma Prostate	Not identified	6.5	21.6	28.2	28.9	6.6	7.3	0.84	0.94	0.55864357	0.52123288	-0.84	-0.94
2136/23	75	Core Biopsy	62.1	3	4+3	Adenocarcinoma Prostate	Not identified	6.8	21.5	28.4	29	6.9	7.5	1.14	1.14	0.45375958	0.45375958	-1.14	-1.14
5075/23	65	TURP Chips	21.5	3	4+3	Adenocarcinoma Prostate	Identified	6.7	21.4	28.3	29.1	6.9	7.7	1.14	1.34	0.45375958	0.3950206 6	-1.14	-1.34
5066/23	62	TURP Chips	55.8	2	3+4	Adenocarcinoma Prostate	Not identified	7.1	21.2	27.8	28.7	6.6	7.5	0.84	1.14	0.55864357	0.45375958	-0.84	-1.14
5388/23	76	TURP Chips	68.5	2	3+4	Adenocarcinoma Prostate	Not identified	7.3	21.9	27.9	28.4	6	6.5	0.24	0.14	0.84674531	0.90751916	-0.24	-0.14

6298/23	73	TURP Chips	70.8	3	4+3	Adenocarcinoma Prostate	Not identified	7.4	21.2	27.8	28.6	6.6	7.4	0.84	1.04	0.558643 57	0.486327 47	-0.84	-1.04
6561/23	80	TURP Chips	55.2	3	4+3	Adenocarcinoma Prostate	Not identified	7.5	21.2	27.4	29	6.2	7.8	0.44	1.44	0.737134 61	0.368567 3	-0.44	-1.44
6882/23	70	Resection	72.3	5	5+4	Adenocarcinoma Prostate	Not identified	7.8	21.3	27.8	28.2	6.5	6.9	0.74	0.54	0.598739 35	0.687770 91	-0.74	-0.54
7304/23	75	TURP Chips	45.8	3	4+3	Adenocarcinoma Prostate	Not identified	8.1	20.2	26.9	27.5	6.7	7.3	0.94	0.94	0.521232 88	0.521232 88	-0.94	-0.94
6573/23	80	TURP Chips	67.1	3	4+3	Adenocarcinoma Prostate	Not identified	8.3	20.1	26.7	27.4	6.6	7.3	0.84	0.94	0.558643 57	0.521232 88	-0.84	-0.94
724/24	75	TURP Chips	75	3	4+3	Adenocarcinoma Prostate	Not identified	7.6	22.4	29.7	30.1	7.3	7.7	1.54	1.34	0.343885 45	0.395020 66	-1.54	-1.34
4592/24	52	TURP Chips	42	3	4+3	Adenocarcinoma Prostate	Not identified	8.1	21.3	27.3	27.9	6	6.6	0.24	0.24	0.846745 31	0.846745 31	-0.24	-0.24
1676/24	76	TURP Chips	35.2	3	4+3	Adenocarcinoma Prostate	Not identified	8.4	21.2	27.5	28.3	6.3	7.1	0.54	0.74	0.687770 91	0.598739 35	-0.54	-0.74

1687/24	72	TURP Chips	55.2	4	4+4	Adenocarcinoma Prostate	Identified	8.6	20.4	27.2	26.9	6.8	6.5	1.04	0.14	0.486327 47	0.907519 16	-1.04	-0.14
1726/24	64	TURP Chips	60	3	4+3	Adenocarcinoma Prostate	Not identified	8.5	20.3	26.9	27.5	6.6	7.2	0.84	0.84	0.558643 57	0.558643 57	-0.84	-0.84
1923/24	65	TURP Chips	112	5	4+5	Adenocarcinoma Prostate	Identified	8.7	20.6	27.1	27.9	6.5	7.3	0.74	0.94	0.598739 35	0.521232 88	-0.74	-0.94
2705/24	80	Core Biopsy	46.2	4	5+3	Adenocarcinoma Prostate	Not identified	8.9	21.9	28	29	6.1	7.1	0.34	0.74	0.790041 31	0.598739 35	-0.34	-0.74
3798/24	84	Core Biopsy	87.4	3	4+3	Adenocarcinoma Prostate	Not identified	9.2	21.2	27.3	28	6.1	6.8	0.34	0.44	0.790041 31	0.737134 61	-0.34	-0.44
4074/24	64	Core Biopsy	55.6	5	5+5	Adenocarcinoma Prostate	Not identified	9	20.1	27.8	28.6	7.7	8.5	1.94	2.14	0.260616 44	0.226879 79	-1.94	-2.14

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