MUTATION SPECTRUM, TUMOR SUBTYPES, AND RISK FACTORS OF PROSTATE CANCER SEEN AT OCEAN ROAD CANCER INSTITUTE AND MUHIMBILI NATIONAL HOSPITAL, DAR ES SALAAM-TANZANIA

BY

FIDELIS CHARLES BUGOYE

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DEPARTMENT OF PATHOLOGY OF MOI UNIVERSITY

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DECLARATION

I hereby declare that this thesis is my original work and has not been submitted for a degree or awards in any other University. No part of this thesis may be reproduced without the prior permission of the author / or Moi University.

Investigator: **Fidelis Charles Bugoye -PhD/IM/6042/22**

Signature: ……... Date ……

Recommendation

This thesis has been submitted for examination with our approval as university supervisors

Dr. Rispah Torrorey

School of Medicine

Department of Pathology

Moi University

Signature:……. Date……..

Dr. Richard K. Biegon

School of Medicine

Department of Pathology

Moi University

Signature:……. Date……..

DEDICATION

First and foremost, I dedicate this work to the Almighty God for guidance and support and as I continuously pray that HIS divine power will enable us to find ways of helping those suffering from prostate cancer. Also, I dedicate this work to my lovely wife Husna and Children Derick, Caren and Elvin and all prostate cancer patients who extremely cooperated.

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ABSTRACT

Background: Prostate cancer (PCa) is a major health problem in Tanzania, with a high age-standardised incidence rate of 10.7 per 100,000 males and a mortality rate of 9.4 per 100,000 males, which are among the highest in Africa and developing regions While studies worldwide have explored gene mutations, tumour molecular types, and risk factors for PCa, little is known about how these apply to Tanzania. Lack of local data makes it hard to understand what influences PCa in Tanzanian men. The study examined gene mutations, classified tumours based on ETS-related gene (*ERG*) fusion and phosphatase and tensin homolog (*PTEN*) expression, and identified PCa risk factors. Knowing whether a tumour is *ERG*-positive or *PTEN*-negative helps identify patients who may benefit from targeted therapies. The absence of localised data on genetic mutations, tumour subtyping and risk factors specific to PCa in Tanzania has led to reliance on findings from other populations despite known regional genetic and environmental differences. This study provides critical findings for developing precise therapeutic strategies tailored to the unique genetic landscape of Tanzanian patients

Objective: To determine the mutation spectrum of *BRCA1/2, ATM, PALB2, TP53, CHEK2, MLH1*, *MSH2, MSH6,* and *PMS2* genes, *ERG/PTEN* tumour subtypes, and risk factors associated with PCa among Tanzanian cancer patients

Methods: This study combined a case-control design to assess PCa risk factors with a cross-sectional study on tumour subtypes and genetic mutations. Ethical approvals for the study were obtained. Consented participants were recruited from Muhimbili National Hospital and Ocean Road Cancer Institute. Risk factors were assessed in 166 participants using a questionnaire. Tumour subtyping was conducted on 83 PCa cases using immunohistochemistry (IHC) staining for *ERG* and *PTEN* on archived tissue samples. Targeted next-generation sequencing (NGS) was performed on dried blood spots from 30 individuals who met the NCCN genetic testing criteria. Logistic regression and Chi-Square were used to examine the association between *ERG* expression and the Gleason class (P=0.05). NGS data underwent quality control, read alignment, variant calling, and pathogenicity prediction using bioinformatic tools.

Results: Significant risk factors included advanced age (AOR = 1.05, 95% CI = 1.01–1.09). alcohol consumption (AOR = 2.75), high red meat intake (AOR = 7.88), family history of cancer (AOR = 6.38), and history of gonorrhea infection (AOR = 6.52). Protective factors were regular consumption of soy products (AOR = 0.285), coffee (AOR = 0.57), and tomatoes (AOR = 0.17), as well as higher income levels (AOR = 0.51), and not being married (AOR = 0.23). The most common tumour subtypes were *ERG-/PTEN-* (68.68%) and *ERG+/PTEN-* (30.12%), with *PTEN* loss linked to aggressive tumour behaviour. Likely pathogenic variants were ATM gene (c.131A>G, c.2289T>A) and *PMS2* gene (c.497T>C), suggesting impaired DNA repair mechanisms. *ERG* expression showed a significant association with Gleason class (p = 0.05), while mutations in *ATM, PMS2, BRCA1,* and MLH1 were associated with high pre-operative PSA levels.

Conclusion and recommendation: Mutations in *ATM* and *PMS2* were identified as key genetic alterations associated with PCa, while the most prevalent tumour subtypes were *ERG-/PTEN-* and *ERG+/PTEN-*. Significant risk factors included advanced age, frequent alcohol consumption, high red meat intake, a history of gonorrhea infection, and a family history of PCa. Also, *ERG* expression was strongly associated with higher Gleason scores and high pre-operative PSA levels, indicating its potential as a prognostic marker. Findings recommend that Genetic testing for pathogenic mutations, especially in *ATM* and *PMS2,* should be prioritised for targeted therapies. Routine IHC staining for *ERG* and *PTEN* subtypes in referral hospitals will aid in molecular classification, while incorporating risk factor assessment can optimise patient management and treatment strategies.

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ABBREVIATIONS

AIC	Akaike Information Criteria
Akt/mTOR	Protein Kinase B (Akt) and Mammalian Target of Rapamycin (mTOR)
AOR	Adjusted Odds Ratio
ATM	Ataxia-Telangiectasia Mutated
AUC	Area Under the Curve
BMI	Body Mass Index
BRCA	Breast Cancer gene.
BWA-MEM	Burrows-Wheeler Aligner - Maximal Exact Matches
CHEK2	Checkpoint Kinase 2.
COR	Crude Odd Ratio
CRAVAT	Cancer-Related Analysis of Variants Toolkit.
DNA	Deoxyribonucleic acid
Dpa	Damaging Pathogenic
ERG	ETS-related gene
FISH	Fluorescence in Situ Hybridisation
GCLA	Government Chemist Laboratory
GLM	Generalized Linear model
HIC	Immunohistochemistry
MLH1	MutL homolog 1.
MNH	Muhimbili National Hospital
MSH2	MutS homolog 2.
MSH6	MutS homolog 6.
NIMR	National Institute for Medical Research
ORCI	Ocean Road Cancer Institute

PALB2	Partner and Localizer of BRCA2
PCa	Prostate Cancer
PI3K/Akt/mTOR	Phosphoinositide 3-Kinase / Protein Kinase B (Akt) / Mammalian Target of Rapamycin
PI3-Kinase	Phosphoinositide 3-Kinase
PIP	Phosphatidylinositol Phosphate
PMS2	PMS1 homolog 2
PTEN	Phosphatase Tensin homology
RR	Relative Risk
SIFT	Sorting Intolerant from Tolerant
Tbe	Torerated Benign
TMPRSS2	Androgen-related transcription factor
TP53	Tumour protein 53
VEP	Variant Effect Predictor
VUS	Variant of Uncertain Significance

DEFINITION OF OPERATIONAL TERMS

Age at diagnosis:	The age at which a patient was diagnosed with prostate cancer. History of Prostate cancer refers to a family of Prostate cancer, thus whether a man
Coefficient of Discrimination:	Quantifies the difference in average predicted probabilities between individuals with and without the disease of interest. It reflects how well a model distinguishes between the two groups, with higher values indicating better separation.
Detection Bias:	Occurs when differences in identifying, diagnosing, or measuring outcomes arise between study groups due to unequal monitoring, awareness of exposure, or inconsistent diagnostic criteria.
Ethnicity:	Someone's cultural background or where one comes from.
Homologous recombination genes(HRR)	Group of genes involved in the homologous recombination repair pathway, a critical DNA repair mechanism that maintains genomic stability.
Maximum likelihood pseudo- R^2:	Measure used in models like logistic regression to evaluate goodness-of-fit. It is based on the likelihood ratio, comparing the log-likelihood of the fitted model to a null model (with no predictors). While it resembles R^2 in interpretation, it is not directly comparable to R^2 from linear regression
McFadden's R^2:	Measure of goodness-of-fit for logistic regression models. It compares the log-likelihood of the fitted model to that of a null model (with no predictors), indicating how well

	the predictors explain the variation in the outcome. Values closer to 1 suggest a better fit.
Modifiable risk factors:	Risk factors that can be changed because they represent the lifestyle
Nagelkerke's R^2:	An adjusted version of Cox and Snell's R^2, used to measure the goodness-of-fit in logistic regression models. It scales the R^2 value to range between 0 and 1, providing a more interpretable measure of how well the model explains the variation in the outcome.
Non-modifiable risk factors:	Risk factors that cannot be changed
Participants:	People who consented to participate in the study
Risk factor:	Anything affecting the chance of getting a disease, risk factors don't tell us everything.
The area under the curve:	Is a summary metric of

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Prostate cancer is the most commonly diagnosed adenocarcinoma of the male prostate gland, which is increasingly becoming an important public health burden among men in the world (Rafael Lozano *et al.*, 2012; Sanjose *et al.*, 2011). Lately reported in 112 countries, the disease ranked fifth as the leading cause of death (6.8%) worldwide in 2020 (Sung *et al., 2021).* This type of cancer is one of the most common solid tumours among men, and it is one of the leading causes of cancer-related deaths among men globally (El Noor and El Noor 2015; Siegel *et al.*, 2011) making it a significant health problem in both developed and developing countries (Baade, *et al.*, 2009). The incidence of prostate cancer differs from country to country, and the proportion of cases varies globally (Sung *et al.*, 2021). The burden of prostate cancer has increased in Europe and Africa, and the mortality rates continue to increase in African people and decrease in the Asian population (Jemal, Bray, and Ferlay 2011; Sung *et al.*, 2021). In Tanzania, prostate cancer is among the leading types of cancer in terms of prevalence, incidence, and mortality rate. The Globocan cancer statistics reported a prevalence of 25.2 per 100,000 population, an incidence rate of 10.4%, and a 9.4% mortality rate for the Tanzanian population (Ferlay, J, *et al.*, 2022)

Though the associated risk factors of prostate cancer among men in sub-Saharan African countries are not well reported, it was previously estimated that disease variation can be associated with genetic factors, environmental factors, and lifestyle factors (Agalliu *et al.*, 2009). Additionally, a family history of prostate cancer and genetic predisposition of pathogenic mutation associated with Homologous recombination and Mismatch repair genes have been identified as important risk factors (Agalliu *et al.*, 2009). Dietary patterns, chemical exposure, smoking, obesity,

alcohol consumption, and low levels of physical activity are all possible risk factors, and it has been suggested that socioeconomic factors, marital status, and other underlying conditions like infections may influence the risk of developing high-grade prostate cancer (Perdana *et al.,* 2016). Unfortunately, there hasn't been enough research done on the risk factors for prostate cancer in the African community, and there isn't enough knowledge about these risks that may be used to lower prostate cancer fatalities. Most reported risk factors associated with prostate cancer were identified through the family background with prostate cancer, environmental exposure, lifestyle, and age-specific factors, which are restricted to European and developed countries' populations. Therefore, these studies are unlikely to reflect risk factors associated with prostate cancer in different populations, including the Tanzanian population.

The management of prostate cancer varies significantly, and evaluating treatment options is often limited by existing prognostic factors and Gleason's score (Lahdensuo *et al., 2016)*. In Tanzania, as in many other countries, the most common approach is the PSA and biopsy, and the reliability of biopsy is challenged by interobserver variability among pathologists and biopsies' under-sampling, which may pose a risk in diagnosis, which may affect therapeutic decision-making (Hoogland *et al.,* 2014). To enhance efficiency in diagnosis, combining Gleason grading using biopsy and additional parameters, such as tumour molecular subtyping based on tumour *ERG* and *PTEN* expression status, could provide valuable insight for guiding treatment decision-making (Hoogland *et al.,* 2014). Certain *ERG/PTEN* tumour molecular subtypes have the potential to predict prognosis and response to recently developed targeted therapies (Giunta *et al.,* 2021). Therefore, the ideal treatment

approach should be precisely personalised by considering each person's tumour status in the context of prognostic and predictive factors (Giunta *et al.*, 2021).

The loss of Phosphatase Tensin homolog (*PTEN)* and *TMPRSS2: ERG* fusion are well-known prognostic biomarkers in prostate cancer (Ahearn *et al.*, 2016; Morais et al., 2019). *PTEN* is a key tumour suppressor gene that plays a central role in regulating cell growth, survival, and metabolism. Its primary function is to counteract the PI3K/AKT/mTOR signalling pathway, a key driver of oncogenic processes. Its loss is associated with poor prognosis and aggressive prostate cancer (Ahearn *et al.*, 2016). Additionally, *PTEN* inactivation may increase the activity of the oncogenic Akt/mTOR signalling pathway, which would suppress the activity of the androgen receptor (AR) transcription factor and inhibit the AR-regulated negative feedback of Akt (Ahearn *et al., 2016)*. These effects together drive the growth of aggressive, high-grade prostate cancer, which is more likely to spread and become harder to treat. Because of this, *PTEN* expression can be a useful biomarker to classify tumour molecular subtypes and identify patients who may benefit from PI3K/Akt/mTOR therapeutic inhibitors (Bugoye *et al.*, 2025). Similarly, the fusion between ETS-related gene (*ERG*) and androgen-regulated transcription factor *TMPRSS2* is the most predominant genomic alteration in prostate cancer that plays a vital role in carcinogenesis, and it occurs in nearly half of prostate cancer patients (Ahearn *et al.*, 2016). This fusion has been associated with high-grade prostate cancer and is considered a biomarker for poor disease outcomes in prostate cancer management (Morais et al., 2019). *TMPRSS2:ERG* fusion alone does not affect prognosis but increases the risk of changes in cancer cell pathways, like *PTEN* loss, which may influence disease progression (Lahdensuo *et al.*, 2016). Though *PTEN* loss alone has been associated with patient outcomes, the combined presence of *TMPRSS2:ERG*

fusion and loss of *PTEN* holds greater clinical significance than either alteration individually (Lahdensuo *et al*., 2016). However, studies investigating the two genes concurrently in the African population are scarce. Specifically, there is a lack of data on the role of the immunohistochemical expression of *ERG* and the loss of *PTEN* in risk stratification among Tanzanian prostate cancer patients. As a result, many prostate cancer patients in Tanzania do not fully benefit from PI3K/Akt/mTOR therapeutic inhibitors. Although the expression of ERG and Loss of *PTEN* and the prevalence of these tumour molecular subtypes vary across populations (Jamaspishvili *et al*., 2018a; Morais *et al*., 2019), none of these markers has been consistently validated for clinical application in Tanzania. Therefore, there was a pressing need to assess the utility of *ERG* expression and loss of *PTEN* in prostate cancer risk stratification and identify candidates who may respond to PI3K/Akt/mTOR therapeutic inhibitors among high-grade patients in Tanzania.

Additionally, mutations in mismatch and homologous recombination repair genes *MLH1, MSH2*, *MSH6, PMS2, and BRCA1/2, ATM, TP53*, *PALB2*, and *CHEK2* has been associated with the development of high-grade prostate cancer (Dominguez-Valentin *et al.*, 2016; Graham and Schweizer, 2022a; Jiang *et al*., 2021). When this mutation occurs, other error-prone and non-conservative pathways repair DNA, resulting in genomic instability and increased cancer risk with worse survival (Amsi *et al.*, 2020; Castro and Eeles, 2012). The occurrence of these Homologous recombination repair (HRR) gene mutations in prostate cancer has been demonstrated to have predictive and therapeutic implications, including susceptibility to poly ADP-ribose polymerase (PARP) inhibition and enhanced sensitivity to platinum-based chemotherapies (Manchana *et al.*, 2020). Similarly, Genetic instability that results from the buildup of errors during DNA replication is caused by a loss of mismatch

repair (MMR) function. Mutations in the MMR genes *MLH1, MSH2, MSH6,* and *PMS2* result in the inactivation of MMR protein functions, defects strongly associated with prostate cancer tumorigenesis in various populations (Dominguez-Valentin *et al*., 2016; Graham and Schweizer, 2022a). Through the identification of HRR and MMR gene mutations, prostate cancer patients may benefit from currently available immune-based therapies. Despite the critical role of HRR and MMR gene mutations in guiding prognosis and therapy selection, there are currently no established genetic testing guidelines for prostate cancer in Tanzania (Manchana, Tantbirojn, and Pohthipornthawat, 2020; Paul *et al.*, 2022). Moreover, there was no information on the mutation status of *BRCA1/2, ATM, TP53, PALB2, CHEK2, MLH1, MSH2, MSH6* and *PMS2* genes among Tanzanians. The frequency of occurrence of these DNA Damage mutations is highly variable (1-6%) and dependent on the number of genes analysed, the technique used, and tumour characteristics (Rebeca Lozano *et al.*, 2021).

However, the frequency of these mutations varies significantly by ethnicity and population (Rebeca Lozano *et al.*, 2021), and genetic profiling is important for optimising treatment selection as patients with known HRR and MMR mutations respond favorably to DNA damage therapies, including PARP and Immunocheckpoint inhibitors (Graham & Schweizer, 2022). However, due to inconsistent data on mutation prevalence in Tanzania, prostate cancer patients may not be fully benefiting from potential life-saving treatments such as platinum-based chemotherapy, PARP inhibitors and Immunocheckpoint inhibitors. To bridge this gap, there was a pressing need to utilise the next-generation sequencing technique targeting exon and splicing regions to determine *BRCA1/2, ATM, TP53, PALB2, CHEK2, MLH1, MSH2, MSH6,* and *PMS2.*

1.2 Statement of the Research Problem

Prostate cancer exhibits a disproportionately high incidence and mortality rate in Tanzania and other sub-Saharan African nations compared to other regions of Africa and most developing countries. Although prostate cancer risk factors have been extensively studied in Western and developing nations, a significant gap remains in understanding how these factors apply to sub-Saharan African populations. In Tanzania, there is no comprehensive data on the genetic risk of oncogenes and their mutation spectrum in prostate cancer. Furthermore, the role of family history, socio-demographic factors, diet, environmental exposures, lifestyle, and medical conditions in prostate cancer incidence is not well understood.

The high mortality rates associated with prostate cancer in Tanzania are attributable to limited access to healthcare services for early detection and treatment. However, these mortality rates also reflect the differential prevalence of aggressive prostate cancer subtypes within the population. Characterising prostate cancer tumours based on *ERG* expression (*ERG+*) and *PTEN* loss (*PTEN-*) is essential for identifying subtypes with poor prognoses and guiding personalised treatment strategies. Yet, in resource-limited settings like Tanzania, genetic testing and immunohistochemical analysis are rarely performed, making it difficult to classify prostate tumours and tailor treatment accordingly.

Research has demonstrated that identifying both modifiable and non-modifiable risk factors is critical for reducing prostate cancer-related mortality. Additionally, molecular classification of prostate cancer into *ERG+* and *PTEN-* subtypes enhances prognostic accuracy and helps identify patients who could benefit from targeted therapies. However, the absence of localised data on risk factors, oncogene mutations, *BRCA1/2, ATM, PALB2, TP53, CHEK2, MLH1, MSH2, MSH6, and PMS2*, and

tumour molecular subtypes has led to an overreliance on data from other populations, despite known genetic and environmental variations across different regions.

To address this critical knowledge gap, this study investigated the mutation spectrum of selected key oncogenes, identified relevant prostate cancer risk factors, and characterised tumour molecular subtypes in Tanzanian prostate cancer patients using immunohistochemical staining for *ERG* and *PTEN*. The findings provide vital insights into the genetic and molecular landscape of prostate cancer in Tanzania, facilitating the development of more effective diagnostic, prognostic, and therapeutic strategies tailored to the Tanzanian population.

1.3 The rationale for the study

Prostate cancer remains a significant health challenge in the world, with high incidence and mortality rates. While absolute prevention may not be possible, understanding key risk factors, tumour molecular subtypes, and gene mutations associated with prostate cancer can significantly reduce its burden. This study addresses these critical gaps by investigating the interplay of risk factors, tumour molecular subtypes, and gene mutations in prostate cancer among Tanzanian patients. The findings reveal that age at diagnosis, dietary habits, gonorrhoea infection, lifestyle factors, and family history are significant predictors of prostate cancer.

Additionally, the *ERG-/PTEN-* and *ERG+/PTEN-* tumour subtypes emerged as the most frequently observed molecular subtypes in this population. Importantly, the study identified the *ATM* gene as a significant genetic risk factor for prostate cancer in Tanzanian men. Furthermore, pre-operative PSA levels and Gleason scores were found to be associated with both gene mutations and *ERG* expression, underscoring their potential role in predicting disease progression and outcomes. By focusing on

these factors, this research contributes to the growing body of knowledge on prostate cancer in Tanzania. It highlights the importance of integrating molecular and genetic insights into clinical practice to improve early detection and targeted treatments. The findings also provide a foundation for developing strategies to reduce prostate cancer fatalities in Tanzania, where late presentation and limited screening remain major challenges.

1.4 Research Questions

The research study consists of the following research questions

i. What are the risk factors associated with prostate cancer among cancer patients at Ocean Road Cancer Institute and Muhimbili National Hospital?
ii. What are the tumour molecular subtypes based on *ERG* and *PTEN* IHC expression among prostate cancer patients at Ocean Road Cancer Institute and Muhimbili National Hospital?
iii. What is the mutation spectrum of *BRCA1/2, ATM, TP53, PALB2, CHEK2, MLH1, MSH2, MSH6,* and *PMS2* genes among cancer patients at Ocean Road Cancer Institute and Muhimbili National Hospital?
iv. What is the association between risk factors, gene mutation, and tumour clinicopathological characteristics in prostate cancer at Ocean Road Cancer Institute and Muhimbili National Hospital?

1.5 Research Objectives

1.5.1 General Objective

To determine the mutation spectrum of *BRCA1/2, ATM, PALB2, TP53, CHEK2, MLH1, MSH2, MSH6,* and *PMS2* selected genes, tumour molecular subtypes, and risk factors associated with prostate cancer among representative cancer patients at Ocean Road Cancer Institute and Muhimbili National Hospital.

1.5.2 Specific Objectives

i. To determine risk factors associated with prostate cancer among cancer patients at Ocean Road Cancer Institute and Muhimbili National Hospital.

ii. To determine tumour molecular subtypes based on *ERG* and *PTEN* IHC expression among prostate cancer patients at Ocean Road Cancer Institute and Muhimbili National Hospital.

iii. To determine the mutation spectrum of *BRCA1/2, ATM, TP53, PALB2, CHEK2, MLH1, MSH2, MSH6,* and *PMS2* genes among cancer patients at Ocean Road Cancer Institute and Muhimbili National Hospital.

iv. To determine the association between risk factors, gene mutation, and tumour clinicopathological characteristics in prostate cancer at Ocean Road Cancer Institute and Muhimbili National Hospital.

CHAPTER TWO

2.1 LITERATURE REVIEW

2.2 Global cancer epidemiology

As the world's population grows and more people adopt lifestyle habits linked to the development of cancer, the burden of the disease rises worldwide. According to the GLOBOCAN report of 2018, there are 9.6 million cancer-related deaths and an estimated 18.1 million new cases worldwide, also one in five men and one in six women will have cancer at some point in their lives. Additionally, one in eight men and one in eleven women worldwide die from cancer. Presently, about 43.8 million people are alive in the globe who have received a cancer diagnosis during the first five years (Cancer, 2018).

2.2.1 Global Prostate Cancer Epidemiology

Prostate cancer is the most common cancer of the male prostate gland, which is becoming a significant global public health burden among males (Lozano *et al.*, 2012; Sanjose *et al.*, 2011). According to recent reports in 2020, prostate cancer is the fifth leading cause of mortality globally 2020 (Sung *et al.*, 2021a). This type of cancer is one of the most common solid tumours in males and one of the main causes of cancer-related deaths in men globally, making it a serious health concern in both developed and developing nations (Baade *et al.*, 2009.; El Noor and El Noor 2015; Siegel *et al.*, 2011). According to a recently published report on global epidemiology, prostate cancer is quite prevalent in Western nations. Australia, New Zealand, and North America, as well as northern and western portions of Europe, have the greatest occurrences of this cancer (Hassanipour *et al.*, 2020). The disease is also common in eastern, and Southern Europe and they are reported to have higher incidence rates compared to other regions like Africa or Asia due to comparable high rates of prostate

cancer screening implementation and acceptance in these regions (Marhold *et al*., 2022). With an age-standardized death rate of 10 per 100,000 males and a ranking of third behind lung and colorectal cancer, prostate cancer continues to be the second most prevalent cancer in eastern European countries. Therefore, it places a heavy financial burden on European healthcare providers. However, due to several recent advancements in therapy, the death rate is anticipated to decrease in European nations (Ferlay *et al.,* 2019; Marhold *et al.,* 2022). In recent decades, it was believed that Asia had significantly lower rates of prostate cancer incidence. However, as a result of socio-cultural differences, economic development, and higher life expectancy, both incidence and death rates of prostate cancer have been on the rise in various Asian nations (Chen *et al.,* 2014). Prostate cancer is projected to progressively become a more severe healthcare and socio-economic concern if adequate precautions are not implemented since the ageing process of the Asian population is still ongoing, and the positive effects of economic growth will continue to accelerate lifestyle changes (Chen *et al.,* 2014).

2.2.2 Prostate Cancer Epidemiology in Africa

The reports that are currently available indicate that African men experience an excessive amount of Prostate cancer compared to men from other parts of the world, even though it is quite challenging to accurately describe the burden of Prostate cancer in Africa due to poor cancer registration systems and it is the main reason for males in sub-Saharan Africa to die from cancer. Recent decades have seen an increase in the number of new cases and deaths in various nations, particularly in low- and middle-income nations (Hassanipour *et al.,* 2020). This has been connected to the region's high frequency of infectious diseases, fast population increase, rising life expectancy, urbanisation with gradually more westernised lifestyles, and urbanisation.

According to the facts and indications available, Africa and other low- and middle-income countries currently account for the majority of new cancer cases. Additionally, it is projected that due to demographic shifts, the number of new cases of prostate cancer will increase more than two times in Africa in the next 20 years. Also, with a growth rate of 2% over the next 20 years, the disease's incidence will more than triple by 2040. (Seraphin *et al.,* 2021). Evidence demonstrates that black African populations have higher mortality rates from prostate cancer than other races (Fletcher *et al.,* 2020). Also, between northern and sub-Saharan African regions, these differences were seen in the patterns and manifestations of prostate cancer. Compared to the average rates in Sub-Saharan Africa (Adeloye *et al.,* 2016; Hamdi *et al.,* 2021). Some of these variations have been attributed to race, relatively higher poverty levels, dietary differences, genetic differences, and the presence of infectious diseases in Sub-Saharan Africa (Adeloye *et al.,* 2016). According to global cancer statistics, East African countries, including Tanzania, are also struggling with the burden of prostate cancer. The report showed a prostate cancer prevalence of 25.2 per 100,000 people, with a prostate cancer incidence rate of 10.7% and a 9.4% mortality rate for Tanzanians.

2.3 Tumorigenesis in Prostate Cancer

The process through which healthy prostate cells develop into malignant cells is known as prostate carcinogenesis. The development of prostate cancer is influenced by a complex combination of genetic, environmental, and lifestyle variables (Maekawa *et al*., 2024; Malik *et al*., 2018). Prostate cell development and division can become uncontrolled due to mutations in certain genes, such as tumour suppressor genes and oncogenes, which can interfere with normal cellular functions. Tumour suppressor gene mutations, such as the one that causes *PTEN* to stop functioning, can

result in unchecked tumour growth in prostate cells and encourage the development of cancer (Jamaspishvili *et al.*, 2018b). Prostate cancer is also linked to the overactivation of oncogenes like *ERG*, which has been mutated, causing uncontrolled cell division. Similarly, mutations in mismatch repair genes (*BRCA*1, *BRCA2*, *ATM*, *CHEK2*, *PALB2*, *MLH1*, *MSH2*, *MSH6*, and *PMS2*) and homologous recombination genes (Bugoye *et al.*, 2023; Maekawa *et al.*, 2024), can cause prostate cancer development. In addition, ageing, inflammation, hormonal imbalances, and exposure to certain carcinogens can all contribute to the development and advancement of prostate cancer (Cannarella *et al.*, 2021; Heinlein & Chang, 2004; Henderson & Feigelson, 2000). Therefore, developing targeted treatments and preventative measures to fight prostate cancer requires an understanding of the risk factors and mechanisms behind the disease's development. The key to controlling prostate cancer risk and enhancing outcomes is early intervention, healthy lifestyle choices, and routine screenings.

2.4 Inflammation and prostate cancer development

Prostate cancer growth and progression have been linked to chronic inflammation, which is becoming more well-recognised (Stark *et al.*, 2015). Prostate inflammation can be brought on by several events, such as autoimmune diseases, infections, environmental exposures, and nutritional variables. Prolonged inflammation can cause cellular stress, tissue damage, and the generation of reactive oxygen species (ROS), chemokines, and pro-inflammatory cytokines (Stark *et al.*, 2015). These elements produce an environment that is favourable for genetic instability, DNA damage, and epigenetic changes, all of which are essential stages in the development of tumours (Bugoye *et al*., 2023). Studies indicate that infections caused by organisms including Trichomonas vaginalis, Chlamydia trachomatis, and Escherichia coli may trigger

inflammatory reactions that lead to cellular changes in the prostate. Also, dietary factors that increase oxidative stress and lipid peroxidation, such as high-fat diets or excessive red meat consumption, may make inflammatory processes worse. Chronic inflammation has also been connected to the proliferation of prostate epithelial cells and the upregulation of carcinogenesis-related genes like COX-2, STAT3, and NF-κB (De Marzo *et al.*, 2007; Nigam *et al.*, 2023).

Additionally, inflammatory diseases such as prostatitis have been linked to an increased risk of prostate cancer, indicating a possible mechanism of action. Although inflammation by itself cannot cause cancer, it can work in concert with genetic predispositions and other risk factors to accelerate the course of the illness (De Marzo *et al.*, 2007). Novel insights into preventative measures, including Managing inflammation through lifestyle changes such as eating a balanced diet, regular exercise, and stress reduction, may mitigate the underlying biological mechanisms that increase the risk of prostate cancer (De Marzo *et al.*, 2007; Nigam *et al.*, 2023). Moreover, treatment therapies that target inflammatory pathways may be obtained by understanding the function of inflammation in the development of prostate cancer (M. Wang *et al.*, 2024).

2.5 Roles of immune cells in Prostate cancer development

The growth and spread of prostate cancer are significantly influenced by immune cells. Through immunosurveillance, the immune system can recognise and eradicate malignant cells (D. Li *et al.*, 2023). However, the tumour microenvironment in prostate cancer can affect the immune system by evading immune detection and encouraging tumour growth (Y. Zhang *et al.*, 2022). Furthermore, immune cells recruited to the prostate gland's chronic inflammation can produce pro-inflammatory

cytokines, which may promote the growth and spread of tumours (Rani *et al*., 2019a). Immune cells, including lymphocytes, neutrophils, and macrophages, are recruited to the site of inflammation when tissue injury or infection occurs to remove pathogens, clear debris, and promote tissue repair (Chen *et al.*, 2018; Rani *et al.*, 2019b). Also, persistent inflammation brought on by dysregulation of immune cell activity has been linked to an increased risk of cancer, including prostate cancer (Herold & Mrowka, 2019). Additionally, although cytotoxic and natural killer cells can identify and eradicate cancer, certain immune cells, including tumour-associated macrophages, myeloid-derived suppressors, and regulatory T cells, can produce an immune-suppressive environment that permits cancer cells to proliferate unchecked (Sfanos & De Marzo, 2012; Zhang et al., 2022). As a result, immunotherapy, which uses the immune system's ability to target cancer cells, has demonstrated promising results in the treatment of advanced prostate cancer (Herold & Mrowka, 2019). Therefore, initiating and managing the inflammatory response requires an understanding of the intricate interactions between cells and prostate cancer.

2.6 Prostate cancer risk factors

Risk factors are any factors that influence a person’s likelihood of developing a disease, such as prostate cancer. However, having a risk factor by itself does not mean that a person with that specific risk or exposure to risk factors will necessarily acquire the disease because some people may have risk factors or be exposed to them but never develop the disease, and some people may not have risk factors but still develop the disease. Prostate cancer is linked with several risk factors. Both modifiable or controllable and non-modifiable risk factors for prostate cancer exist (Adjakly *et al.*, 2015; Ni Raghallaigh & Eeles, 2022; Safe *et al.*, 2023).

2.7 Non-Modifiable Prostate Cancer Risk Factors

2.7.1 Genetic factors

The significance of genetic diversity in the risk of prostate cancer has gained tremendous understanding because of advancements in DNA technology. Together, common germline mutation variations imparting risk and high-risk prostate cancer risk genes account for more than a third of the family prostate cancer risk (Ni Raghallaigh & Eeles, 2022). Men with extra risk factors, such as genetic mutations or favourable family history, should be identified since they are more likely to develop prostate cancer and may also be diagnosed earlier and have worse outcomes (Ni Raghallaigh & Eeles, 2022). The presence of these *BRCA1/2, ATM, TP53, PALB2,* and *CHEK2* genes has been shown to have therapeutic implications, including susceptibility to poly ADP-ribose polymerase (PARP) inhibition and enhanced sensitivity to platinum-based chemotherapies (Ni Raghallaigh & Eeles, 2022). The proteins encoded by the *BRCA1/2, ATM, TP53, PALB2,* and *CHEK2* genes are also linked to the repair of chromosomal damage with a significant role in preventing apoptosis (Amsi *et al.*, 2020; Manchana *et al.*, 2020). Similar to this when the mismatch repair function is lost, errors in DNA replication accumulate, leading to genetic instability. The functions of the mismatch repair protein are rendered inactive by mutations in the mismatch repair genes *MLH1, MSH2, MSH6,* and *PMS2,* and defects of MMR are strongly linked to tumorigenesis and prostate cancer in various populations.

Although identifying mutations in genes such as *BRCA1/2, ATM, PALB2, TP53, CHEK2, MLH1, MSH2, MSH6,* and *PMS2* is crucial for therapeutic decisions and prognosis, there are currently no genetic testing guidelines for prostate cancer patients in Tanzania, which limits the potential for personalised treatment and effective

management (Manchana *et al.,* 2020; Paul *et al.*, 2022). While studies conducted in western and developed countries have reported *BRCA1/2, ATM, PALB2, TP53, CHEK2, MLH1, MSH2, MSH6,* and *PMS2* genes and various genes have been linked to the prostate cancer across different ethnicities (Giri *et al.*, 2022), there is limited evidence regarding the occurrence of mutations in genes such as *BRCA1/2, ATM, CHEK2, PALB2, TP53, MLH1, MSH2, MSH6,* and *PMS2,* and their association with prostate cancer grade in the Tanzanian (Bugoye *et al.*, 2023).

2.7.1.2 Mutation spectrum of Mismatch Repair and Homologous recombination genes in Prostate cancer

The genesis and progression of prostate cancer have been linked to DNA repair mechanisms, specifically the mismatch repair and homologous recombination processes, according to genomic research (Pritchard *et al.,* 2016; Roy *et al.,* 2011). The mismatch repair pathway and homologous recombination genes, composed of key genes including *MLH1, MSH2, MSH6, PMS2,* and *BRCA1, BRCA2, ATM, TP53, CHEK2, and PALB2,* fix nucleotide mismatches, minor insertions/deletions and involved in repairing double-strand DNA breaks and therefore maintaining genomic instability (Bugoye *et al.,* 2023; Roy *et al.*, 2011). Germline mutations of these genes have been shown to increase the risk of prostate cancer (Castro *et al.,* 2013a; Castro & Eeles, 2012). Additionally, a thorough investigation of mutations in homologous recombination and mismatch repair genes can lead to improved detection and development of targeted therapies in prostate cancer (Bugoye *et al.,* 2023; Mateo *et al.,* 2015). The clinical significance of these Homologous recombinations and Mismatch repair genes in prostate cancer are outlined below.

2.7.1.3 Breast Cancer gene (*BRCA)*Mutation and Prostate Cancer Immunity

The direct effects of *BRCA* mutations on prostate tumour immunity are not well understood, but it is well known that these mutations can affect the behaviour of the tumour cells' microenvironment, which may have consequences for immunotherapy approaches used to treat prostate cancer (Bugoye *et al.,* 2023; Castro *et al*., 2013b). According to research, tumours bearing harmful *BRCA* mutations may differ from tumours without them in terms of their molecular characteristics and immunological profiles. Increased genomic instability in prostate tumours caused by *BRCA* mutations increases the tumour's immunogenicity and consequently increases the infiltration of immune cells (Castro *et al.,* 2013b). Accordingly, as previously documented (Abida *et al*., 2019; Kantoff *et al.,* 2010), tumours with *BRCA* mutation and enhanced genomic instability may be candidates for immune-based therapy. For example, Sipuleucel-T, an autologous active cellular vaccine used in immunobased therapy, has been demonstrated to be effective in reducing the risk of death in patients with metastatic castration-resistant prostate cancer (Kantoff *et al*., 2010). Research is still being done to determine the precise relationship between *BRCA* mutations and immunity to prostate cancer, but it is clear that these mutations are important for the carcinogenesis of prostate cancer. Knowing the molecular features of prostate cancers with both *BRCA1* and *BRCA2* mutations may help with the development of new treatment approaches, such as immunotherapy (Bugoye *et al.*, 2023).

2.7.1.4 Breast cancer Gene 1(*BRCA1*) Mutation

One of the most important genes in our knowledge of hereditary propensity to breast and ovarian cancers is the Breast Cancer gene 1, or *BRCA1* (Castro *et al*., 2013a; Roy *et al.,* 2011). Encoding a large protein implicated in several physiological functions such as transcriptional regulation, cell cycle regulation, and DNA repair, *BRCA1* is a

gene found on chromosome 17q21 (Castro *et al.*, 2013a; Roy *et al.*, 2011). Contrary to *BRCA2,* which has received more research, *BRCA1* mutations have also been linked to an increased risk of prostate cancer (Castro *et al.*, 2013a). An elevated risk of prostate cancer, especially aggressive forms of the illness, is associated with germline *BRCA1* mutations (Castro *et al.*, 2013a; Roy *et al.*, 2011). Though at lesser frequencies than *BRCA2* mutations, these gene mutations have been found in a subgroup of patients with familial prostate cancer (Bugoye *et al.*, 2023; Castro *et al*., 2013a; Roy *et al.*, 2011). Among the cancers that affect men most frequently is prostate cancer. Research findings indicate that variations in DNA repair genes, such as *BRCA1,* may heighten the likelihood of getting prostate cancer. Various authors have documented varying *BRCA1* mutation spectra across various groups. These populations include;

i. BRCA1 c.181T>G(5382insC)

This frameshift insertion mutation, which affects exon 20 of the *BRCA1* gene, is also referred to as the 5382insC mutation. This mutation type has been reported in many cases of prostate cancer, the *BRCA1* c.181T>G (5382insC), and described as a major genetic component associated with a higher likelihood of prostate cancer. Individuals having family or first-degree relatives harbouring this gene mutation, are recommended to undergo genetic testing for *BRCA1* mutations, including c.181T>G (5382insC), to help (Bugoye *et al.*, 2023; Castro *et al*., 2013b; Pritchard *et al*., 2016).

ii. *BRCA1* c.68_69delAG(185delAG)

The *BRCA1* gene's exon 2 contains a frameshift deletion mutation that truncates the *BRCA1* protein (Bugoye *et al.*, 2023; Castro *et al*., 2013b; Pritchard *et al*., 2016). The *BRCA1* c.68_69delAG (185delAG) mutation has been observed in sporadic cases of prostate cancer, especially in families with a history of multiple cancer types (Bugoye

et al., 2023; Castro *et al.*, 2013b; Pritchard *et al.*, 2016), although it is primarily linked to breast and ovarian cancers (Castro *et al*., 2013a; Leongamornlert *et al.*, 2012b). A higher risk of prostate cancer has been linked to this frameshift mutation, which involves the deletion of two nucleotides (AG) at positions 68 and 69 of the *BRCA1* gene (c.68_69delAG), which has been identified in African populations (Bugoye *et al*., 2023; Castro *et al*., 2013b; Pritchard *et al*., 2016).

iii. *BRCA1* c.3331_3333delCAAG(3450delCAAG) Mutation

In this mutation type, the four nucleotides (CAAG) are deleted in the *BRCA1* nucleotide sequence between the *BRCA1* gene sequence at locations 3331 and 3334 (D. Thompson & Easton, 2002). The *BRCA1* c.3331_3334delCAAG (3450delCAAG) mutation is thought to be harmful, though this mutation is not frequently reported in African populations, it has been observed in European patients with prostate cancer (Pritchard *et al*., 2016). To improve patient management in African prostate cancer patients, more research is required to determine the frequency and significance of the *BRCA1* c.3331_3333delCAAG mutation in African Americans with prostate cancer diagnoses, to better understand this mutation and its link to the disease in these populations (Bugoye *et al*., 2023). Consequently, to uncover genetic risk factors specific to a given group and to further individualized approaches to prostate cancer prevention, screening, and treatment, genetic-based molecular testing and research initiatives targeting different populations will be essential (Bugoye *et al*., 2023).

iv. *BRCA1* c.1953_1956delGAAA Mutation

A particular deletion mutation within the *BRCA1* gene, the *BRCA1* c.1953_1956delGAAA mutation is sometimes referred to as the *BRCA1* 1953delGAAA mutation. Research findings reported that exon 10 of the *BRCA1* gene

contained the initial mutation c.1953 1956delGAAA at the coding DNA level, which corresponds to p.Lys653SerfsX47 at the protein level (Salmi *et al.,* 2021). According to previous research, this mutation is caused by the loss of four nucleotides (GAAA) between nucleotide positions 1953 and 1956 in the *BRCA1* gene sequence (Bugoye *et al*., 2023; Pritchard *et al.*, 2016). In the Moroccan population, it has been reported that Men with germline *BRCA1* mutations, such as the *BRCA1* c.1953_1956delGAAA mutation, were found to have a higher chance of aggressive and early-onset prostate cancer in earlier research (Bugoye *et al*., 2023; Pritchard *et al.*, 2016).

2.7.1.5 Breast cancer Gene 2(*BRCA2*) Mutation

Concerning breast and ovarian malignancies, breast cancer gene 2 (*BRCA2*) has also been thoroughly investigated as a tumour suppressor gene. Nonetheless, the significance of *BRCA2* mutations in prostate cancer susceptibility has come under more and more scrutiny in recent times (Bugoye *et al.*, 2023). The authors have documented several nucleotide *BRCA2* mutations from various populations, which are important for producing data to enhance risk assessment, early identification, and individualised prostate cancer treatment (Bugoye *et al*., 2023). The following *BRCA2* nucleotide mutations have been linked to prostate cancer:

i. *BRCA2* c.2808_2811delACAA nucleotide Mutation

Particularly in people with a family history of cancer or hereditary cancer syndromes, the *BRCA2* c.2808_2811delACAA(p.Ala938Profs) mutation is a type of nucleotide deletion mutation that has been reported within the *BRCA2* gene and linked to the development of prostate cancer susceptibility (de Juan *et al*., 2015; Isaacsson Velho *et al.*, 2018). People who carry this mutation are at risk of developing prostate cancer because it involves the deletion of four nucleotides (ACAA) from positions 2808 to

2811 of the *BRCA2* gene. However, there is very little data on this type of mutation in the African population, so more research is warranted (Bugoye *et al.*, 2023; de Juan *e*t *al.*, 2015). Based on the available information, the most common frameshift BRCA2 mutation in Spain is *BRCA2* c.2808_2811del ACAA, which is also the second most often reported mutation (Diez *et al.*, 2010; Huszno *et al.*, 2019). This particular mutation type has also been documented in premenopausal breast cancer in African women (Huszno *et al.*, 2019; van der Luijt *et al.*, 2001), and in Western European countries (Neuhausen *et al.*, 1998). However, little is known about this mutation in prostate cancer in the African population.

ii. *BRCA2* c.5946delT Mutation

In this *BRCA2* c.5946delT Mutation, the *BRCA2* gene's position 5946 has a thymine (T) nucleotide deleted. In certain populations, the *BRCA2* c.5946delT mutation is rather frequent and has been linked to an elevated risk of prostate cancer. This increased risk is often compounded by a familial history of breast and ovarian malignancies (Castro *et al.*, 2013a; Na *et al.*, 2017)**.** Given that the *BRCA2* protein is essential to DNA repair mechanisms and that its normal function is disrupted by the *BRCA2* c.5946delT mutation, the mutation is regarded as harmful. The discovery of this mutation through genetic molecular testing can help guide focused treatment methods and early detection in addition to individualized screening and management plans for patients with prostate cancer and their families (Bugoye *et al.*, 2023; Na *et al.*, 2017). Studies have shown a correlation between a higher risk of prostate cancer and the *BRCA2* c.5946delT mutation. In comparison to people without the mutation, men who inherit this gene are more likely to develop prostate cancer, frequently at a younger age and with more aggressive features (Bugoye *et al.*, 2023).

iii. *BRCA2* c.7234_7235insG (7234insG)

In BRCA2 c.7234_7235insG mutation, the guanine (G) nucleotide that is inserted between positions 7234 and 7235 of the gene, is sometimes referred to as 7234insG nucleotide mutation (Leongamornlert *et al.*, 2012c). The pathogenic mutation causes a frameshift in the *BRCA2* gene's coding sequence, which in turn alters the *BRCA2* protein's structure and functionality. Individuals having this kind of mutation have been linked to a higher risk of developing early-onset prostate cancer (Leongamornlert *et al.*, 2012c).

iv. *BRCA2* c.6174delT mutation

The c.6174delT mutation, which is referred to as the *BRCA2* 6174delT mutation, is a particular genetic modification found in the *BRCA2* gene that has been linked to an increased risk of developing prostate cancer (Gallagher *et al.*, 2010). In this mutation, a thymine (T) nucleotide at position 6174 of the *BRCA2* gene sequence is deleted (Gallagher *et al.*, 2010). A higher chance of acquiring many malignancies, including prostate cancer, is associated with the *BRCA2* c.6174delT mutation. According to research, males who carry hereditary *BRCA2* mutations, such as c.6174delT, have a higher chance of getting prostate cancer than people in general (Kote-Jarai *et al.*, 2011). The *BRCA2* c.6174delT mutation in African men with prostate cancer, however, is not well understood.

v. *BRCA2* c.6710delACAA mutation

A particular genetic mutation known as the *BRCA2* c.6710delACAA mutation affects the *BRCA2* gene and results in the deletion of four nucleotides (ACAA) at position 6710 in the gene sequence. This loss causes a frameshift mutation, which modifies the gene's reading frame and produces a shortened or non-functional *BRCA2* protein (Edwards *et al.*, 2003). As previously documented in the UK and other populations

(Mitra *et al.*, 2008; Salmi *et al.*, 2021), the *BRCA2* c.6710delACAA mutation can be inherited in an autosomal dominant fashion, meaning that individuals with this mutation have a probability of passing it on to their offspring. Additionally, patients with prostate cancer who have the *BRCA2* c.6710delACAA mutation have particular clinical traits, such as an earlier age of onset, more aggressive tumour features, and probable resistance to a particular therapy (Edwards *et al.*, 2003). Thus a tailored strategy for treatment, such as targeted medicines like PARP inhibitors, can be guided by knowledge of the mutation status (Bugoye *et al.*, 2023; Pritchard *et al.*, 2016).

vi. *BRCA2* c.1813_1814insA Mutation

Adenine (A) nucleotide insertion at positions 1813 and 1814 of the *BRCA2* gene causes this *BRCA2* frameshift mutation. Such insertion mutations can modify both the structure and the function of the resultant *BRCA2* protein by altering the regular gene sequence (Bugoye *et al.*, 2023). In Germany, a higher Gleason score has been linked to an increased risk of aggressive prostate cancer caused by this *BRCA2* c.1813_1814insA mutation, and similar has been reported in different populations (Maier *et al.*, 2014; Salmi *et al.*, 2021). However, further research is necessary because this mutation has not been extensively documented in the African population, particularly in areas where prostate cancer is more common.

vii. *BRCA2* c.3847delGT Mutation

In this frameshift mutation, the *BRCA2* gene's nucleotide GT at position 3847 is deleted, and as a result of this deletion mutation, the gene sequence changes, leading to a truncated or non-functional *BRCA2* protein (Salmi *et al.*, 2021). According to research, carriers of the pathogenic c.3847delGT mutation had a higher chance of having prostate cancer with advanced features, including grade Tumours with a Gleason score >8 and metastasis characteristics as compared to non-carriers(Maier *et*

al., 2014; Salmi *et al.*, 2021). Since individuals harbouring *BRCA2* mutation can benefit from tailored treatment, therefore, the determination of *BRCA2* c.3847delGT Mutation is also important for personalized medicine(Bugoye *et al*., 2023).

viii. *BRCA2* c.4449delA Mutation

In this mutation, an adenine (A) nucleotide at position 4449 of the *BRCA2* gene is deleted and it is denoted as *BRCA2* c.4449delA mutation (Castro *et al.*, 2013b; Gallagher *et al.*, 2010). Similar to other deletion mutations, this mutation type may have an impact on the gene's reading frame. As a result, the protein product changes and has been linked to hereditary cancer syndromes as well as prostate cancer (Castro *et al.*, 2013b; Gallagher *et al*., 2010; Salmi *et al.*, 2021).

ix. *BRCA2* c.6037A>T mutation

This mutation causes thymine (T) to replace adenine (A) at location 6037 of the *BRCA2* gene. An encoded protein's function may be impacted by substitution mutations, which can alter the protein's amino acid composition (Maier *et al.*, 2014). previously reported in the genomics of prostate cancer, the c.6037A>T mutation in *BRCA2* has consequences for cancer susceptibility, including prostate cancer (Maier *et al.*, 2014).

x. BRCA2 c.7495C>T Mutation

The *BRCA2* c.7495C>T mutation occurs when thymine (T) is substituted for cytosine (C) at position 7495 of the *BRCA2* gene. As with *BRCA2* c.6037A>T, this substitution mutation can change the *BRCA2* protein's amino acid sequence, which could affect how the protein functions in DNA repair and cancer suppression (Mitra *et al*., 2008)It has been documented previously that the c.7495C>T mutation is linked to an increased risk of cancer, particularly prostate cancer (Mitra *et al*., 2008).

xi. *BRCA2* c.7234_7235insG Mutation

There have been findings linking this frameshift insertion mutation to a higher risk of prostate cancer in Moroccans (Salmi *et al.*, 2021). By inserting one guanine nucleotide into exon 14 of the *BRCA2* mRNA (c.7234_7235insG), a frameshift pathogenic mutation occurs that results in a reading frame shit at codon 2413. This raises the chance of developing several forms of breast, ovarian, and prostate cancer by causing an early translational stop signal 2 amino acid residues, which in turn causes a missing or disordered protein product (Salmi *et al.*, 2021). Patients with prostate cancer who have a history of breast cancer in Morocco have also been shown to carry this *BRCA2* c.7234_7235insG mutation. Even though Tanzanian and Moroccan communities with 17-YSTRs makers reported Y chromosomal mutation rates that were comparable, this form of mutation has not been adequately reported in sub-Saharan Africa (Bugoye *et al., 2018; Laouina et al., 2013)*. Thus, more research is required in sub-Saharan African nations. Broadly speaking, genetic counselling, risk assessment, and customized cancer care techniques may be beneficial for people with germline *BRCA1* and *BRCA2* mutations, including the ones mentioned above. Since little information is available about *BRCA2* nucleotide mutations reported from the African population, more research is necessary to determine the efficacy of targeted therapies, like PARP inhibitors, in treating prostate cancer with specific *BRCA2* mutations (Bugoye *et al.*, 2023).

2.7.1.6 ATM Gene Mutation and Prostate Cancer Immunity

Several cancers, including prostate cancer, have been found to contain mutations in the *ATM* (ataxia telangiectasia mutated) gene in various populations under study. As was already established, the *ATM* gene is essential for both DNA repair and the preservation of genomic stability. A mutation in the *ATM* gene can result in

compromised DNA repair pathways, genomic instability, and a higher chance of developing cancer (Bugoye *et al.,* 2023). Based on available findings, tumours become more immunogenic, immune recognition-susceptible, and capable of inducing an immune response because of the increased genomic instability caused by *ATM* mutation, which results in generations of neoantigens proteins that change the prostate tumour microenvironment (M. Jiang *et al.,* 2021). Researchers have demonstrated that immune checkpoint blockade immunotherapies are effective in treating prostate cancer patients who exhibit ATM-mutated genes and metastatic castration resistance (Abida *et al*., 2019; Bugoye *et al.,* 2023). Furthermore, to assist individuals who carry an *ATM* gene mutation, combination therapy has been investigated due to the heightened immunogenicity of prostate tumours. Targeting underlying DNA repair deficiencies linked to *ATM* gene mutations, these combination treatments aim to improve the immune response to tumour cells (Kwok *et al.,* 2016). While not every patient with prostate cancer who carries an *ATM* mutation will benefit from immune-based treatments, more studies in various populations are advised to identify the various forms of *ATM* gene mutations and to find predictive biomarkers for the response of prostate cancer patients to immunotherapy (Su *et al.,* 2023). The following are a few examples of *ATM* gene mutations that have been identified in people with prostate cancer:

i. *ATM* c.7271T>G Missense Mutation

Prostate cancer patients have been shown to have the *ATM* c.7271T>G missense mutation (Nguyen-Dumont *et al.,* 2020). This missense mutation occurs at nucleotide position 7271, where it causes a T to G substitution. The resultant amino acid, glycine, replaces valine at codon 2424, despite having different characteristics (Goldgar *et al.,* 2011; Nguyen-Dumont *et al.,* 2020). The deletion occurs in exon 48

of the *ATM* gene coding area. In addition to being extensively documented in cases of prostate cancer, this change has also been observed in cases of ataxia-telangiectasia and other known cancers (Goldgar *et al.*, 2011; Hall *et al.*, 2021; Pearlman *et al.*, 2017). Nguyene and colleagues identified *ATM*: c.7271T>G in aggressive prostate cancer patients (Nguyen-Dumont *et al.*, 2020). Other studies have reported *ATM* mutation in breast and ovarian cancer as well as non-aggressive prostate cancer. Although this missense mutation has been extensively documented in different populations, little is known about its prevalence in Africa.

ii. *ATM* c.7630-2A>C Splice site Mutation

There is a recognized splice site for *ATM* c.7630-2A>C, also referred to as IVS53-2A>C. It has an impact on mRNA splicing, which leads to changed protein as a consequence of exon skipping, shortening, or intronic material inclusion (Sandoval *et al.*, 1999), as a result, it is associated with a higher risk of prostate cancer, which is marked by a higher Gleason score and a poor prognosis (Pritchard *et al.*, 2016). Though certain African Americans with this mutation may benefit from personalized treatment, this alteration type has not been substantially described, similar to other *ATM* harmful variants connected to prostate cancer Bugoye *et al*., 2023).

iii. *ATM* c.7010 _7011del mutation

A deletion mutation in the *ATM* nucleotide gene sequence occurs between positions 7010 and 7011 and is known as *ATM* c.7010_7011del. Two nucleotides are lost as a result of this deletion, which may result in a frameshift mutation and altered protein function (Wokołorczyk *et al.*, 2020). According to research findings research, this particular mutation type is linked to aggressive and early-onset prostate cancer in Poland, as indicated by higher Glasson scores (Bugoye *et al.*, 2023; Wokołorczyk *et al., 2020).*

2.7.1.7 TP53 Gene Mutation in Prostate Cancer

The *TP53* gene plays a key role in controlling apoptosis, DNA repair, and the cell cycle. In human cancers, particularly prostate cancer, *TP53* mutations are among the most frequently found genetic changes. Research shows that *TP53* mutations in prostate cancer include missense, insertion, deletion, and point mutations and that these mutations are particularly common in advanced or metastatic stages. Prostate cancer treatment results have a negative association with *TP53* mutations (Su *et al.*, 2022). Studies reveal that these mutations are more common in castration-resistant prostate cancer and high-grade tumours, suggesting a role in the aggressiveness of the illness. The cancer genomic research has increased our understanding of *TP53* mutation patterns by identifying a high frequency of single nucleotide variations (Kaur *et al.*, 2019).

Several hotspot mutations restricted to the DNA-binding domain of the *TP53* gene are among the common nucleotide variations of the gene found in prostate cancer. These mutations frequently change how well the tumour suppressor controls downstream genes that are involved in cell cycle regulation and apoptosis (Barnoud *et al.*, 2019). *TP53* c.524G>A, *TP53* c.743G>A, *TP53* c.743G>T, *TP53* c.818G>A, and *TP53* c.818C>T) are the most commonly found variations in prostate cancer (Martin *et al.*, 2002; Shirts *et al.*, 2016). The function of the protein is disrupted by these mutations, which are primarily missense and cause the substitution of essential amino acids. For example, the *TP53* c.524G>A mutation is one of the most frequent mutations involving *TP53* protein loss of function brought on by structural instability. Similarly, mutations such as *TP53* c.743G>T and *TP53* c.818G>A affect the protein's ability to bind DNA, which accelerates the growth of tumours (Chiang *et al.*, 2021). In advanced stages of prostate cancer, such as castration-resistant prostate cancer, these

mutations are especially common and frequently linked to poor clinical outcomes. Though they are less common than missense mutations, nonsense mutations and frameshift insertions or deletions are frequently seen. These results highlight the potential of *TP53* as a therapeutic target and prognostic biomarker, as well as its crucial involvement in the biology of prostate cancer (Barbieri *et al.*, 2013).

2.7.1.8 Checkpoint Kinase 2 (*CHEK2*) Mutation in Prostate Cancer

Damage to DNA response and repairing pathways rely on the essential gene *CHEK2*. By controlling cell cycle checkpoints and encouraging DNA repair, it significantly contributes to the preservation of genomic stability (Antonarakis, 2018; Bugoye *et al.*, 2023). *CHEK2* mutations are not as common as other mutations in DNA damage repair genes, but they are nonetheless important for the carcinogenesis and development of prostate cancer (Antonarakis, 2018; Leongamornlert *et al.*, 2012c).

2.7.1.9 Checkpoint Kinase 2 (*CHEK2*) Mutation and Prostate Cancer Immunity

According to previous findings, *CHEK2* mutations frequently impact DNA repair mechanisms and raise the risk of prostate cancer (Bugoye *et al*., 2023; Wokołorczyk *et al.*, 2020). Because DNA repair pathways and immune signalling pathways are closely related, research findings point to possible connections between DNA repair genes like *CHEK2* and the immune response in cancer (Bugoye *et al.*, 2023; Le *et al.*, 2017). Increased genomic instability, a higher tumour mutational burden, and the production of neoantigens which the T cell-mediated immune system can identify as foreign can result from mutations in DNA repair genes like *CHEK2* (Le *et al.*, 2017). Thus, mutations affecting DNA repair genes, including *CHEK2*, may affect the immune response to prostate cancer as well as the tumour's immunological microenvironment (DLe *et al.*, 2017). Furthermore, immune checkpoint mechanisms that regulate the immune system's response and prevent autoimmunity include T-

lymphocyte-associated protein 4 (CTL-4) and programmed cell death protein 1 (PD-1)/PD-L1 pathways (Bishop *et al.*, 2015; Bugoye *et al.*, 2023). However, according to research, tumours with a high burden of tumour mutations, such as those with *CHECK2* mutation, may be more amenable to immune checkpoint blockade therapy because of their higher propensity to produce neoantigens, which can trigger the immune system's antitumor response (Pritchard *et al.*, 2016). Diverse groups have reported cases of prostate cancer with *CHEK2* mutations; people with this mutation can benefit from more frequent and earlier screenings, as well as the possibility of targeted therapy (Bugoye *et al.*, 2023; Wokołorczyk *et al.*, 2020). The following are among the known *CHEK2* mutations in prostate cancer.

i. *CHECK2* c.592+3A>T Mutation

The CHEK2 gene's A to T alteration occurs 3 nucleotides after coding exon 3 and causes the check2 c.592+3A>T intronic variation. This mutation has been linked to a higher risk of prostate cancer, but it has also been found in some cohorts with breast cancer(Hauke *et al.*, 2018) . Individuals with this type of mutation may be candidates for, and benefit from, the available personalized treatment, similar to many other homologous recombination repair gene mutations (Bugoye *et al.*, 2023).

ii. *CHEK2* c.1100delC Mutation

This mutation type is a well-publicized form of the *CHEK2* mutation that has been associated with multiple malignancies, including prostate carcinoma. This particular mutation type results in a frameshift mutation and premature protein termination due to the deletion of cytosine (C) at position 1100 of the *CHEK2* gene (Hale *et al.*, 2014). The c.1100delC (p. Thr367fs) mutation in *CHEK2* has been linked to an elevated risk of prostate cancer, among other cancers, according to earlier research findings (Hale 14). According to meta-analyses, there is a 2-4 odds ratio for prostate cancer, along

with breast, colorectal, and potentially a somewhat lesser increased risk for malignant melanoma (Wang *et al.,* 2015; Weischer *et al.,* 2008). Prostate cancer risk has been linked to the *CHECK2* c.1100delC (p. Thr367fs) mutant variation, which has been extensively reported in various populations (Hale *et al.,* 2014). Further research is necessary because there is insufficient information available about this mutant variant in the African population (Bugoye *et al*., 2023; Wokołorczyk *et al.,* 2020).

iii. *CHECK2* c.T470C Mutation

One of the frequent germline variants found in the *CHEK2* gene is the T470C mutation. According to the literature, the *CHEK2* gene's position 470 has a shift from thymine (T) to cytosine (C), which is known as the T470C mutation. Though its contribution to an elevated risk of prostate cancer in varied groups is low, this missense mutation has been frequently documented and linked to an increased susceptibility to prostate cancer. Consequently, more research is required (Dong *et al*., 2003; Wokołorczyk *et al.,* 2020).

iv. *CHECK2* c.I157T Mutation

The *CHEK2* gene's I157T mutation is a missense mutation variant that results from a substitution of isoleucine (I) for threonine (T) at position 157. Research has revealed links between the *CHEK2* c.I157T mutation and prostate tumour risk, especially in certain populations, despite the variant's initial classification as low-penetrance (Bugoye *et al.,* 2023; Wokołorczyk *et al.,* 2020). Although findings of this variant have been made in a variety of populations, few African populations have been used in those reports (Bugoye *et al.,* 2023; Dong *et al.,* 2003).

2.7.1.10 Partner and Localizer of *BRCA2* (*PALB2*) Mutation in prostate cancer

By interacting with *BRCA2*, the gene *PALB2* is also implicated in the DNA repair pathway (Bugoye *et al.,* 2023; Leongamornlert *et al.,* 2012c). According to research,

mutations in *PALB2* can hinder the repair of DNA damage, resulting in genomic instability and perhaps promoting the onset of cancer (Pritchard *et al.*, 2016). Researchers have linked the *PALB2* mutation to an elevated risk of prostate cancer in those who carry the modification (Bugoye *et al.*, 2023; Leongamornlert *et al.*, 2012c). Although there are fewer reports of *PALB2* mutation variants in prostate cancer than in breast cancer, new research points to the possible involvement of *PALB2* mutations in the initiation and spread of prostate cancer, hence justifying additional studies (Leongamornlert *et al.*, 2012c).

2.7.1.11 Partner and Localizer of *BRCA2* (*PALB2*) Mutation and prostate cancer immunity

Among the genes involved in DNA damage repair, namely in the homologous recombination process, is *PALB2* (Bugoye *et al.*, 2023; Yadav *et al.*, 2020). Prostate cancer cells may exhibit elevated genomic instability and a high tumour molecular burden as a result of mutations in the *PALB2* gene that impair DNA repair capacity. Improved neoantigen accumulation is associated with high tumour molecular load, and this may improve the immune system's ability to recognise tumour cells (Wu *et al.*, 2018). Tumours with defective DNA repair mechanisms, such as those associated with *PALB2* mutations, have been shown to display altered immune cell infiltration into the tumour microenvironment as a result of an elevated tumour molecular burden (Mateo *et al.*, 2020). Furthermore, different forms of pathogenic *PALB2* gene mutations and other DNA repair deficits in prostate tumours may show distinctive immunological landscapes that enhance the response to immunobased therapy (Bugoye *et al.*, 2023; Topalian *et al.*, 2015). Treatments with checkpoint inhibitors that target either cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) or program cell death 1 (PD-1)/PD-L1 have shown promise in tumours with DNA repair deficiencies; this may be because these tumours are more immunogenic and activate T

cells (Topalian *et al.*, 2015, 2016). Additionally, *PALB2* mutant variants are being studied as predictive biomarkers for the response to immunotherapy in prostate cancer, along with other DNA repair gene mutations (Bugoye *et al.*, 2023; Topalian *et al.*, 2016).. Thus, it is imperative to explore these variants from various populations and utilise the current data on *PALB2* mutations to assist those who are afflicted with prostate cancer. Some of the reported *PALB2* mutations in prostate cancer include the following;

i. *PALB2* c.509_510del (del157ins5 Mutation

The mutation that results in a frameshift mutation and premature termination of the protein is caused by the loss of guanine and adenine (GA) nucleotides at positions 509 and 510 of the *PALB2* gene (Park *et al.*, 2012). A higher risk of prostate cancer has been linked to the *PALB2* c.509_510delGA mutant variant, primarily in families with a history of cancer (Park *et al.*, 2012). However, there's a paucity of information regarding the role of this mutation in diverse populations, therefore it calls for further research.

ii. **PALB2 c.2993G>A (p.Trp998Ter) Mutation**

The missense mutation variant PALB2 c.2993G>A, also known as the p. Trp998Ter variant, is a nonsense mutation that causes glycine (G) to replace adenine (A) at position 2993 of the *PALB2* gene. It has been linked to an increased risk of prostate cancer and causes early termination of the protein (Leongamornlert *et al.*, 2012a; Risch *et al.*, 2006). Research on gene mutations related to the *PALB2* gene is scarce in the African context; therefore, pathogenic PALB2 c.2993G>A is not well reported and should be investigated further (Bugoye *et al.*, 2023). In addition, Individuals carrying pathogenic mutations involving homologous recombination repair genes benefit from customized treatments, as previously reported (Bugoye *et al.*, 2023).

iii. ***PALB2* c.2982+1G>A Mutation**

Within the *PALB2* gene, a splice site mutation known as *PALB2* c.2982+1G>A occurs. This mutation results in faulty RNA splicing and may have an impact on the function of the *PALB2* protein by altering the consensus splice donor site at intron 7's +1 position (Soemedi *et al.,* 2017). Although the *PALB2* c.2982+1G>A mutation type has been extensively documented in pancreatic, ovarian, and breast cancers (Jones *et al.*, 2009), little is known about its contribution to prostate cancer, so more research in this area is necessary (Bugoye *et al.*, 2023; Leongamornlert *et al.*, 2012a).

iv. ***PALB2* c.1592delT (p. Leu531fs) Mutation**

The frameshift mutation identified as *PALB2* c.1592delT, which results from the deletion of a thymine (T) base at position 1592, causes a frameshift in the structure of the nucleotide sequence of the *PALB2* gene (Casadei *et al.,* 2011). It is located in coding exon 4 of the *PALB2* gene. This frameshift causes the altered site to terminate prematurely, which results in the truncated *PALB2* protein. The resulting protein cannot interact with *BRCA2* in DNA repair pathways correctly, and as a consequence, it can be linked to an increased risk of cancer, including prostate cancer (Casadei *et al.*, 2011; Erkko *et al.*, 2007). Although there is little information about this mutation type in the African population, more research is required even though it has been linked to several well-known malignancies, including ovarian, breast, and prostate cancers in different groups (Casadei *et al.*, 2011; Erkko *et al*., 2007).

v. *PALB2* c.3113G>A (p.Trp1038*) Mutation

Specifically, the pathogenic *PALB2* c.3113G>A mutation, which is located in coding exon 10 of the *PALB2* gene sequence and is caused by a G to A substitution at nucleotide position 3113 (Casadei *et al.*, 2011), is a nonsense mutation that results in the premature termination of the *PALB2* protein. It was previously reported that this

mutation, which occurs in the final base pair of coding exon 10, results in three defective mRNA isoforms: one that completely skips exon 10 (deleting 117 bp), another that uses an alternate splice site within exon 10 (deleting 31 bp out of frame), and a third that uses tryptophan as the amino acid source (Casadei *et al.*, 2011). In certain populations, this frameshift mutation has been linked to a higher risk of prostate cancer development (Southey, Winship, *et al.*, 2016; Thompson *et al.*, 2015), however, due to the paucity of available data, their impact on the elevated prostate prevalence and mortality rates in African populations is minimal.

2.7.1.12 MutL homolog 1 (*MLH1*) Mutation in prostate cancer

Maintaining genomic stability and preventing the accumulation of mutations are two important functions of *MLH1*, one of the major DNA mismatch repair genes. A harmful *MLH1* mutation can lead to errors in DNA repair mechanisms, which can accelerate the progression of certain cancers, such as prostate cancer (Bugoye *et al.*, 2023). Furthermore, the *MLH1* gene plays a crucial role in the DNA mismatch repair (MMR) pathway, which corrects mistakes that arise during the replication process of DNA (Li, 2008). To detect and fix mismatched nucleotides and provide tiny insertion-deletion loops in DNA, the *MLH1* protein interacts with other protein molecules, such as *PMS2*, to form a complex (Li, 2008). Research has shown that there is a link connecting *MLH1* mutations and prostate cancer and that those who carry this mutation have an increased likelihood of acquiring prostate cancer than those who do not have the *MLH1* pathogenic mutations (Raymond *et al.*, 2013). The early detection and management of prostate cancer are greatly impacted by molecular genetic testing for *MLH1* mutations. Individuals with *MLH1* mutations may benefit from more frequent and earlier screening for prostate cancer, as well as personalized methods and possible targeted treatments depending on their genetic mutation landscape

(Bugoye *et al.*, 2023; Pritchard *et al.*, 2016). Thus, understanding the significance of *MLH1* mutations is crucial for screening plans, personalized treatment plans, and risk evaluation for prostate cancer in individuals with *MLH1* mutations (Bugoye *et al.*, 2023; Pritchard *et al., 2016).* To precisely understand how *MLH1* mutations affect the onset and course of prostate cancer, more investigation is necessary. The following are a few of the many populations' previously reported *MLH1* mutations:

2.7.1.13 MutL homolog 1 (*MLH1*) Mutation and prostate cancer Immunity

One of the essential components of the DNA mismatch repair mechanism, which fixes mistakes made during DNA replication, is *MLH1*. A faulty DNA repair process and instability in the genome can be caused by mutations in *MLH1* and other mismatch repair genes, which can contribute to the genesis of prostate cancer (Gryfe *et al.*, 2000). Cancer cells accumulate more mutations and neoantigens due to defects in the mismatch repair system, which are frequently brought on by *MLH1* mutations. This effect is referred to as tumour immunogenicity (Le *et al.*, 2015), whereby the immune system recognizes cancer cells more readily. Although there are few specific studies connecting *MLH1* mutations to immunity against prostate cancer, it is plausible that these tumours may have changed immune microenvironments (Bugoye *et al.*, 2023), making them possibly more immunogenic and sensitive to immune-based therapies than *MLH1*-proficient tumours (Le *et al.*, 2015). Some of the *MLH1* mutations connected to increased risk of prostate cancer include the following;

i. *MLH1* c.655A>G (p.Ile219Val) Mutation

The mutation *MLH1* c.655A>G results in the replacement of guanine (G) for adenine (A) at position 655 of the *MLH1* gene. This causes an amino acid change from isoleucine (Ile) to valine (Val) at codon 219 of the *MLH1* protein (Fredriksson *et al.*, 2006). Research has shown that people with Lynch syndrome, especially those with

MLH1 mutations such as *MLH1* c.655A>G, are more likely than those in the general population to acquire prostate cancer (Fredriksson *et al.,* 2006). Germline *MLH1* mutations have the potential to enhance prostate carcinogenesis, underscoring the significance of molecular genetic testing and risk assessment in prostate cancer patients (Fredriksson *et al.,* 2006). Prostate cancer detection regularly can help persons with the *MLH1* c.655A>G (p.Ile219Val) mutation detect illnesses early when treatment options are more favourable. To precisely understand how *MLH1* mutations affect the onset and course of prostate cancer in various groups, more study is necessary (Bugoye *et al.,* 2023).

ii. ***MLH1* c.1165C>T (p.Arg389Trp) Mutation**

The structural cause of the *MLH1* c.1165C>T mutation is a nucleotide substitution at nucleotide position 1165, which occurs in coding exon 12 of the *MLH1* gene. This nucleotide alteration results in the amino acid tryptophan, which has distinct properties, replacing the arginine at codon 389 (Rosty *et al*., 2014). Although this genetic variant has been linked to several cancer types, scientists have also linked it to a higher risk of prostate cancer (Samowitz *et al*., 2001; Win *et al.,* 2012). Since there is little evidence linking these mutant types to an increased risk of prostate cancer, understanding the roles played by *MLH1* mutations in prostate cancer has significant therapeutic implications, particularly for cancer risk assessment, genetic counselling, and monitoring. To establish a more certain correlation, more research is required to precisely examine the mutation landscape and implications of the c.1165C>T mutation in prostate cancer (Bugoye *et al.,* 2023).

2.7.1.14 MutS Homolog 2 (*MSH2*) Mutation in Prostate Cancer

A protein that is involved in DNA mismatch repair processes is encoded by several genes, including *MSH2* (Boland & Goel, 2010; Bugoye *et al.,* 2023). Prostate cancer

is one of the many malignancies that arise from mutations in the *MSH2* gene that impact DNA repair pathways (Boland & Goel, 2010; Bugoye *et al.*, 2023). Because it corrects errors that arise during DNA replication, *MSH2* is essential for maintaining genomic stability. Furthermore, mutations in *MSH2* genes have been linked to the accumulation of mutations that cause cancer and microsatellite instability (Boland & Goel, 2010). *MSH2* mutations have been linked in the past to an increased risk of prostate cancer, especially in tumours that exhibit microsatellite instability (Giri *et al.*, 2018). Thus, opportunities for genetic counselling, risk evaluation, and therapeutic options may arise from the identification of *MSH2* mutations in people with prostate cancer. Additionally, patients with *MSH2* alterations may benefit from personalised immunotherapies that make use of defects in DNA repair (Pritchard *et al.*, 2016).

2.7.1.15 MutS Homolog 2 (*MSH2*) Mutation and Prostate Cancer Immunity

Previous studies have shown that *MSH2* mutations lead to defective DNA repair, which can increase the burden of tumour mutations (Le *et al.*, 2017). Increased neoantigen accumulation is associated with a higher tumour mutation burden, and this improves the immune system's ability to recognize tumours (Le *et al.*, 2017). Furthermore, increased genomic instability linked to mismatch repair deficit associated with *MSH2* mutations increases immunological tumour-infiltrating lymphocytes (TILs) and upregulates immune checkpoint markers such as PD-1/PD-L1 (Marcus *et al.*, 2019).Thus, this implies that individuals with prostate malignancies harbouring *MSH2* mutations may be susceptible to immune checkpoint blockage such as anti-PD-1/PD-L1 therapy (Luchini *et al.*, 2019). The following are some of the *MSH2* mutations that have been previously identified in patients with prostate cancer:

i. *MSH2* c.942+3A>T Mutation

The harmful nucleotide mutation known as *MSH2* c.942+3A>T occurs when thymine (T) is substituted for adenine (A) at the +3 position of intron 5 in the *MSH2* nucleotide gene sequence. The splice acceptor site gets affected by this nucleotide alteration, and it plays an important role in appropriate mRNA splicing during gene expression (Dominguez-Valentin *et al.,* 2016b). Normal splicing is typically disrupted by these pathogenic mutations at splice sites, which leads to faulty mRNA transcripts and potentially altered protein products (Dominguez-Valentin *et al.*, 2016a). Similarly, to several other mismatch repair genes, nucleotide alterations in *MSH2* c.942+3A>T are linked to faulty DNA repair processes, microsatellite instability, and the accumulation of mutations that aid in prostate cancer (Dominguez-Valentin *et al.,* 2016b). Even though this mutation has been linked to prostate cancer, more investigation is needed to determine the exact effects of the *MSH2* c.942+3A>T mutation in a variety of populations including unreported African populations (Bugoye *et al.*, 2023; Dominguez-Valentin *et al.*, 2016b).

ii. *MSH2* c.1906G>C Mutation

The deleterious *MSH2* c.1906G>C mutation, which results in a nucleotide substitution at position 1906 from G to C, is located in coding exon 12 of the *MSH2* gene sequence. Proline, an amino acid, substitutes the amino acid alanine at codon 636. These alterations represent a founder mutation linked to several well-known malignancies that affect Ashkenazi Jews (Wu *et al.,* 2020; Yuan *et al.,* 1999). Additionally, reports have linked this mutation to prostate cancer in many ethnic groups (Laish *et al.,* 2021; Wu *et al.,* 2020). Because the *MSH2* mutation benefits from individualized treatment due to the presence of numerous other mismatch repair genes, it is crucial to carry out genetic research, investigate this mutation in a variety

of populations, and identify those who have the pathogenic *MSH2* c.1906G>C mutation for personalized treatment (Bugoye *et al.,* 2023).

2.7.1.16 MutS Homolog 6 (*MSH6*) mutation in prostate cancer

Pathogenic mutations in the *MSH6* gene can impair the function of the DNA mismatch repair mechanism, which in turn can contribute to prostate cancer. *MSH6* is a crucial gene involved in DNA mismatch repair and a component of the MutSα heterodimer (Jiricny, 2006). Prostate tumours with mutations in the *MSH6* gene, like many other alterations involving DNA mismatch repair genes, may show particular molecular and clinical characteristics as well as microsatellite instability associated with the emergence of aggressive prostate cancer (Pritchard *et al.*, 2016). According to earlier research, patients with prostate cancer who have *MSH6* mutations may benefit from customized targeted therapies. For this reason, molecularly detecting *MSH6* mutations in prostate cancer patients has a big influence on genetic counselling and familial cancer risk assessment (Haraldsdottir *et al.*, 2014).

2.7.1.17 MutS Homolog 6 (*MSH6*) mutation and prostate cancer immunity

Mismatched DNA mismatch repair insufficiency is caused by *MSH6* mutations, just like it is by many other mismatch repair genes (Le *et al.*, 2017). In prostate tumours with *MSH6* mutations, this deficiency typically leads to increased tumour mutational burden, accumulation of neoantigens that improve immune detection, increased tumour-infiltrating lymphocytes (TILs), and activation of immunological checkpoint markers (Le *et al.*, 2017). Accordingly, individuals with an *MSH6* pathogenic mutation may be sensitive to immune checkpoint blockage based on this immune checkpoint overexpression (Antonarakis *et al*., 2020). Studies on *MSH6* mutations in patients with prostate cancer are rare, however, some of the previously documented *MSH6* Mutations have been reported elsewhere. These include the following:

i. *MSH6* c.3261dupC Mutation

According to its structural rearrangement, the c.3261dupC harmful mutation is found in coding exon 5 of the *MSH6.* It is a frameshift alteration in the gene nucleotide sequence that results from the duplication of a cytosine (C) nucleotide at position 3261. This mutation consequently alters the gene's normal reading frame, resulting in an *MSH6* protein product that is not functional (Shin *et al.,* 2004). A limited amount of information is available regarding individuals of African origin. This frameshift gene mutation has been revealed elsewhere in various families and individuals with Lynch syndrome-associated cancers, including prostate cancer (Bugoye *et al.,* 2023; Matejcic *et al.,* 2020; Shin *et al.,* 2004).

ii. *MSH6* c.3799_3800del Mutation

A translational frameshift with a predicted alternate stop codon (p.M1267Gfs*7) is caused by the deletion of two nucleotides at nucleotide positions 3799 to 3800, which results in the pathogenic mutation known as *MSH6* c.3799_3800delAT, which is located in coding exon 8 of the *MSH6* nucleotide gene sequence (Klarskov *et al.,* 2011). According to research mutations causing defects in the mismatch repair pathway through *MSH6* modification lead to genomic instability, mutation accumulation, and enhanced carcinogenesis (Bugoye *et al.,* 2023). Additionally, this mutation has been found in people with a variety of cancer forms, including prostate cancer. However, it is unclear how significant of a role it plays in African males who get prostate cancer (Bugoye *et al.,* 2023; Pritchard *et al.,* 2014).

iii. *MSH6* c.1444C>T Mutation

This harmful mutant variation arises from a single nucleotide alteration in exon 4 of the *MSH6* gene sequence. This alteration contributes to an early translation stop signal, which in turn produces a nonfunctioning *MSH6* protein (Bugoye *et al.,* 2023).

resulting in abnormalities in the DNA repair system and heightened vulnerability to cancer *MSH6* c.1444C>T has been linked to Lynch syndrome in addition to the prostate, like many other *MSH6* mutations (Hendriks *et al.*, 2004). Even though people who have this mutation can benefit from tailored treatment, more research is necessary because there isn't enough knowledge on this particular mutation type.

2.7.1.18 Postmeiotic Segregation Increased 2(*PMS2*) Mutation in Prostate cancer

One of the major genes involved in DNA mismatch repair, a crucial process that corrects errors during DNA replication, is *PMS2*. Prostate cancer is among the carcinogenesis-causing factors that result from mutations in the *PMS2* gene, which impair the mismatch repair system (Dominguez-Valentin *et al.*, 2020). During DNA replication, the *PMS2* gene directs the synthesis of a protein that heterodimerizes with *MLH1* to create the MutLα complex. This complex is responsible for detecting and correcting short insertion-deletion loops and DNA mismatches (Dominguez-Valentin *et al.*, 2020). Maintaining genomic stability and preventing the accumulation of mutation events are made possible by the mismatch repair pathway mechanism's appropriate operation. On the other hand, when the *PMS2* mutation takes place, it causes microsatellite instability and raises the chance of developing several cancers, including Prostate cancer(Bugoye *et al.*, 2023).

2.7.1.19 Postmeiotic Segregation Increased 2(*PMS2*) mutation and Prostate cancer immunity

Research on the intricate and developing relationship between *PMS2* mutations and the immune response in prostate cancer is ongoing. Due to defective DNA repair mechanisms brought on by mutations in *PMS2* and other mismatch repair genes, there is an increased generation of neoantigens, which in turn leads to high levels of microsatellite instability. Ultimately, this enhances immune detection and promotes

antitumor immunity (Schulz *et al.*, 2012). To fully understand the relationship between *PMS2* gene mutations and immunity to prostate cancer, it is important to take into account several factors, such as tumour antigenicity, immune cell infiltration, and immunotherapy response (Le *et al.*, 2015). *PMS2* gene mutations affect the immune response within the tumour microenvironment. Immune checkpoint pathways, including cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) and programmed cell death protein (PD-1)/PD-L1, may be enhanced by defective mismatch repair mechanisms linked to *PMS2* mutations (Bugoye *et al.*, 2023; Dudley *et al.*, 2016). According to research, these immunological checkpoints contribute to immune cell escape by inhibiting T-cell-mediated antitumor immunity (Dudley *et al.*, 2016). The information that is now available indicates that tumours with dysfunctional mismatch repair mechanisms like those containing *PMS2* mutations increase immune cell infiltration, especially that of cytotoxic T cells. Prostate cancer is one of the cancer types for which the presence of tumour-infiltrating lymphocytes (TILs) has been linked to favourable responses to immunotherapy (Bugoye *et al.*, 2023; Dudley *et al.*, 2016). The following are a few significant *PMS2* mutations:

i. ***PMS2* c.736C>T (p.Arg246Ter) mutation**

PMS2 c.736C>T nucleotide mutation, which results from a nucleotide substitution at nucleotide position 736 from C to T, is structurally located in coding exon 7 of the *PMS2* gene sequence. As a result of these alterations, it replaces the amino acid serine at codon 246 with proline (Senter *et al.*, 2008). According to research, this mutation has been found in cases of prostate cancer and is linked to the truncation of the *PMS2* protein. This leads to a dysfunctional DNA repair process that is functionally defective, causing microsatellite instability and an elevated risk of many cancers, including prostate cancer (Senter *et al.*, 2008). Though the *PMS2* c.736C>T mutation

has been linked to hereditary prostate cancer, as has been reported in the literature, the role of *PMS2* c.736C>T mutation in the African population is limited (Bugoye *et al*., 2023).

ii. ***PMS2* c.137G>T (p.Gly46Val) mutation**

The harmful gene mutation known as *PMS2* c.137G>T is located in coding exon 2 of the *PMS2* gene. It results in the substitution of nucleotide G to T at nucleotide position 137 (Andini *et al.*, 2023; Pandey *et al.*, 2022). As a result, this type of mutation contributes to genetic instability and has been connected with defective mismatch repair activity, which raises the risk of developing cancer susceptibility (Clendenning *et al.*, 2006). Further research across various populations is necessary although this mutation has been linked to an elevated likelihood of prostate cancer and other types of cancers. This is because the currently available data is limited to a few populations (Bugoye *et al.*, 2023; Pandey *et al.*, 2022).

2.7.2 Age at diagnosis

The risk of developing prostate cancer increases with age and is proven to play a factor in treatment options and overall survival (Darré *et al*., 2022; Pernar *et al*., 2018). The average age for prostate cancer diagnosis varies among racial groups, ethnicity, family history of prostate cancer, physical activity, and socioeconomic demographic characteristics (Darré *et al.*, 2022; Hassanipour *et al.*, 2020; RIGOTTI, 2018). Prostate cancer is not commonly diagnosed in males under the age of 40 but it's more prevalent in men above the age of 55 (Darré *et al.*, 2022; Perdana *et al*., 2016). Hassanipour and colleagues reported that males over 65 are more likely than younger individuals to be diagnosed with prostate cancer. Similarly, the average age at diagnosis reported in selected Sub-Saharan African countries is above 60 years of age (Seraphin, Joko-Fru, Manraj, *et al.*, 2021), while in the US, less than 10% of men

are diagnosed with prostate cancer at ages under 55 (Pernar *et al.*, 2018) as compared to the UK where 36% are found with prostate cancer in males over 75, but only 1% of cases were found in males the age 50 at diagnosis (Hassanipour *et al.*, 2020). As Age at diagnosis determines cancer-specific mortalities, there was little information on specific risk ages in the Tanzanian population, and it is still unknown what age Tanzanians are most at risk for developing prostate cancer. There was a need to determine the average risk age at diagnosis of Tanzanian prostate cancer patients seen at Ocean Road Cancer Institute and Muhimbili National Hospital in Dar es Salaam, Tanzania.

2.7.3 Ethnicity and Prostate Cancer

An in-depth knowledge of the gaps between different ethnic groups in prostate cancer incidence, aggressiveness, and mortality is essential for improving prevention, diagnosis, and treatment strategies. Ethnicity plays a major role in the epidemiology, incidence, and outcomes of prostate cancer. In comparison to males from other racial or ethnic origins, African men especially those of sub-Saharan African descent have greater incidence rates of prostate cancer. Prior studies have indicated that worldwide prostate cancer incidence rates are higher among African American men (Kensler & Rebbeck, 2020; Rawla, 2019). According to research findings, data from the International Agency for Research on Cancer and other sources indicates that the incidence of prostate cancer is rising in several African nations, including Tanzania, Lagos, South Africa, and Zimbabwe (Bugoye *et al.*, 2019). Globally, African American males have the highest incidence of prostate cancer and they are more likely to receive an aggressive diagnosis and a younger age of diagnosis (DeSantis *et al.*, 2019). These differences are influenced by a variety of factors, including socioeconomic status, biology, and healthcare access (Chornokur *et al.*, 2011). On the

other hand, men of Hispanic descent have intermediate incidence rates of prostate cancer when compared to both non-Hispanic White men and African American men. The differences in lifestyle variables, genetic predisposition, and healthcare accessibility that affect the prognosis of prostate cancer have contributed to this variation (Siegel *et al.*, 2019). Previous research showed that men of Hispanic descent have a higher risk of prostate cancer than men of other racial or ethnic groups, such as Asian Americans and American Indian men, but a lower total incidence of the disease than non-Hispanic White men as previously reported (Siegel *et al.*, 2019). Prostate cancer incidence rates among Hispanic males, however, differ depending on where they are from. Research indicates that men of Puerto Rican and Jamaican descent experience greater incidence rates than men of Mexican American descent (Siegel *et al.*, 2019). Additionally, compared to other ethnic groups, Asian men often have lower incidence rates of prostate cancer (DeSantis *et al.,* 2019). These discrepancies are caused by variations in food practices, lifestyle variables, and genetic predisposition (Cheng *et al.,* 2009) Previous research on cancer epidemiology has reported males of African descent to have worse survival rates and greater death rates from prostate cancer than males of several other ethnic backgrounds (DeSantis *et al.,* 2019). Research indicates that differences in advanced-stage presentation, late diagnosis, and inadequate access to high-quality healthcare all contribute to differences in survival rates and survival outcomes (Bugoye *et al.*, 2019; Chornokur *et al.,* 2011). Prostate cancer risk factors should, therefore be identified and mitigated in various populations to lower the chance of prostate cancer development and mortality from prostate cancer.

2.7.3.1 Family history of prostate cancer

Prostate cancer is one of the most heritable cancers, and a family history of prostate cancer has long been recognized as a significant risk factor for acquiring the disease. Understanding the known risk of getting prostate cancer has significant therapeutic implications for the identification of higher-risk groups for screening and subsequent therapy of individuals thought to be at elevated risk and lower prostate cancer mortality rates (Madersbacher *et al.*, 2011). Additionally, a man's risk may be further impacted by his degree of relationship with the affected family member (s), their age at prostate cancer diagnosis, and the total number of affected family members (Bratt *et al.*, 2016). Prostate cancer lifetime absolute risk is estimated to range from 12 per cent for men with a first-degree relative affected at or under 60 years to 35-45 per cent for men with three or more affected male relatives, compared with an 8 per cent absolute risk in men without a family history of the disease field (Bratt *et al.*, 2016). There are currently no studies being conducted specifically to identify family history and patients' relatives as risk factors associated with prostate cancer using the Tanzanian population, so there was no information on family history as a risk factor for developing prostate cancer in Tanzania. There was a need to determine the prevalence of family history as a risk factor for prostate cancer in the proposed study.

2.8 Modifiable Prostate Cancer Risk Factors

2.8.1 Marital status

According to epidemiological evidence, there is a link between marital status and health (Robards *et al.*, 2012; Salmon *et al.*, 2021). It has been discovered that being in a committed relationship is universally linked to leading a healthier lifestyle, including consuming less alcohol and tobacco, eating better, engaging in more physical activity, and maintaining healthy body weight, all of which have been linked

to the prevention of prostate cancer (Schoeppe *et al.*, 2018). Additionally, the social support that comes with marriage may serve as a stress reliever and encourage sustaining these healthy lifestyle practices as well as frequent health care activities, including early screening and preventive care visits (Salmon *et al.*, 2021). Although being in a committed relationship is linked to a better prostate cancer prognosis, little is understood about the association between marital status and the occurrence of the disease. Marriage and relationships can give social support, which may encourage healthy living and more frequent use of healthcare services (Salmon *et al.*, 2021). As a result, research is required to establish a link between prostate cancer incidence and married status in the Tanzanian population.

2.8.2 Social determinants

Studies have demonstrated that a person's socioeconomic status, which includes income, education, and employment, influences their risk of developing prostate cancer. Men who have low monthly incomes, low levels of education, and certain types of occupational exposure are more likely to have a range of adverse health outcomes, including prostate cancer, and are more likely to die prematurely (Coughlin, 2020). The relationship between socioeconomic factors and the risk of developing prostate cancer has been studied in several ethnic populations, and preventative efforts are advised to lessen social disparities to lower the risk and mortality rates from prostate cancer (Coughlin, 2020). There is limited information on the impact of societal variables on prostate cancer in the African continent, even though sub-Saharan countries have higher total prostate cancer death rates than other African nations (Hamdi *et al.*, 2021). Also, it was not unclear how socioeconomic variables affect prostate cancer in the Tanzanian population. Thus, the purpose of this

study was to explore how socioeconomic factors related to prostate cancer in Tanzania, aiming to understand their impact on cancer risk and outcomes.

2.8.3 Dietary pattern

Food composition and variety as a whole, as opposed to concentrating just on certain nutrients or food ingredients, are considered in dietary patterns. There is mounting evidence that suggests certain dietary patterns can affect the incidence and spread of prostate cancer as discussed below (Bouvard *et al*., 2015). However, conflicting results were also observed with different food intakes observed in different populations and there was limited information on the association between food intake and prostate cancer association in Tanzanian Population. Therefore, this study aimed to determine the association between different types of food intake and the occurrence of Prostate cancer using the Tanzanian population.

2.8.3.1 Tomato and lycopene consumption

A negative correlation has been reported by epidemiological research between the risk of prostate cancer and increased tomato consumption. Lycopene is primarily found in tomatoes, and it is thought that tomatoes can prevent prostate cancer by acting as a natural antioxidant carotenoid (lycopene) on genes that control androgen stimulation of prostate growth, cytokines, and enzymes that produce reactive oxygen species (Stacewicz-Sapuntzakis & Bowen, 2005). Lycopene modulates anti-inflammatory pathways by downregulating TNF-α, IL-1β, and IL-6, which are pro-inflammatory cytokines. It also lowers the expression of proinflammatory mediators such as inducible nitric oxide synthase (iNOS) and cyclooxygenase 2 (COX-2) and therefore may influence the development of prostate cancer(Kulawik *et al.*, 2024). Other phytochemicals found in tomatoes, whether eaten raw, cooked or in sauce form, may

work in conjunction with lycopene to fight against free radicals and enhance the protective effects that help stop prostate cells from turning cancerous (Petyaev, 2016; Rowles *et al.*, 2018). Cooking increases lycopene's bioavailability (Stahl & Sies, 1992), and eating fat in the same meal encourages the development of carotenoid-containing micelles that enhance lycopene absorption in the intestine (Furr & Clark, 1997). Similarly, the prevalence of aggressive prostate cancer is reduced with increasing blood levels of lycopene (Gann *et al.*, 1999). While eating tomatoes lowers the risk of prostate cancer, other authors have noted that the bioavailability of lycopene varies with age, genetics, lifestyle, and ethnicity (Ganji & Kafai, 2005; Zubair *et al.*, 2015). Additionally, there have been some inconsistent findings regarding the relationship between tomato consumption and a lower risk of prostate cancer (Diallo *et al.*, 2016; Petyaev, 2016). Given that Tanzania and other sub-Saharan African nations have high incidence rates of prostate cancer, research was required to ascertain whether tomato consumption can lower the risk of prostate cancer.

2.8.3.2 Coffee or coffee product consumption

Coffee is a widely consumed drink in the world, and as lifestyles become more Westernized, the amount of coffee consumed daily increases (Safe *et al.*, 2023). The link between coffee consumption and prostate cancer has been studied in several studies, but outcomes are still mixed (Lu *et al.*, 2014; Yu *et al.*, 2011). While coffee consumption is linked to higher serum levels of sex hormones and total testosterone, which influences the risk of prostate cancer (Svartberg *et al.*, 2003), it is also believed that coffee could avert prostate cancer due to its antioxidant, anti-inflammatory, and anti-carcinogenic effects (Murai & Matsuda, 2023). Numerous compounds known to have anti-cancer properties have been found in coffee (Murai &

Matsuda, 2023). The compounds that were evaluated recently included cafestol and kahweol acetate, both of which inhibited the migration and proliferation of prostate cancer cells (Murai & Matsuda, 2023; Svartberg *et al.,* 2003). Together, these substances have the synergistic effect of preventing proliferation and migration through the induction of apoptosis, the inhibition of the epithelial-mesenchymal transition, and a reduction in androgen receptors. This ultimately leads to a decrease in nuclear androgen receptors in cells that are positive for androgen receptors (Murai & Matsuda, 2023; Svartberg *et al.,* 2003). Prostate cancer is associated with dysregulation of chemokines. The development and spread of prostate cancer entail a complex network of chemokine signalling pathways that control immune cell recruitment and trafficking (Hao *et al.,* 2020; R. Huang *et al.,* 2021) Prostate cancer advancement is linked to the up-regulation of chemokine ligand 2 (CCL2) and chemokine receptor 2 (CCR2), chemokine ligand 5 (CCL5) and chemokine ligand 5 receptor (CCR5), and various immune microenvironment (Q. Hao *et al.,* 2020). Coffee compounds, such as cafestol and kahweol acetate, have been shown in studies to negatively regulate CCR2 and CCR5 without increasing their ligands, CCL2 and CCL5 (R. Huang *et al.*, 2021), ultimately limiting the growth of prostate tumours (Iwamoto *et al.,* 2019). Yet the most current cohort studies involving a variety of populations found an inverse correlation between coffee consumption and prostate cancer. However, the African population is underrepresented, and the data cannot be broadly applied to the African population in general due to differences in lifestyle and culture, which warrant additional study (Chen *et al.*, 2021).

2.8.3.3 Red Meat Consumption and Risk of Prostate Cancer

In addition to other known tumour-causing agents like N-nitroso compounds (NOCs), polycyclic aromatic hydrocarbons (PAHs), and heterocyclic amines (HCAs), red and processed meat contains heme iron and has been linked to prostate cancer (Rodriguez

et al., 2006; Sinha *et al*., 2009). These compounds are generated when red meat is cooked for an extended period or at high temperatures (Domingo & Nadal, 2017). Eating red meat has been linked to an elevated risk of prostate cancer in a variety of populations, and the correlation is stronger for individuals with aggressive disease types (Sinha *et al.*, 2009). The International Agency for Research on Cancer (WHO-IARC) defines red meat consumption as likely carcinogenic to humans and cooked meat intake as carcinogenic to humans (Bouvard *et al.*, 2015). Nevertheless, there is a paucity of information regarding the relationship between the intake of red and processed meats and prostate cancer in the African population, and some of the data based on other ethnic groups have contradictory findings (Nouri-Majd *et al.*, 2022). For understanding, and incorporating African perspectives into research is necessary to fully understand the risk of prostate cancer in the African population and to suggest preventive measures based on genetic and cultural differences.

2.8.3.4 Consuming food with saturated fats and Prostate Cancer Risk

The easiest way to define saturated fats is as foods that are mostly composed of lipids and solids at the temperatures at which they are typically stored and consumed (Astrup *et al.*, 2020). Consuming saturated fat has been linked in numerous studies to an increased likelihood of prostate cancer (Di Sebastiano & Mourtzakis, 2014; Pelser *et al.*, 2013). Alteration in the androgen signalling pathway, the insulin-like growth factor (IGF) signalling system and the processes of angiogenesis and cell proliferation pathways are some of the processes through which dietary fat influences the onset and spread of prostate cancer. Research findings reported dietary consumption of saturated fat enhances IGF-1 signalling pathways and thus raises blood levels of IGF-1(Holmes *et al.*, 2002; Kaklamani *et al.*, 1999). Reduced levels of insulin-like growth factor binding protein-3, which are linked to a higher risk of prostate cancer, are the

outcome of rising IGF-1 levels. Additionally, there exists an inverse correlation between the consumption of saturated fat and insulin-like growth factor binding protein-3 (IGFBP-3), which is the predominant binding protein of IGF-1 in the plasma (Holmes *et al.*, 2002; Kaklamani *et al.*, 1999), Furthermore, IGFBP-3 is independently linked to the risk of prostate cancer (Allott *et al.*, 2017). Accordingly, the IGF signalling pathway, which is disturbed in malignant cells, can be stimulated more by rising levels of IGF-1 in the blood and falling levels of IGFBP-3 in the serum. IGF-1 mostly interacts with the IGF-1R or IGF-2R receptors in malignant cells (Singh *et al.,* 2013). The IGF-1R tyrosine kinase receptors contribute to cell proliferation and differentiation in normal circumstances, they are crucial for the initiation and spread of cancer cells in transformed cells (Singh *et al*., 2013). Although several studies have shown a direct link between consumption of saturated fat and prostate cancer, variability occurs across populations, and conflicting findings on this association have been reported, indicating the need for more research (Allott *et al.*, 2017).

2.8.3.5 Leguminous food intake and Risk of Prostate cancer

The Leguminosae family is one of the biggest and most significant families of flowering and podded fruit plants (S. Ahmed & Hasan, 2014). Legumes comprise Alfalfa, carob, clover, copaifera, fenugreek, indigo, lentil, liquorice, lupin, mesquite, natto, soybean, peanut, rosewood, and tamarind (Schneider, 2002). Their anti-cancer properties stem from the presence of bioactive compounds, and they are the richest source of protein, starch, minerals, and vitamins. The legume compounds with anti-carcinogenic properties include lectins, saponins, and isoflavones (S. Ahmed & Hasan, 2014). Legumes have also been shown to decrease cell proliferation, trigger apoptosis, bind to tumour cell membranes, and strengthen the immune response

(Gautam *et al.*, 2020). These nutritional benefits have also been linked to a lower risk of prostate cancer in several ethnic groups (Schneider, 2002; Schuurman *et al.*, 1998), though other studies produced contradicting findings (Li & Mao, 2017). The significance of legumes in reducing the incidence of prostate cancer needs further research, particularly in underrepresented populations, as men's intake of legumes varies with geographic location among ethnic groups (Schneider, 2002).

2.8.3.6 Soya Consumption and Risk of Prostate Cancer

Soy comprises a variety of constituents that may have positive impacts on health. Soybeans contain soy isoflavones and soy protein, which may help lower inflammation and oxidative stress, two factors that are important in the development of prostate cancer (Fan *et al.*, 2022). There is indication that isoflavones can prevent prostate cancer, this protective mechanism is thought to be mediated by hormone-like effects that arise from the binding of competitive estrogen receptors α and β (Kuiper *et al.*, 1997), and blocking tyrosine kinases, modulation of cell proliferation, regulation of the cell cycle, apoptosis, and angiogenesis, as well as tumour cell metastasis (Sivoňová Kmetová *et al.*, 2019). Isoflavones function as weak hormones to prevent the development of prostate cancer by acting as a protective physiological impact (Applegate *et al.*, 2018). According to studies, soy isoflavones genistein, and daidzein build up in prostatic tissue and eventually become cytotoxic to malignant cells. Soy isoflavone genistein has several impacts on cancer cells, including increased histone acetylation and suppression of NF-κB activation and DNA methylation and upregulating tumour suppressor gene function in prostate cancer cells (Applegate *et al.*, 2018; Bektic *et al.*, 2005; Gardner *et al.*, 2009). In addition, it has antiangiogenic, anti-inflammatory, and antioxidant properties that can lower the risk of prostate cancer. It also inhibits cell development and metastasis (Bektic *et al.*,

2005). Several studies have also reported a significant inverse relationship between a decrease in prostate cancer risk and an increase in soy consumption (Fan *et al*., 2022). There has been evidence of a lower incidence of prostate cancer among individuals regularly consuming soy-containing food materials (Applegate *et al.*, 2018). The relationship between soy consumption and the risk of prostate cancer in the African population is still unclear, despite inconsistent findings reported by other investigators elsewhere.

2.8.3.7 Tea consumption and Risk of Prostate cancer

Green and black tea are the two types most commonly consumed. Tea is made from the leaves of the Camellia sinensis plant and is typically drunk as either unfermented green tea or fermented black tea (Jankun *et al.*, 1997). Polyphenols are the main components of the tea (Y. Lin *et al.*, 2014), and the Green tea's catechins, which include epicatechin, epicatechin-3-gallate, epigallocatechin, and epigallocatechin-3-gallate, have potent antioxidant properties and are important in their ability to combat cancer (Sharifi-Zahabi *et al.*, 2021). The regulation of cell signalling pathways for neoplastic transformation, inflammation, apoptosis, and angiogenesis is also considered to be one of tea's possible anticancer capabilities (Greef *et al.*, 2022). Contradictory findings about the role of drinking green tea in reducing the risk of prostate cancer have been reported by epidemiological studies using various populations (Lin *et al.*, 2014; Zheng *et al.*, 2011). However, those findings, which have shown an inverse relationship between green tea and prostate cancer (Miyata *et al.*, 2019), have not included a significant portion of the African population, consequently, they cannot be generalized and call for further studies

2.8.3.8 Fruit and vegetable consumption and Risk of Prostate cancer

Sufficient intake of fruits and vegetables has been linked to protection against several illnesses, including prostate cancer (Hosseini *et al*., 2018). Various immune-protective compounds, including fibre, folate, vitamins, and non-nutrient phytochemicals like carotenoids and flavonoids, such as β-carotene, anthocyanins, flavanols, and flavanones, are found in fruit and vegetables (Hosseini *et al.*, 2018). The immune system requires these compounds to function at their best because they have a significant impact on cellular proliferation and differentiation (Wintergerst *et al*., 2006). Furthermore, there is evidence linking these fruit and vegetable compounds to reduced deoxyribonucleic acid (DNA) oxidation, cell damage, low-grade inflammation, and enhanced detoxification (Key, 2011). Numerous bioactive substances with varying effects on prostate cancer prevention can be found in a variety of fruits and vegetables (Perez-Cornago *et al.*, 2017). Many Fruits are high in vitamins, especially A and C, and phytochemicals such as carotenoids and phenolic compounds, which may explain the correlation between fruit consumption and prostate cancer risk. These vitamins and compounds possess antioxidant properties that are essential for pathways that prevent cancer (Chan & Giovannucci, 2001; Key, 2011). While there is a dearth of research on the relationship between fruit and vegetable consumption and the likelihood of preventing prostate cancer in the African population, the data that is available is inconsistent across different geographical areas (Perez-Cornago *et al.*, 2017). Thus, more research is needed to show the importance of fruits and vegetables.

2.8.3.9 Milk, Dairy product Consumption and Risk of Prostate cancer

Milk and its byproducts are essential components of nutritious diets because the Essential amino acids, vitamins, and calcium found in milk have several health advantages (Sargsyan & Dubasi, 2021). Even though milk and dairy products are healthy, they have been linked to elevated levels of c-peptide and insulin growth-like factors in the blood, which increases the risk of prostate cancer (Giovannucci *et al.,* 2006; McGreevy *et al.,* 2007). As noted earlier, a high calcium diet associated with milk and dairy products may accelerate the development of prostate cancer by lowering blood levels of 1,25-dihydroxyvitamin D, which causes apoptosis and differentiation and has anticancer properties (Chan & Giovannucci, 2001). Furthermore, consuming low-fat milk has been associated with a higher risk of non-aggressive prostate cancer, whereas whole milk is linked to the aggressive type of the disease (Sargsyan & Dubasi, 2021). A higher risk of prostate cancer has also been linked to milk consumption's elevated blood levels of the hormones IGF-1 and estrogen, however, the precise mechanism is still unclear (Qin *et al.,* 2004). These results, however, contradict a lower risk of colorectal cancer associated with increased milk intake (Aune *et al.,* 2012), which calls for more research to be carried out on different populations with various dietary habits and cultural backgrounds.

2.8.3.10 Fish Consumption and Risk of Prostate Cancer

There have been numerous studies conducted on the connection between prostate cancer and the consumption of fish. Prostate cancer rates are lower in populations that eat more fish compared to populations that take less (Szymanski *et al.,* 2010). High levels of long-chain omega-3 polyunsaturated fatty acids discovered in fish have beneficial anti-inflammatory effects that lessen the likelihood of developing prostate

cancer (Qin *et al.,* 2004). Though varied sources of fish and how it is prepared may provide explanations for the inconsistent results, other research findings have shown the opposite effect (Joshi *et al.,* 2012).Omega-6 and omega-3 fatty acids, which belong to the two families of polyunsaturated fats, are transformed by the body into eicosanoids, which are short-lived lipids that resemble hormones prostaglandins and thromboxanes (Hedelin *et al.,* 2007). These thromboxanes and prostaglandins have a variety of biological functions, such as controlling immunological and inflammatory responses, promoting cell differentiation, and promoting cell proliferation (Hedelin *et al.,* 2007). Suppression of synthesis of eicosanoids derived from arachidonic acid, which have proinflammatory effects and may promote carcinogenesis, is one of the believed processes by which omega-3 fatty acids may influence cancer (Hedelin *et al.,* 2007; Larsson *et al.,* 2004). However, eicosanoids generated from eicosapentaenoic acid have anti-inflammatory properties and may prevent the development of prostate cancer (Hedelin *et al.,* 2007; Larsson *et al.,* 2004). Eating foods high in the ratio of omega-3 to omega-6 fatty acids, such as fish, causes a shift in the synthesis of eicosapentaenoic acid-derived eicosanoids instead of arachidonic acid-derived eicosanoids, which may reduce the risk of prostate cancer (Hedelin *et al.,* 2007; Larsson *et al*., 2004). Even though fish is a common food in Africa, there is not much evidence to support its ability to lower the risk of prostate cancer, thus more research is required.

2.8.3.11Vitamins or Mineral Elements Consumption and Risk of Prostate Cancer

A link between vitamin and mineral supplements and the risk of prostate cancer as well as mortality, has already been documented (Zhang *et al.,* 2009). For example, vitamin E is a class of fat-soluble antioxidant micronutrients that are essential as chemo-preventive agents because of its antioxidative properties that scavenge radicals

(Loh *et al.*, 2022). Unluckily, there is insufficient data on its ability to prevent prostate cancer by chemoprevention or to lower the risk of prostate cancer (Loh *et al*., 2022; Lonn *et al*., 2005).

Folate is important for the methylation and nucleotide synthesis of DNA. It also helps metabolize methionine into S-adenosylmethionine and is referred to as the body's main methyl donor. Insufficient amounts of folate in the human body, however, may promote DNA hypomethylation, trigger proto-oncogenes, and lower S-adenosylmethionine levels. In addition, a deficiency in folate intake can cause uracil misincorporation during DNA synthesis, resulting in chromosome breakage in humans and potentially raising the risk of prostate cancer (Duthie, 1999; Pelucchi *et al.*, 2005). Folate consumption through diet has therefore been associated with a lower incidence of prostate cancer (Pelucchi *et al.*, 2005).

Zinc contributes to the preservation of genome integrity because it is an antioxidant and regulates cell signalling pathways. However, when consumed over an extended period through multivitamins or individual supplements (Loh *et al.*, 2022), zinc negatively impacts prostate cancer carcinogenesis. Although available data suggests zinc can inhibit prostate cancer cells' ability to proliferate and spread, conflicting findings regarding zinc consumption have been reported (To *et al*., 2020).

Within peroxidase enzymes, Selenium is a key component of the three proteins; Glutathione peroxidase, albumin proteins, and three selenoproteins P. This antioxidant enzyme system has been reported to increase apoptosis and inhibit the production of many prostate cancer-related oncogenes (Brigelius-Flohé *et al.*, 2002; Cui *et al.*, 2017). Furthermore, selenium has been linked to similarly scavenging reactive nitrogen oxide species to Vitamin E, inhibiting cell peroxidation and

reducing inflammation (Brigelius-Flohé *et al.*, 2002; Cui *et al.*, 2017). Given the inconsistent findings to date about the effects of vitamin or mineral supplements on prostate cancer, more study is advised. (Loh *et al.*, 2022; Pelucchi *et al.*, 2005).

2.8.3.12 Grains Consumption and Risk of Prostate Cancer

The whole grains are made up of caryopsis that is either whole, crushed, cracked, or flaked, and its main elements are present in the same relative amounts as they are in the intact caryopsis (Okarter & Liu, 2010). However, during processing, some or all of the bran layers are removed from the refined grains, which lowers the amount of several physiologically active chemicals like fibre, antioxidants, minerals, micronutrients, and phytoestrogens that have been shown to have positive impacts on carcinogenesis. Phytoestrogens are especially crucial in the prevention of malignancies that are dependent on hormones, such as prostate cancer (Hsing *et al.*, 2003; Okarter & Liu, 2010). Eating whole grains has been associated with a lower incidence of aggressive prostate cancer because they are high in fibre, which increases the globulin that binds to sex hormones and improves insulin sensitivity (Hsing *et al.*, 2003; Tabung *et al.*, 2012). Very little research has been done on the correlation between dietary fibre intake, grain consumption, and prostate cancer aggressiveness by race, similarly, dietary fibre and the risk of prostate cancer in African people have not been the subject of many studies (Tabung *et al.*, 2012).

2.8.4 Lifestyle Behaviors

The research findings in prostate cancer have reported numerous lifestyle variables that have been found to affect the development and occurrence of prostate cancer in various populations (Leitão *et al.*, 2022). According to several studies, engaging in physically demanding activities lowers the chance of acquiring prostate cancer, but riding a bicycle, or motorbike riding, sexual activities smoking, and drinking alcohol

have been connected to elevated risk of prostate cancer (Al Qadire *et al*., 2019; E.C *et al.*, 2017; Koupparis *et al*., 2020). Prostate cancer incidence is also negatively correlated with a range of lifestyle behaviours (Van Hoang *et al.*, 2018). Although these modifiable risk factors affect the development and incidence of prostate cancer, their contributions to occurrence have inconsistent results, thus further research employing the Tanzanian population is still required.

i. Alcohol Use and Risk of Prostate Cancer

Ethanol, acetaldehyde, aflatoxins, and ethyl carbamate are among the compounds found in alcoholic beverages that are likely to raise the risk of prostate cancer (Lachenmeier *et al.*, 2012). Although the exact biological process linking alcohol intake to an increased risk of prostate cancer remains indistinct, acetaldehyde's genotoxic effect, the induction of microsomal cytochrome P450 2E1, and increased oxidative stress have all been linked to the development of prostate cancer (Khandrika *et al*., 2009). The key enzyme that converts ethanol to acetaldehyde is cytosolic alcohol dehydrogenase. Then, the enzyme aldehyde dehydrogenase in mitochondria converts acetaldehyde to acetate. By interacting with proteins and DNA to produce adducts that cause mutations, acetaldehyde triggers the carcinogenic pathway (Díaz Gómez *et al.*, 1999). Acetaldehyde can impede the activity of O6-methylguanine transferase, an enzyme involved in the repair of DNA alkylation caused by carcinogens (Agarwal, 2021; Macke & Petrosyan, 2022). Additionally, exposure to acetaldehyde has been linked to the generation of reactive nitrogen and oxygen species, altered immunological responses, disruption of folate metabolism, and an increased risk of prostate cancer (Agarwal, 2021; Macke & Petrosyan, 2022). Furthermore, taking in too much alcohol may trigger the second metabolic pathway of ethanol, which involves the cytochrome P450 2E1 (CYP2E1)

enzyme oxidizing ethanol in microsomes. This produces reactive oxygen species, which in turn promotes oxidative stress and ultimately DNA damage, both of which are crucial for the development and spread of prostate cancer (Khandrika *et al*., 2009). Alcohol consumption is linked to an increased risk of prostate cancer in a variety of populations**.** Even though alcohol is a major risk factor for many different types of cancer (Hong *et al.,* 2020)**, t**he question of whether alcohol consumption increases the risk of prostate cancer is still being debated in the field of cancer research (Macke & Petrosyan, 2022)**.** More research is necessary because some authors have reported contradictory findings despite studies from many regions utilizing diverse ethnic populations showing a positive correlation between the development of prostate cancer and heavy and moderate drinking (Dickerman *et al.,* 2016; Hong *et al*., 2020)**.**

ii. Physical Activities and Risk of Prostate Cancer

Previous research has indicated that men who engage in high-intensity physical exercise have a decreased risk of prostate cancer (Lee *et al.,* 1992). Workout affects many different biological systems, such as the insulin, hormone, and anti-inflammatory pathways, and it also lowers the levels of insulin growth factor and testosterone which are linked to the development of advanced prostate cancer (Lee *et al.,* 1992). Vigorous exercise also strengthens the immune system, energy balance, and antioxidant defence mechanisms which are vital for reducing prostate cancer development (Johnsen *et al*., 2009). In addition, regular physical activity has been linked to significant anti-inflammatory effects (B. Luo *et al.,* 2024). The mechanism by which exercise improves inflammation is by promoting the release of hormones like cortisol and adrenaline as well as myokines that help immune cells produce fewer pro-inflammatory cytokines (B. Luo *et al.,* 2024). In line with this, Exercise also mobilises immune cells that are involved in modulating immune responses, such as

regulatory T cells and natural killer cells, which release anti-inflammatory cytokines like IL-10. (Gleeson *et al.*, 2011; B. Luo *et al.*, 2024). However, there is inconsistency in the data regarding the relationship between physical activity or exercise and prostate cancer, leading the expert panel to conclude that more research is necessary as there is not enough evidence to make a formal conclusion (Johnsen *et al.*, 2009)

iii. Cigarette Smoking and Risk of Prostate Cancer

Though tobacco use and cigarette smoking are known risk factors for several cancers, there is little consensus about their relationship to the incidence of prostate cancer (De Nunzio *et al.*, 2015; Karam-Hage *et al.*, 2014). Smoking cigarettes releases about 7000 compounds, comprising at least 70 carcinogens including cadmium and polycyclic aromatic hydrocarbons (Yang *et al.*, 2023). There is evidence that polymorphisms or alterations to genes related to metabolism, and detoxification of aromatic polycyclic hydrocarbons elevate the likelihood of developing prostate cancer (Nock *et al.*, 2007). Glutathione-S-transferases (GSTs) are enzymes that can detoxify various mutagens, such as polycyclic aromatic hydrocarbons. However, its subtypes, GSTP and GSTM, have been linked to a higher risk of prostate cancer in smokers and have a lessened ability to detoxify mutagens (Behrens *et al.*, 2019; Nock *et al.*, 2007). Additionally, smoking can raise testosterone levels by stimulating Leydig cells to secrete more testosterone or by inhibiting the aromatase enzyme (Shiels *et al.*, 2009). Since cadmium has androgen-like activity, it binds with the androgen receptor to promote prostate cancer. When combined with androgen, cadmium also increases androgen-mediated transcriptional function (Ye *et al.*, 2000). In addition, cigarette smokers have a higher risk of prostate cancer than nonsmokers due to changes in the P53 and CYP1A1 genes respectively. Additional research is

necessary because the effects of smoking on the risk of prostate cancer in African populations have not been thoroughly studied. However, smoking and sensitive genotypes may have a synergistic effect on prostate cancer risk (Quiñones *et al.*, 2006).

iv. Sexual Activity, Erectile Dysfunction, and Prostate Cancer Risk

Studies have linked low ejaculation frequency, retained carcinogens within the prostatic cells, and an increased risk of prostate cancer and STDs. These findings suggest that sexual activity can impact prostate cancer pathogenesis in multiple ways (Jian *et al.,* 2018; Lozano-Lorca *et al.,* 2023). The possibility of getting prostate cancer is reduced in men who engage in sexual activity due to the release of intraluminal prostate and retained toxins, however, the precise mechanism behind this is unclear (Kotb *et al.,* 2015). Additionally. Prostate cancer and Erectile dysfunction have common associated risk factors, including age, smoking, and obesity. Though several research findings have reported the link between erectile dysfunction and Prostate cancer (Lin *et al.,* 2017), there is limited information describing this relationship in African populations. Therefore, it is necessary to conduct additional studies because there have been inconsistent results reported by multiple authors and few findings employing the African population.

v. Bicycle cycling, Motorcycle riding, and Prostate Cancer Risk

The epithelial cells of the healthy prostate gland secrete a glycoprotein known as Prostate Specific Antigen (PSA). While several therapeutic procedures have been found to raise blood PSA levels, benign prostatic hyperplasia, prostatitis, and prostate malignant cells have all been linked to elevated blood PSA levels (Mejak *et al.,* 2013). Despite the procedures varying throughout countries, the serum PSA screening test is frequently used to screen people for prostate cancer (Mejak *et al.,* 2013).

Motorbikes are used as a cost-effective means of transport, and cycling is seen as a physical activity that can be utilised as one of the preventative measures for many illnesses and is linked to a lower risk of mortality from various causes (Logan *et al.*, 2023). There is limited information regarding the correlation between long-distance cycling, motorbike riding, and prostate cancer. Yet, it has been reported that men who ride long distances experience a 9.5% increase in PSA (E.C *et al.,* 2017; Heger *et al.*, 2016; Mejak *et al.,* 2013).

2.8.5 Environmental Risk Factors

i. Agriculture chemicals or pesticides and the Risk of prostate cancer

It has been shown in recent research that exposure to various pesticides may account for some of the greatest rates of prostate cancer among patients with prostate cancer. Pests such as moulds, insects, and undesired plants can be controlled with the use of pesticides (Okarter & Liu, 2010). In addition to being classed according to their chemical composition, pesticides can also be classified according to the target of their action, for example, herbicides, fungicides, and insecticides. Among the most often used pesticides include carbamates, triazines, organochlorine, and organophosphorus compounds. Several explanations attempt to shed light on how pesticides cause cancer. However, pesticides can induce oxidative stress in cells, leading to the production of reactive oxidative radicals that can damage cells (Krstev & Knutsson, 2019; NELSON *et al*., 2001). Prostate cancer has been connected with exposure to pesticide substances classified as organochlorine and organophosphate. These organochlorides include dichlorodiphenyltrichloroethane (DDT), hexachlorocyclohexanes, and fonofos and terbufos; these organophosphate substances include malathion and fonofos (Pardo *et al., 2020)*. Furthermore, while the majority of pesticide compounds do not cause mutagenicity, they might contain endocrine

disruptors, which work by either stimulating or inhibiting hormone receptors to raise testosterone levels and cause prostate cancer. Several research studies have connected the use of pesticides in farming to a higher risk of prostate cancer; nevertheless, mixed results have been found (Krstev & Knutsson, 2019; NELSON *et al.*, 2001; Pardo *et al.*, 2020). Since the studies on a variety of pesticides, including exposure to various agriculture organic chemicals, have been conducted in-depth; however, the conclusion has not been definitively established, therefore additional study including varied populations is necessary.

ii. Exposure to Organic chemical and Risk of Prostate Cancer

Research has demonstrated the connection between men's increased risk of prostate cancer and exposure to several organic compounds. Polycyclic aromatic hydrocarbons, polychlorinated biphenyls, and persistent organic pollutants are examples of organic compounds (Lim *et al*., 2017). However, polycyclic aromatic hydrocarbons are most well known for their propensity to form adducts with DNA. The formation of adducts with polycyclic aromatic hydrocarbons promotes damage to DNA in prostate cells and raises the risk of prostate cancer (Rybicki, Nock, *et al*., 2006).

iii. Polycyclic aromatic hydrocarbons and Risk of Prostate cancer

Polycyclic aromatic hydrocarbons can form DNA adducts through their ultimate metabolite’s interactions with DNA, and their antiestrogenic properties play a role in the initiation or promotion of prostate cancer (Nadon *et al.,* 1995; Rybicki, Neslund-Dudas, *et al.,* 2006). Polycyclic aromatic hydrocarbons are the environmental carcinogenesis that results from incomplete combustion of carbon-containing fuels like crude oil, tar, tobacco, wood, diesel, or charbroiled meat and they are involved in prostate carcinogenesis (Rybicki, Nock, *et al.,* 2006; Tang *et al.,* 2013). Humans are

exposed to cancer-causing Polycyclic Aromatic Hydrocarbons (PAHs) through their diet, which includes foods like barbecued meats, green vegetables, and grains (Shimada *et al.*, 1998), Other sources of exposure include smoke from cigarettes and workplace exposure, such as working in environments like gasoline stations, wood dust, and diesel emission machines (Rybicki, Nock, *et al.*, 2006; Scherer *et al.*, 2000). Previous research indicates that grilled meats contain benzo[a]pyrene, a polycyclic aromatic hydrocarbon that raises the likelihood of advanced prostate carcinoma (Sinha *et al*., 2009; Tang *et al*., 2013). Though some studies have documented the impact of polycyclic aromatic hydrocarbons on tumour growth and racial variations in the genetic variation associated with PAH-metabolizing enzymes (Tang *et al.*, 2013), there is a paucity of evidence linking occupational exposure to PAHs and the risk of prostate cancer, with inconsistent results (Nadon *et al.*, 1995; Rybicki, Neslund-Dudas, *et al.*, 2006)

iv. Exposure to Organic Solvents benzene, Toluene, Xylene, and Styrene and Risk of Prostate Cancer

The compounds benzene, toluene, xylene, and styrene are among the monocyclic aromatic hydrocarbons that are categorised as potential human carcinogens. In certain populations, these aromatic volatile organic chemicals have been linked to a higher risk of developing prostate cancer (Aino Siltari Teemu J. Murtola & Auvinen, 2018; P. Hao *et al.*, 2022). These compounds are commonly found in traffic, paint, rubber, adhesive solvents, and tobacco smoke (Mögel *et al.*, 2011). Also, petroleum is the primary source of benzene, toluene, and xylene utilised in industrial production. These compounds are often used as solvents and intermediates in paints, inks, dyes, thinners, adhesives, and coatings (Blanc-Lapierre *et al.*, 2018). Thus, those who engage in the industrial manufacture of polymers, rubber products, shoes, and

synthetic fibres, as well as in printing, painting, and leather tanning, as well as drivers and petrol station attendants, may also be exposed to these compounds (Blanc-Lapierre *et al.*, 2018; Weisel, 2010). There are a few potential mechanisms that could link prolonged exposure to organic volatile compounds and the development of prostate cancer. These possible mechanisms include endocrine disruption and direct action through the oxidation process of metabolites of catechols of estrogens or benzene to quinones, which are capable of producing DNA adducts and, through defects in DNA repair, initiate alterations and subsequently prostate cancers (Blanc-Lapierre *et al.*, 2018). Furthermore, the generation of reactive oxygen species and oxidative stress-induced DNA damage are two additional ways that various metabolites of benzene and toluene cause toxicity, which raises the risk of prostate cancer (Blanc-Lapierre *et al.*, 2018; Moro *et al.*, 2012; Uzma *et al.*, 2010). There is little information available regarding potential pathways linking exposure to styrene and prostate cancer, and benzene itself is thought to possess no mutagenic or cytotoxic activity Uzma *et al.*, 2010). Though studies have found a favourable correlation between exposure to certain volatile organic chemicals and prostate cancer (Aino Siltari Teemu J. Murtola & Auvinen, 2018; P. Hao *et al*., 2022), some findings remain unclear. Further research is necessary because there is limited information on the relationship between African residents' risk of prostate cancer and exposure to benzene, toluene, xylene, and styrene in the environment.

v. Working in X-rays or harmful radiation condition

A lower incidence of prostate cancer has been found among men when exposed to ultraviolet light (Hanchette & Schwartz, 1992; Studzinski & Moore, 1995), and there is an inverse relationship between ultraviolet light exposure and prostate cancer. The protective effects of ultraviolet light exposure against prostate cancer are associated

with an increase in vitamin D synthesis in the skin upon exposure to Ultraviolet light. High vitamin D levels prevent distinct tumour cells from proliferating and dedifferentiating (Bodiwala *et al.,* 2003; Luscombe *et al.,* 2001). However, it has been shown that exposure to ionising radiation increases the chance of developing several malignancies (Myles *et al*., 2008; Preston *et al.*, 2003). Exposure to hip X-rays and barium enemas administered to a general male population at least five years before diagnosis has been strongly linked to an increased risk of prostate cancer (Myles *et al*., 2008). In addition, elevated risk of prostate cancer is linked to hip X-ray exposure in men with a family history of cancer as well as exposure to hip X-rays between 10 and 20 years before diagnosis (Myles *et al.*, 2008). Yet, family history and genetic variables associated with prostate cancer were reported to modify the effects of X-rays on the risk of acquiring prostate cancer (Grönberg, 2003). It is difficult to generalize the results due to the lack of information on lower X-ray ionizing doses (Brenner *et al.,* 2003; Myles *et al.*, 2008), and the inadequate representation of African populations in the available data.

2.8.6 Medical Condition and Risk of Prostate Cancer

Frequent ejaculation is thought to reduce the concentration of carcinogenic substances within the prostatic fluid (Hyun Jae Saog, 2012) or reduce the production of intraluminal prostatic crystalloids (Although prostate cancer by itself decreases the sexual demand and the frequency of sexual intercourse (Hyun Jae Saog, 2012), the frequency of sexual intercourse is reduced with increasing age, therefore posing the risk of developing prostate cancer, however, these findings are contradictory with other researchers who theorize the increase in sexual desire is linked with increasing sex hormones such as androgens that are associated with prostate cancer risk elevation (Richards, 2009). According to previous findings, Men with High to moderate

ejaculation frequency per week and those with fewer female sex partners are linked to reduced risk of prostate cancer (Jian *et al.*, 2018)

i. Sexual transmitted Diseases and risk of Prostate cancer

Although the precise causes of prostate cancer are still unknown, the information that is currently available suggests the possibility that infectious organisms may play a significant role in disease risk (Joung *et al.*, 2015). Prostate cancer is likely to occur as a result of sexually transmitted infections that produce persistent, chronic inflammation (Lawson & Glenn, 2022). Prostate cancer risk is further increased by high-frequency sexual activity with several partners, which is linked to an increased risk of syphilis, gonorrhoea, and human papillomavirus infections (Rosenblatt et al., 2001). Exposure to microbiological infectious agents causes chronic inflammation in the prostate and can trigger the release of proinflammatory cytokines (De Marzo *et al*., 2007; Joung *et al., 2015; Lawson & Glenn, 2022),* however, the development of prostate cancer linked to inflammation is not well-known, the process encompassing both innate and adaptive immune responses (De Marzo *et al.*, 2007; Sawaya *et al.,* 2024). In the course of the process, the innate immune system's triggered phagocytic inflammatory cells release reactive chemicals like superoxide, hydrogen peroxide, singlet oxygen, and nitric oxide, which can damage epithelial cells' DNA due to oxidative or nitrosative stress or react with other cellular components like phospholipids to start a free-radical chain reaction that damages DNA. This raises the risk of developing prostate cancer (Ames *et al.,* 1995; De Marzo *et al.,* 2007). Additionally, noteworthy correlations between sexually transmitted infections such as gonorrhoea, syphilis, and the human papillomavirus and an increased likelihood of acquiring prostate cancer have been reported worldwide (Joung *et al.,* 2015; Lawson & Glenn, 2022). Therefore, reducing sexually transmitted diseases can lower the

number of cases of prostate cancer and the deaths associated with it (Caini *et al.*, 2014). However, different study populations show differing associations between sexually transmitted Diseases and prostate cancer, and the majority of data presented has been based on data from the Western population, more research involving African individuals residing in Africa is necessary (Chung *et al*., 2013; Joung *et al.*, 2015; Lawson & Glenn, 2022; Sawaya *et al*., 2024),

ii. Diabetic Condition and Risk of Prostate Cancer

Prostate cancer risk is negatively correlated with type 2 diabetes mellitus, a condition that affects metabolism. Insulin resistance and hyperinsulinemia are the earliest symptoms of the disease (Grossmann & Wittert, 2012; Rodriguez *et al.*, 2005). The pancreas eventually loses its ability to secrete insulin, but an increase in insulin can increase the release of insulin-like growth factor 1 (IGF1), which in turn encourages the proliferation of primary prostate cells (Grossmann & Wittert, 2012; Rodriguez *et al*., 2005). Reduced blood levels of circulating insulin are caused by the pancreas' inability to produce enough insulin as a result of damage to the pancreatic B cells (Barnard *et al.*, 2002; Rodriguez *et al.*, 2005). According to previous research, Proinflammatory cytokines TNF-α and IL-6 have been reported to be involved in chronic inflammation in poorly controlled diabetes (Joung *et al*., 2015; Olatunde *et al.*, 2021). The persistent inflammation is linked to genetic stability and thus increases the likelihood of prostate cancer (Xu *et al.*, 2014). The likelihood of developing prostate cancer has been associated with elevated levels of circulating insulin and insulin resistance in males recently diagnosed with diabetes (Barnard *et al.*, 2002; Rodriguez *et al*., 2005). As insulin levels drop, the chance of developing prostate cancer is lowered (Giovannucci, 2001; Rodriguez *et al.*, 2005). While research has shown that diabetic patients have a lower risk of prostate cancer across various

populations (Bansal *et al.,* 2013), little is known about the relationship between diabetes and prostate cancer risk in African populations, which calls for more investigation (Brenner *et al.,* 2003).

2.8.7 Medication and Risk of Prostate Cancer

i. Antihypertensive Drugs and Risk of Prostate Cancer

Antihypertensive medications are frequently prescribed to help individuals with hypertension control their blood pressure. Commonly utilized drug types include diuretics, alpha- and beta-blockers, calcium channel blockers, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, and others (Cao *et al*., 2018; Thomopoulos *et al.,* 2015). These prescription drugs also disrupt regular biological processes, including fluid homeostasis, the sympathetic nervous system, and the renin-angiotensin system, all of which may have an impact on the development of prostate cancer (Siltari *et al.,* 2020). Constant use of these hypertension medications has, however, sparked worries about men's increased risk of prostate cancer. Antihypertensive medication use has been linked by several authors to an increased risk of prostate cancer (Aino Siltari Teemu J. Murtola & Auvinen, 2018; Jick *et al*., 1997), but other researchers have generated conflicting findings, necessitating further study (Cao *et al.*, 2018; Lever *et al.*, 1998).

ii. Anti-diabetic Drugs and Risk of Prostate Cancer

The regularly prescribed oral antidiabetic medication biguanide metformin is used to re-sensitize diabetic patients with moderately high HbA1c levels to insulin (Cannata *et al., 2010; Pircher et al., 2018).* Metformin not only re-sensitizes the body to insulin, but it also has anti-tumour effects that prevent growth by activating the AMPK pathway, inhibiting the mTOR pathway downstream, blocking the progression of the cell cycle, and reducing inflammation (Pircher *et al.,* 2018). Metformin also

reduces the development of prostate cancer by suppressing the androgen receptor's function (Wong *et al.*, 2014). Like many other drugs, antidiabetic effects on prostate cancer have been shown to produce inconsistent results, and studies reporting antidiabetic effects on prostate cancer have not included a sufficient number of African Americans, necessitating additional research.

2.9 Histological Prostate Cancer subtypes

Prostatic cancer classification and diagnosis have undergone many modifications. As part of the World Health Organization's fifth edition of the Classification of Tumours of the Urinary and Male Genital Systems (Kench *et al.*, 2022), the growth structures, histological types, and subtypes have also been used to further categorize prostate cancer in the fifth-edition categorization system (Marra *et al.*, 2023). As indicated by Table 1 below, there are two categories of prostate cancer tumours: epithelial tumours and mesenchymal tumours specific to the prostate. Two main families of neoplasms fall under the epithelial tumour category: glandular and squamous.

2.9.1 The glandular neoplasm

Six tumour types belong to the glandular family: prostatic acinar adenocarcinoma, prostatic ductal adenocarcinoma, high-grade prostatic intraepithelial neoplasia, intraductal carcinoma of the prostate, treatment-related neuroendocrine prostatic carcinoma (Surintrspanont & Zhou, 2022).

2.9.2 High-grade prostatic intraepithelial neoplasia

The high-grade prostatic intraepithelial neoplasia is the typical prostate cancer precursor lesion. High-grade PIN consists of abnormal cells within the prostate glands that look atypical under the microscope but have not invaded surrounding tissues (Andras et al., 2020; Norman et al., 2023). It shares several physical characteristics with prostatic adenocarcinoma, including hyperchromasia in the secretory epithelial

cells and larger nuclei. Though race and geographic location influence the occurrence in the population under the study, the presence of large nucleoli in the epithelial cells is a crucial diagnostic hallmark of high-grade prostatic intraepithelial neoplasia (Bostwick *et al.,* 2004; Guo & Czerniak, 2012.)

2.9.3 Intraductal carcinoma of the prostate

The World Health Organisation Blue Book recognised intraductal carcinoma of the prostate as a separate tumour category in 2016 (Surintrspanont & Zhou, 2023). Intraductal carcinoma of the prostate is currently defined as neoplastic epithelial proliferation characterised by architectural and cytological atypia beyond what is acceptable for high-grade prostatic intraepithelial neoplasia" (Surintrspanont & Zhou, 2023).

2.9.4 Ductal adenocarcinoma

Ductal adenocarcinoma is a unique subtype of prostatic adenocarcinoma, which has been defined by large glands bordered by tall pseudostratified columnar cells (Melicow & Tannenbaum, 1971). Initially, this subtype was proposed to originate from remnants of the Mullerian duct, but currently, it is thought to originate from prostatic epithelial cells (Surintrspanont & Zhou, 2023). The majority of ductal adenocarcinomas are located in the peripheral zone; a tiny fraction originates from the transition zone around the prostatic urethra, leading to hematuria and urinary obstruction. Although ductal adenocarcinoma has an increased PSA level, it frequently has a lower level than acinar adenocarcinoma (Surintrspanont & Zhou, 2023).

Prostate cystadenoma

Prostate cystadenoma is an extremely uncommon benign tumour originating from the prostate. It usually manifests as a big multilocular cystic tumour and is located between the rectum and the urinary bladder (Wang *et al.,* 2022). In addition to lower

urinary tract symptoms and difficulties in defecation, the patient complains of discomfort associated with prostate cystadenoma, also known as gigantic multilocular prostate cystadenoma (Wang *et al.*, 2022).

2.9.5 Prostatic acinar adenocarcinoma

It is estimated that 95% of all prostate malignancies are prostatic acinar adenocarcinomas, and a wide range of morphologies are seen in prostate acinar adenocarcinomas (Epstein *et al.*, 2021; Morais *et al.*, 2015; Sung *et al.*, 2021a). It has been reported by previous authors that there are different criteria for prostate cancer diagnosis, and the main microscopic criteria for diagnosis of prostate carcinoma include loss of the basal cell layer, nuclear atypia, and an infiltrative growth pattern (Surintrspanont & Zhou, 2023). Moreover, atrophic, foamy gland, microcystic, and pseudo-hyperplastic development patterns are all observed in prostatic acinar adenocarcinoma. These development patterns complicate the diagnosis since they resemble benign illnesses (Guo & Czerniak, 2012). Also, very aggressive clinical behaviour is linked to histologic subtypes, including pleomorphic, sarcomatoid, and signet-ring-cell-like (Surintrspanont & Zhou, 2022, 2023).

2.9.6 Treatment-related neuroadenocarcinoma of the prostate

Due to its unique clinical and biological characteristics, the treatment-related neuroendocrine prostatic carcinoma has been discussed in the fifth edition of the WHO classification of prostate cancer (Board, 2022; Surintrspanont & Zhou, 2023). Following androgen restriction therapy, treatment-related neuroendocrine adenocarcinoma of the prostate shows either full or partial neuroendocrine differentiation (Surintrspanont & Zhou, 2023). This type of cancer is present in 10–15% of castration-resistant prostate cancers and is the result of castration-resistant prostate cancer transdifferentiating through lineage plasticity (Sommariva *et al.*, 2016;

Surintrspanont & Zhou, 2023). Furthermore, it is believed that genetic alterations involving the PTEN and TP53 genes have a role in the trans differentiation of castration-resistant prostate cancer to neuroendocrine adenocarcinoma of the prostate associated with treatment (Sommariva *et al.*, 2016; Surintrspanont & Zhou, 2023).

2.9.7 Squamous neoplasms

Three tumour types belong to the squamous family: prostate adenoid cystic carcinoma, squamous cell carcinoma, and adeno squamous carcinoma. Furthermore, only the stromal tumour family, which comprises prostatic stromal tumours of unknown malignant potential and prostatic stromal sarcoma, is included in the mesenchymal tumour category (Surintrspanont & Zhou, 2023).

2..9.8 Stromal tumour of the prostate

The stromal tumour family includes two prostatic stromal tumours of unknown malignant potential and prostatic stromal sarcoma. All of these are included in the mesenchymal tumour category (Surintrspanont & Zhou, 2023).

Table 2.1: Taxonomic Classification of Tumours of the Prostate

Category	Family	Type
Epithelial tumours of the prostate	Glandular neoplasms of the prostate	Prostatic cystadenoma
		High-grade prostatic intraepithelial neoplasia
		Intraductal carcinoma of the prostate
		Prostatic acinar adenocarcinoma*
		Prostatic ductal adenocarcinoma
		Treatment-related neuroendocrine prostatic carcinoma
	Squamous neoplasms of the prostate	Adenosquamous carcinoma of the prostate
		Squamous cell carcinoma of the prostate
		Adenoid cystic (basal cell) carcinoma of the prostate
Mesenchymal tumours are unique to the prostate	Stromal tumours of the prostate	Prostatic stromal tumour of uncertain malignant potential
		Prostatic stromal sarcoma

2.10 Grading of Prostate Cancer

The shape and clinical characteristics of prostate adenocarcinomas vary widely, ranging from low-volume, physiologically inert tumours to high-grade, aggressive malignancies. A suitable grading system is therefore required to characterise the differences in appearance between cancerous and healthy cells, as this presents both opportunities and challenges (Baraban & Epstein, 2022). Therefore, an accurate prostate cancer grading system is required. The grading system quantifies and helps patients with tumors unlikely to cause morbidity or death from receiving overtreatment, and it also makes clinical intervention for high-risk tumours easier (Baraban & Epstein, 2022).

2.10.1 Gleason Pattern in Prostate Cancer

Men with prostate cancer can benefit from the Gleason grading system as a useful prognostic and therapy tool (Truong *et al.,* 2018). Despite its shortcomings, the Gleason grading system remains the gold standard for determining the prognosis of prostate cancer through microscopic tissue morphology assessment. The most physiologically indolent types of illness correspond to the lowest grade patterns of prostate cancer, which are identified in normal practice, while a greater Gleason pattern denotes a more advanced stage of prostate cancer (Truong *et al.,* 2018). Prostate cancer cells can be classified into five Gleason patterns according to their differentiation and appearance, and cancer cells resemble normal cells more when the pattern number is lower (Baraban & Epstein, 2022). There is a correlation between the pattern number and the prostate cancer grade. Gleason pattern 3 is the lowest pattern of low-grade adenocarcinoma since Gleason patterns 1 and 2 appear to be healthy cells and are less useful, and they are no longer diagnosed (Fletcher *et al.*, 2020; Shah & Zhou, 2016). In prostate needle biopsies, Gleason pattern 3 is the most frequently observed. Its formal definition is cancer glands that are distinct and well-

formed. The typical example of Gleason pattern 3 is represented by individually sized glands invading between benign glands (Fletcher *et al.,* 2020). Furthermore, compared to normal cells, Gleason pattern 5 is highly aberrant, whereas Gleason patterns 3 and 4 are in the middle (Fletcher *et al.,* 2020).

2.10.2 Gleason score calculation and grade groups in prostate cancer

The Gleason score is determined by classifying the growth patterns of adenocarcinomas (van Leenders *et al.,* 2020). When determining the Gleason score, the total Gleason score, which varies from 2 to 10, is obtained through the addition of the two patterns of prostate cancer cells that were detected during the microscopic examination of tissues (Fletcher *et al.,* 2020; Short *et al.,* 2019). The grade of the most prevalent pattern of cancer cells is also added to the pattern with the highest grade if a third pattern is found in the tissue samples, however, to facilitate comprehension and interpretation, the grade grouping system was put in place (Fletcher *et al.,* 2020; Short *et al.,* 2019). The Gleason Group is the most widely used approach for prognosticating prostate cancer and is the most helpful predictor of prostatic adenocarcinoma prognosis (Pudasaini & Subedi, 2019). Though the Gleason score is also frequently used in Tanzania, but still faces several challenges, such as interobserver variability in pathologist Gleason grading and biopsies taken during sampling that may put therapeutic decision-making at risk (Hoogland *et al.,* 2014). Therefore, the combination of Gleason grading and other factors such as tumour molecular characterisation may provide additional information that would help in therapeutic decision-making. As indicated in the table below, the grading group system uses a five-point scale, where 1 denotes well-differentiated tissue morphology and 5 denotes poorly-differentiated tissue morphology (Pudasaini & Subedi, 2019).

Table 1.2. Details on the Grade group system in prostate cancer

Grade group	Gleason score	Description	References
1	6 or less	Tissues are well differentiated, and the cancer is likely to grow and spread very slowly	(Fletcher *et al.*, 2020)
2	3+4 =7	Tissues are moderately or intermediately differentiated, and the cancer is likely to grow and spread slowly	(Fletcher *et al*., 2020; Wright *et al.*, 2009)
3	4+3 =7	Tissues are moderately or intermediately differentiated, and the cancer is likely to grow and spread slowly. Unlike Grade Group 2, Grade Group 3 is more likely to grow and spread than Grade Group 2.	(Fletcher *et al*., 2020; Wright *et al*., 2009)
4	8	The cancer is likely to grow and spread quickly (called poorly differentiated or high grade).	(Fletcher *et al.*, 2020; Ginsburg et al., 2020)
5	9 or 10	The cancer is particularly aggressive and likely to grow and spread quickly. Grade Group 5 is likely to grow and spread more quickly than Grade Group 4.	(Egevad *et al.*, 2024; Fletcher et al., 2020)

2.11 Genetic and Molecular Drivers of Prostate Cancer

In prostate cancer, a complex interaction of genetic and molecular alterations underlies the disease's initiation, progression, and resistance to treatment, adding to its inherent heterogeneity (Jamaspishvili *et al.*, 2018b; Naderali *et al.*, 2019). Two of the most well-researched and clinically important molecular processes in prostate cancer are the *TMPRSS2:ERG* gene fusion and *PTEN* loss. A major role in cancer invasion and progression, the *ERG* transcription factor is abnormally overexpressed as a result of *TMPRSS2:ERG* fusion (Jamaspishvili *et al.*, 2018b; Naderali *et al.*, 2019). Similar to this, *PTEN*, a crucial tumour suppressor gene that controls the PI3K/AKT signalling pathway, is often inactivated or deleted, especially in cancers that have spread and progressed. When *PTEN* loss and *TMPRSS2:ERG* fusion occur together, it is linked to poor clinical outcomes, aggressive tumour behaviour, and resistance to traditional therapy. These alterations affect the biology of tumours and offer important information to develop personalised and targeted treatments. For example, the discovery of *TMPRSS2:ERG* fusion opened up opportunities for treatments meant to interfere with *ERG*-driven carcinogenesis, and the knowledge of *PTEN*'s function in

controlling the PI3K/AKT pathway has driven the investigation of inhibitors that target this system (Jamaspishvili *et al.*, 2018b; Naderali *et al.*, 2019). When taken as a whole, these developments have greatly improved the understanding of prostate cancer pathogenesis and opened the door to more successful therapeutic approaches.

2.11.1 Phosphatase and TENsin Homolog (PTEN)

A crucial tumour suppressor gene, *PTEN*, is found on chromosome 10 at the 10q23 locus. With nine exons and eight introns, it is roughly 200 kb long and codes for a multifunctional protein with lipid phosphatase activity that has 403 amino acids (Jamaspishvili *et al.*, 2018b; Naderali *et al.*, 2019). *PTEN* regulates the PI3K/AKT signalling pathway by dephosphorylating phosphoinositide substrates in its dual-specific role as a protein phosphatase. Important biological functions such as apoptosis, cell cycle regulation, cancer suppression, and cell invasion inhibition depend on this regulation (Jamaspishvili *et al.*, 2018b; Naderali *et al.*, 2019). *PTEN* mutations, deletions, and inactivation are commonly seen in prostate cancer, especially in castration-resistant prostate cancers, which have them in almost half of cases (Jamaspishvili *et al.*, 2018b; Naderali *et al.*, 2019). These alterations result in dysregulated PI3K/AKT signalling, which is linked to the development, spread, and poor clinical outcomes of tumours (Jamaspishvili *et al.*, 2018b; Naderali *et al.*, 2019).

2.11.1.1Nuclear Functions of PTEN

In addition to its well-known function in the cytoplasm, *PTEN* plays important roles in the nucleus. *PTEN*, the most frequently mutated tumour suppressor gene in prostate cancer, is essential for preserving genomic integrity (Ho *et al.*, 2020). Although *PTEN* is best known for its capacity to block the oncogenic PI3K pathway utilizing its lipid phosphatase activity, new research indicates that its tumour-suppressive properties go beyond this mechanism (Maehama & Dixon, 1999). Because of the presence of PI3K,

AKT, and pyruvate dehydrogenase kinase 1 (PDK1) in the nucleus, early research indicated that *PTEN* functions as a lipid phosphatase in this compartment (Déléris *et al., 2006)*. However, since *PTEN*'s main substrate, PIP3, is not substantially available in the nucleus, later studies have shown that *PTEN*'s nuclear functions are independent of its lipid phosphatase activity (Lindsay *et al.,* 2006). Nuclear *PTEN* uses phosphatase-independent pathways to support chromatin shape and genomic integrity (Amor *et al.,* 2004). Where it localises, it maintains its tumour-suppressive functions and improves structural stability in heterochromatin. The failure of numerous anti-PI3K medications to completely suppress tumour growth in *PTEN*-deficient malignancies makes these findings especially pertinent, indicating that *PTEN*'s nuclear functions are essential for its overall tumour-suppressive effect (Juric *et al.,* 2015; Le *et al.,* 2016).

2.11.1.2 Cytoplasmic Functions of PTEN

As a dual-specificity phosphatase in the cytoplasm, *PTEN* dephosphorylates phosphoinositol (3,4,5)-trisphosphate (PIP3) to prevent AKT activation and PI3K signalling (Jamaspishvili *et al.,* 2018b). Growth factors usually trigger the PI3K pathway by binding to receptor tyrosine kinases, which changes PIP2 into PIP3 and makes it easier for PDK1 to phosphorylate AKT and to enhance cell growth, proliferation, and survival, activated AKT affects downstream targets, such as mTOR (J. O. Lee *et al.,* 1999). This regulatory system is disrupted by *PTEN* loss, which results in unregulated PI3K/AKT signalling. Resistance to androgen deprivation therapy in Prostate cancer is a result of this imbalance, which encourages androgen-independent activation of the androgen receptor (AR) pathway. The loss of control over vital cellular functions necessary for cancer progression, such as cell survival,

energy metabolism, proliferation, and structural integrity, is another consequence of *PTEN* inactivation (Vidotto *et al.*, 2020).

2.11.1.3 Loss of PTEN and Immunosuppression in Prostate Cancer

The primary cause of *PTEN* loss in prostate cancer is copy number variations, which sets prostate cancer apart from other cancers (Poluri & Audet-Walsh, 2018). *PTEN* deficiency causes metabolic reprogramming, which encourages rapid cell proliferation and aggressive tumour growth (Jamaspishvili *et al.*, 2018b; Zhou *et al.*, 2019). *PTEN* loss also triggers the PI3K-AKT signalling pathway, which is closely linked to unfavourable oncological outcomes In line with this, when chromatin architecture is disturbed, heterochromatic foci are lost, chromatin compaction is decreased, heterochromatic genes are amplified, heterochromatin protein 1 becomes unstable, and eventually, genomic instability results (Gong *et al.*, 2015).

Particularly in patients with clinically localised prostate cancer, *PTEN* has become a promising genetic marker for differentiating between aggressive and indolent disease (Jamaspishvili *et al.*, 2018b; Steelman *et al.*, 2004). In addition to its function in tumour suppression, *PTEN* affects the growth of tumours by modifying the immune system and the tumour microenvironment (TME) (Jamaspishvili *et al.*, 2018a). *PTEN* deletions in prostate cancer have been linked in studies to advanced tumour stages and higher Gleason scores. However, discrepancies in results caused by participant selection, methodological variations, and population heterogeneity underscore the necessity of more study (Geybels *et al.*, 2017; Jamaspishvili *et al.*, 2018b).*PTEN* depletion has also been found to vary by ethnicity. For example, compared to European American men, African American men have lower rates of *PTEN* loss (Huang *et al.*, 2017; Tosoian et *al.*, 2017). Despite these realisations, it is still unclear how ethnic background, *PTEN* loss, and a bad prognosis are related, and further

research is necessary (Tosoian *et al.*, 2017). The development of an immunosuppressive TME, which is defined by increased cytokine and chemokine signalling, is intimately associated with *PTEN* loss. Immunosuppressive cells that impair antitumor immune responses, such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), and M2-polarized macrophages, are prevalent in this milieu (F. Cheng & Eng, 2019). Furthermore, *PTEN*-deficient tumours exhibit elevated expression of immune evasion markers, such as indoleamine 2,3-dioxygenase 1 (IDO1) and programmed death-ligand 1 (PD-L1), which hinder immune cell cytotoxicity and promote immunological escape (Vidotto *et al.*, 2020). The loss of *PTEN*'s nuclear activities contributes to an immunosuppressive TME by exacerbating cytokine signalling and inflammation (Cheng & Eng, 2019). This environment inhibits antitumor immunity and is dominated by M2 macrophages, MDSCs, and regulatory immune cells. Additionally, increased genomic instability linked to *PTEN* loss can produce neoantigens that can activate CD8+ T cells and stimulate an immunological response (Vidotto *et al.*, 2020). The immunological landscape may become even more complex in cancers with significant genetic instability, though, as infiltrating tumour-associated macrophages can neutralise the neoantigen-driven immunity (Vidotto *et al.*, 2020).

2.11.1.4 PTEN, Interleukin-6 (IL-6), and PI3K-AKT Pathway Interplay in Prostate Cancer

Prostate cancer pathophysiology is significantly influenced by the interaction of PI3K-AKT pathway activation, *PTEN* loss, and Interleukin-6 (IL-6) cytokine signalling. These molecular interactions promote cell survival, drive tumour progression, and promote treatment resistance, highlighting the importance of integrated approaches to prognostic evaluation and targeted therapy development

(Culig & Puhr, 2012; Smith *et al*., 2013) . The processes and genetic changes that underlie prostate cancer are intimately related to chronic inflammation, a major factor in the disease's pathogenesis (Georgescu *et al.*, 2000; Haverkamp *et al.*, 2008). Out of all the inflammatory mediators, IL-6 is particularly important for PCa pathogenesis because it promotes angiogenesis, tumour growth, and disease advancement (Blum *et al.*, 2008). In addition to its inflammatory attributes, increased production of IL-6 is closely linked to poor clinical outcomes, advanced disease stages, and resistance to treatment. The PI3K-AKT pathway is activated by IL-6 receptor (IL-6R) signalling, which promotes PCa cell proliferation and survival and mediates the carcinogenic effects of IL-6 (Culig & Puhr, 2012; Smith *et al.,* 2013). This creates a positive feedback loop in which the loss of *PTEN* and the PI3K-AKT pathway's subsequent hyperactivation increase the expression of IL-6 and intensify its carcinogenic effects. Targeting pathways mediated by IL-6 have great therapeutic potential. Monoclonal antibodies that are directed against IL-6 or IL-6R are examples of anti-IL-6 therapy that may interfere with IL-6 signalling and reduce the development of prostate cancer (Culig & Puhr, 2012; Smith *et al.,* 2013). The dynamic interplay between IL-6, *PTEN* loss, and PI3K-AKT signalling highlights the potential of combination therapies. Strategies that concurrently inhibit IL-6 signalling, block the PI3K-AKT pathway, and address *PTEN* loss are likely to enhance therapeutic outcomes in prostate cancer. Such integrated approaches offer a promising avenue to overcome treatment resistance and improve patient prognosis (Culig & Puhr, 2012; Smith *et al.,* 2013).

2.11.2 TMPRSS2: ERG Gene Fusion in Prostate Cancer

The *TMPRSS2: ERG* gene fusion is one of the most frequent and well-characterised structural variations in prostate cancer. The androgen-regulated gene *TMPRSS2* is structurally situated on chromosomal band 21q22 and is mostly expressed in the

prostate (Hermans *et al.*, 2009). The *ERG* gene, which is similarly located on 21q22, is situated about 3 Mb downstream of *TMPRSS2*. The most prevalent molecular change in PCa is the *TMPRSS2:ERG* fusion, which has been seen in about 50% of cases (Hägglöf *et al.*, 2014a; Omar *et al.*, 2023). Given that it is common in both the early and severe stages of the disease, this fusion highlights its importance in the pathophysiology of prostate cancer (Barros-Silva *et al.*, 2013; Cerveira *et al.*, 2006; Tomlins *et al.*, 2005). The *TMPRSS2: ERG fusion* occurs when the first exon or exons of *ERG* come into contact with the androgen- and prostate-specific regulatory domains of *TMPRSS2*, resulting in androgen-driven overexpression of the fusion transcript. This is due to an intronic loss on chromosome 21 that eliminates the genomic region between *TMPRSS2:ERG* and *ERG* in about 50–60% of fusion-positive tumours. Alternatively, this fusion may potentially result from complex genomic rearrangements involving chromosome 21q22 and other chromosomes (Hermans *et al.*, 2006; Lorenzin & Demichelis, 2022; Perner *et al.*, 2006). The frequent occurrence of this fusion is explained by a shared deletion site that connects *TMPRSS2* and *ERG* (Kohaar et al., 2020). As a result of this fusion, the androgen-responsive promoter of *TMPRSS2* drives constitutive expression of *ERG*, which increases prostate cancer cell invasion, proliferation, and angiogenesis (Giunchi *et al.*, 2020; Kobelyatskaya *et al.*, 2021). Additionally, the *TMPRSS2:ERG* fusion activates downstream oncogenes, further amplifying its carcinogenic effects (Yamoah *et al.*, 2021).

2.11.2.1Mechanisms and Therapeutic Implications *TMPRSS2:ERG* fusion

Androgen receptor (AR) activation plays a central role in driving *TMPRSS2* promoter activity, resulting in the production of an *ERG* mRNA fusion transcript. This transcript is translated into the *ERG* transcription factor, which subsequently activates

downstream oncogenic signalling pathways (Manzar *et al.*, 2023; St John *et al.*, 2012). This molecular cascade presents two key therapeutic targets. First, the Androgen Receptor Inhibitors, Agents such as enzalutamide and abiraterone, block *TMPRSS2* activation, reducing fusion transcript production and limiting *ERG* overexpression (Semaan *et al.*, 2019). Second Bromodomain Inhibitors: Compounds like JQ1 interrupt the transcriptional machinery necessary for *ERG*-mediated gene activation, mitigating its oncogenic effects (Blee, 2018; Manzar *et al.*, 2023). These targeted approaches, which interrupt key nodes in the *TMPRSS2:ERG* fusion signalling pathway, offer promising opportunities for personalised therapy in PCa (Assadi *et al.*, 2020; J. C. Brenner, 2012).

2.11.2.2 Functional *ERG* Overexpression in Prostate Cancer

The progression from prostatic intraepithelial neoplasia (PIN) to invasive prostate cancer is marked by metastases, advanced tumour stages, shorter survival times, and higher Gleason scores. The ongoing overexpression of the *ERG* oncogene is what drives this process (Carver, Tran, Chen, *et al.*, 2009; Hägglöf *et al*., 2014b). *ERG* exhibits only moderate expression in normal prostate cells, in contrast to other members of the ETS family, including SAM-pointed domain-containing ETS transcription factor (SPDEF) and ETS2 repressor factor (ERF), which are crucial for preserving the identity of normal prostate epithelium (Consortium *et al.*, 2015). However, ERG has a variety of carcinogenic effects when it is overexpressed in prostate cells. For example, ERG contributes to carcinogenesis through its interaction with the PI3K oncogenic signalling pathway (Carver, Tran, Chen, *et al.*, 2009). Furthermore, ERG increases the transcription of the androgen receptor (AR) and improves its binding, especially in PCa patients with PTEN loss or malfunction (Chen *et al.*, 2013). Notably, cells with ERG overexpression have exhibited different AR

binding patterns (Chen *et al.*, 2013; Yu *et al.*, 2010). ERG also boosts β-catenin activation and promotes PCa growth and progression by activating the Wnt signalling pathway (Iljin *et al.*, 2006; Leshem *et al.*, 2011; L. Wu *et al.*, 2013).

Although these carcinogenic functions, there is evidence that ERG overexpression is not always linked to the course of the disease or the prognosis of patients and that it is not enough to start cancer (Carver, Tran, Chen, *et al.*, 2009; Chen *et al.*, 2013; Hoogland *et al.*, 2012; Minner *et al.*, 2011; Pettersson *et al.*, 2012; B. Xu *et al.*, 2014). There have also been reports of geographic and ethnic differences in *ERG* overexpression and rearrangements (Table 1). *ERG* expression is found in 50% to 55% of American populations overall, with 28% of Caucasian Americans exhibiting this expression. ERG expression rates in African American populations vary considerably depending on the population under study. Sub-Saharan Africa still has a dearth of data. ERG rearrangements are found in 50–55% of Asian populations; rates are 28% for Indian patients and 49% for Chinese PCa patients (Xiao *et al.*, 2018).

2.11.2.3Clinical Implications of *ERG* Overexpression

The relevance of ERG overexpression in the progression of prostate cancer is further highlighted by its interaction with other regulatory networks, such as *PTEN* loss, and its potential as an indication of disease aggression (Carver, Tran, Gopalan, *et al.*, 2009; Krohn *et al.*, 2014). The clinical importance of *ERG* as a possible biomarker for disease classification and risk assessment is shown by the greatly increased risk of prostate cancer for those with *ERG*-positive high-grade prostatic intraepithelial neoplasia (HGPIN) (K. Park *et al.*, 2014).

2.11.2.4 ERG and PTEN Crosstalk in Prostate Cancer

Recent findings demonstrate that high *ERG* expression, caused by the *TMPRSS2:ERG* fusion, is ineffective for predicting biochemical recurrence or overall survival in prostate cancer. However, adding *PTEN* status into molecular assessments gives a stronger predictive structure, improving our understanding of tumour aggression and enabling the development of targeted therapy methods (Blee *et al.,* 2018; Brady *et al*., 2021). Although the PI3K signalling pathway is directly regulated by functional *PTEN*, its loss in several cancers, including prostate cancer, causes PI3K signalling to become hyperactivated, which aids in the growth of tumours and resistance to treatment (Brady et al., 2021). According to new research, when *ERG* activation and *PTEN* loss occur together, prostatic intraepithelial neoplasia (PIN) may progress to invasive cancer (Abou-Ouf *et al.,* 2021). Even while some research has looked at the connection between Gleason score and *ERG* expression, the results are still unclear, which emphasises the need for more study. Conversely, there is more proof that a poor prognosis and higher Gleason scores are associated with decreased *PTEN* expression (Spinos *et al.,* 2023). Although the relationship between *ERG* overexpression and PTEN loss has not been thoroughly examined, research examining their potential as therapeutic targets has produced encouraging findings (Spinos *et al.*, 2023).

Poor overall survival, adverse clinical characteristics, and the emergence of castration-resistant and metastatic prostate cancer are all closely linked to *PTEN* function loss (Krohn *et al.,* 2012; Mithal *et al.,* 2014). These results imply that, in contrast to the *TMPRSS2:ERG* fusion, *PTEN* loss might be a more clinically relevant molecular change in prostate cancer. Understanding the molecular processes behind prostate cancer progression and developing successful treatment strategies depend on a knowledge of the interplay between *ERG* and *PTEN*.

2.12 Tumor Molecular Characterization of Prostate Cancer

Tumor molecular characterization in personalized treatment for prostate cancer entails the categorization of prostate tumours based on molecular traits and genetic testing to understand the biology of the tumour better and inform treatment choices (Figiel *et al.,* 2023; Giunta *et al*., 2021a; Randall *et al.*, 2019). Identification of particular genetic mutations, variations in gene expression, and other molecular modifications that promote tumour development and advancement are usually steps in this process (Ge *et al.,* 2023; Vlajnic & Bubendorf, 2021). Medical professionals can identify the best course of action for treating a tumour by analyzing its molecular profiles. These options include immunotherapies or individualized treatments that precisely target the tumour's molecular vulnerabilities (Bugoye *et al.*, 2023; Figiel *et al.*, 2023; Renard-Penna *et al.,* 2015). The response of prostate cancer patients to specific therapies and the possible mechanism of resistance can both be predicted with the aid of molecular profiling (Ge *et al.,* 2023; Vlajnic & Bubendorf, 2021). Tumour molecular classification is essential to personalized therapy because it enables the development of more accurate treatment plans based on the distinct genetic signatures of individual tumours (Bugoye *et al.,* 2023). Homologous recombination and mismatch repair genes, such as *BRCA1, BRCA2, ATM, CHEK2, PALB2, TP53, MLH1, MSH2, MSH6, PMS2,* and changes impacting *ERG* and *PTEN* expression, are some of the often-occurring molecular changes in prostate cancer (Bugoye *et al.,* 2023).

2.13 Methods in Tumor Molecular Characterization

Prostate cancer is characterized using a variety of techniques (Barnes *et al.*, 2022), all of which offer important insights into the genetic and molecular characteristics of the illness. Typical techniques include those that follow:

2.13.1 Next-Generation sequencing

By thoroughly analyzing the gene fusions caused by genetic mutations and other molecular changes found in prostate tumours, next-generation sequencing techniques like whole-genome, whole-exome, and targeted panel sequencing have completely changed the field of prostate cancer genomics (Giunta *et al.,* 2021b). As a result, homologous recombination genes and mismatch repair genes linked to prostate cancer can be specifically identified by Next Generation Sequencing (Bugoye *et al.,* 2023). Moreover, unique genetic alterations that could affect tumour behaviour and treatment response can be identified by employing next-generation sequencing. However, the technique has several disadvantages (Cheng *et al.*, 2023), which include the following:

i. **Cost of Investment and Data Analysis Complexity:**

Next-generation sequencing generates high-throughput data requiring bioinformatics workflow, expertise, and resources for data processing, alignment, variant calling, and interpretation (Cheng *et al.,* 2023). The method can be expensive which may limit its wide application, especially for resource-limited healthcare systems or research groups (Skorheim *et al*., 2014).

ii. **Sample Requirement and Prostate Tumor Heterogeneity**

DNA samples of high quality are necessary for next-generation sequencing, but they are not always readily available, particularly in clinical settings where sample quality varies. The precision and dependability of the results of molecular subtyping using next-generation sequencing might be impacted by variations in sample quality and quantity (Cheng *et al*., 2023).

iii. **Occurrence of subclonal population with distinct genetic profiles**

Genomic instability is frequently observed in prostate tumours due to a higher frequency of mutations, chromosomal rearrangements, and copy number alterations

(Baca *et al.,* 2013). Intratumor heterogeneity may result from this genomic instability as it could give rise to subclonal populations that have unique genetic alterations (Baca *et al.,* 2013). Comparably, the clonal makeup of prostate tumours is greatly enhanced by the prostate tumour microenvironment. Variations in oxygenation, nutrient availability, and immune cell infiltration are examples of heterogeneous microenvironmental conditions that produce selective pressures that support the survival and growth of subclonal populations with adaptive advantages (Gundem *et al*., 2015). Prostate tumours also experience clonal evolution, which is the gradual acquisition and selection of genetic variations over time (Cooper *et al.,* 2015). Due to this evolutionary process, subclonal populations with unique genetic profiles emerge, which represents the continuous evolution and adaptation of tumours (Cooper *et al.,* 2015). Therefore, the heterogeneity of tumours may not be fully captured by Next Generation Sequencing molecular subtyping, which could result in an incomplete or inaccurate characterization of tumour subtypes (Cooper *et al.,* 2015).

2.13.2 Gene Expression Profiling

Gene activity levels in prostate tumours can be determined through gene expression profiling methods like RNA sequencing and microarray analysis. Researchers can predict outcomes, identify molecular subtypes of prostate cancer tumours (Durand *et al., 2007),* and identify potential therapeutic targets by analyzing the patterns of gene expression (Durand *et al.,* 2007). However, as with other PCR-based molecular techniques, the intratumoral heterogeneity of prostate tumours and the requirement for complex data-handling skills limit the broad applicability of gene expression profiling. However, variations in sample preparation, RNA extraction procedures, microarray workflow, or RNA-sequencing methodologies can lead to technical variability in gene expression profiling approaches (Irizarry *et al.,* 2003). These

variations can affect the repeatability and dependability of molecular subtyping of prostate cancer results by adding noise and complicating the interpretation of information on gene expression (Irizarry *et al.,* 2003).

2.13.3 Fluorescence in Situ Hybridization (FISH)

A molecular method called fluorescence in situ hybridization can identify particular genetic anomalies in prostate cancer cells, like the fusion of the *TMPRSS2-ERG gene.* Fluorescence in situ hybridization analysis can support the confirmation of known genetic changes and help direct treatment decisions (Chen *et al.,* 2018). To facilitate the molecular subtyping and clinical correlation of prostate cancer, the technique can be applied to preserved tissue samples and integrated with histopathological data for formalin-fixed paraffin-embedded (FFPE) tissues, which are frequently used in pathology laboratories (Zhu *et al.,* 2017). But compared to other molecular profiling techniques like multiplex immunohistochemistry or RNA sequencing, it might not be as capable of multiplexing. Furthermore, various RNA targets may need to be simultaneously detected within a single tissue segment, making the approaches technically demanding and possibly requiring optimization (Kagan *et al.,* 2010).

2.13.4 Immunohistochemistry (IHC)

Prostate tumour tissue samples can be stained with an immunohistochemistry approach to see which proteins are expressed (Barnes *et al.,* 2022). The method makes it possible to see and characterize the patterns of protein expression in prostate tumour tissues by staining tissue sections using monoclonal antibodies. Hence, IHC serves as a resource for tumour molecular subtyping in prostate cancer since it targets particular proteins linked to molecular subtypes, signalling pathways, or therapeutic targets. IHC additionally provides important information on molecular features and biological behaviour (Zhao *et al.,* 2020). The difficulties in employing rely on covering a small

number of antigens, even though multiplex immunohistochemistry may be able to partially address this problem (Gevaert *et al.,* 2021). However, when working with samples that present limited foci of atypical glands, immunohistochemistry helps in a correct diagnosis of prostate cancer. This is particularly important in cases that are borderline when staining the basal cell layer, or in advanced cases where confirming the prostatic origin of a metastatic deposit is necessary (Magi-Galluzzi, 2018). Furthermore, immunohistochemistry offers the ability to evaluate the expression of several makers, such as *PTEN* and *TMPRSS2: ERG*, on formalin-fixed paraffin-embedded tissues. This allows for the identification of tumour protein expression resulting from mechanisms other than genomic deletion, using different makers (Vlajnic & Bubendorf, 2021). Immunohistochemistry analysis could be conveniently included in the diagnosis process for patients with prostate cancer that has spread due to the affordability of the test (Gurel, *et al.*, 2011).

2.13.5 ERG and *PTEN* Immunohistochemistry-based Tumour Molecular Subtypes in Prostate Cancer

Immunohistochemistry (IHC) analysis of *ERG* and *PTEN* expression reveals considerable molecular heterogeneity in prostate cancer, which may be classified into four subtypes. Normal *ERG* with loss of *PTEN* expression (*ERG-/PTEN*-), rearranged *ERG* with normal *PTEN* expression (*ERG+/PTEN+*), normal ERG with normal *PTEN* expression (*ERG-/PTEN+*), and rearranged *ERG* with loss of *PTEN* expression (*ERG+/PTEN*-) are some of these subtypes (Brady *et al.*, 2021; Lahdensuo *et al.*, 2016; Reid *et al.,* 2010). The prevalence of these tumour molecular subtypes differs across populations, emphasising the impact of both environmental and genetic factors on disease biology. Also, these subtypes may demonstrate different therapeutic potential, especially when considering immunotherapy and personalised treatment. For example, *ERG+/PTEN+* tumours might react differently to treatments that target

the androgen receptor (AR) than *ERG-/PTEN*-tumors, which are frequently linked to more aggressive illness and resistance to standard therapy (Lotan *et al.,* 2011; Yoshimoto *et al.,* 2012). It is essential to understand these molecular differences to establish individualised treatment plans and improve prostate cancer patients' clinical results.

2.13.6 ERG-/PTEN- Tumour Molecular Subtypes

The molecular subtype of prostate cancer that is distinguished by *PTEN* loss and lack of *ERG* expression (*ERG-/PTEN*-) has important biological and therapeutic potential (Spinos *et al.,* 2023). This tumour molecular subtype contributes to resistance to AR-targeted therapies such as androgen deprivation therapy (ADT) because it is less dependent on androgen receptor (AR) signalling than *ERG*-positive tumours (Cai *et al*., 2009). *ERG-/PTEN*-tumors are linked to a poor prognosis and are frequently more aggressive due to their reliance on PI3K/AKT signalling and increased genomic instability (Esteban-Villarrubia *et al.*, 2024; Haney *et al.*, 2020; Krohn *et al.*, 2012). These tumours are more common in some groups, like African American men, who have lower frequencies of *TMPRSS2-ERG* fusion and greater rates of *PTEN* loss, suggesting that there may be genetic and ethnic differences in prostate cancer subtypes (C. K. Zhou *et al.*, 2017). These tumour genetic subgroups present therapeutic hurdles and offer potential candidates for novel approaches, despite demonstrating reduced susceptibility to traditional AR-targeted therapy (Ma *et al.*, 2024)To address cross-pathway interactions, medications that target the PI3K/AKT/mTOR pathway are particularly helpful, especially when used in combination with AR inhibitors (Li *et al.,* 2016). Immunotherapy, which includes immune checkpoint inhibitors, is a potential therapy option for *PTEN* loss related to immune evasion (Esteban-Villarrubia *et al.*, 2024).

2.13.7 ERG+/PTEN+ Tumour Molecular Subtypes

ERG rearrangements, most commonly the *TMPRSS2:ERG* fusion and intact *PTEN* function are hallmarks of the *ERG+/PTEN+* molecular subtype of prostate cancer (Reid *et al.*, 2010). However, because *PTEN* mutations are heterogeneous, the occurrence of this subtype varies: *PTEN* loss has been reported in 20–50% of patients. Approximately 40–70% of prostate tumours have *ERG* rearrangements (Al Bashir *et al.*, 2024; Shan *et al*., 2018). *ERG* rearrangements biologically drive oncogenesis by promoting tumour invasion and progression through the enhancement of androgen receptor (AR)- regulated transcriptional pathways (Silva *et al.*, 2024). This subtype is distinguished from the more aggressive *ERG+/PTEN* subtype by its intact *PTEN* function, which aids in maintaining control over the PI3K/AKT pathway (Glaviano *et al.*, 2023; Imada *et al*., 2021). Clinically, *ERG+/PTEN+* tumours remain sensitive to AR-targeted therapies, such as androgen deprivation therapy (ADT), abiraterone, and enzalutamide, which form the cornerstone of treatment for this subtype (Graff *et al.*, 2015). Emerging therapeutic strategies, including precision medicine, approaches that target vulnerabilities in ERG-driven pathways and DNA repair mechanisms, such as PARP inhibitors, may benefit a subset of these tumours, particularly those with additional alterations in DNA damage repair genes (Brenner *et al.*, 2011). Immune checkpoint inhibitors also represent a potential therapeutic avenue for *ERG+/PTEN+* tumors, as this subtype may maintain a more immunologically active tumour microenvironment compared to *PTEN*-deficient subtypes. However, the efficacy of these treatments remains under investigation (Shakfa *et al.*, 2023; Vidotto *et al.*, 2023).

Compared to *PTEN*-deficient subtypes, *ERG+/PTEN+* cancers may have a more immunologically active tumour microenvironment, making immune checkpoint

inhibitors a potential substitute. Nonetheless, research on their efficacy is still ongoing (Shakfa *et al.*, 2023; Vidotto *et al.*, 2023). Utilising new therapeutics that target ERG-associated signalling pathways is another novel approach to developing future treatments (Shao *et al.*, 2020). Despite these advancements, the biology and clinical diversity of this subtype in various groups, as well as the hunt for biomarkers to guide personalised treatment, remain open questions. Understanding the specific sensitivities of *ERG+/PTEN+* tumours is necessary for improving treatment strategies and outcomes for patients with this tumour molecular subtype of prostate cancer (Al Bashir *et al.*, 2024; Shan *et al*., 2018; Shao *et al.*, 2020).

2.13.8 ERG-/PTEN+ Tumour Molecular Subtypes

ERG rearrangements, including *TMPRSS2:ERG* fusions, do not exist in the *ERG-/PTEN+* molecular subtype of prostate cancer, and *PTEN* function, which is essential for controlling the PI3K/AKT signalling pathway, is retained (Reid *et al.*, 2010). This subtype is a subset of *ERG*-negative prostate tumours, which make up 30–60% of cases of prostate cancer (Reid *et al.*, 2010; Yoshimoto *et al*., 2008). Studies have shown that the *ERG-/PTEN+* subtype's prevalence varies by population, demonstrating that the disease biology varies by population. There may be ethnic and genetic differences in prostate cancer, as evidenced by the greater frequency of *PTEN*-positive tumours and lower prevalence of *ERG* rearrangements in African American men (Sedarsky *et al.*, 2017; Zhou *et al.*, 2017). *ERG-/PTEN+* tumours differ from more aggressive *PTEN*-deficient subtypes in that they retain *PTEN* function, which aids in controlling cellular survival and proliferation (Ferraldeschi *et al.*, 2015; Reid *et al.*, 2010) . As these tumours still rely on androgen receptor (AR) signalling, they can be treated with androgen deprivation therapy (ADT) and AR-targeted medications such as abiraterone and enzalutamide. Furthermore, the absence

of *PTEN* loss might result in a tumour microenvironment that is more immunologically active, which would increase the likelihood of investigating immunotherapies, such as immune checkpoint inhibitors, possibly in conjunction with AR-targeted treatments (Graff *et al*., 2015; Yoshimoto *et al*., 2007).

2.13.9 ERG+/PTEN- Tumour Molecular Subtypes

The lack of *PTEN* function and the presence of *ERG* rearrangements, most frequently *TMPRSS2:ERG* fusions, are characteristics of the *ERG+/PTEN*- a molecular subtype of prostate cancer (Fallahabadi *et al.,* 2016; Reid *et al.*, 2010). As demonstrated by more invasive tumours, higher Gleason scores, and an increased risk of developing castration-resistant prostate cancer, this subtype is associated with aggressive illness (Fallahabadi *et al.,* 2016; Repka *et al.*, 2024). *PTEN* loss interferes with the PI3K/AKT signalling pathway, resulting in uncontrolled cell proliferation and resistance to apoptosis, whereas *ERG* rearrangements increase androgen receptor (AR)-mediated transcriptional activity (Goel *et al.*, 2021). The frequency of *ERG+/PTEN* tumours varies by ethnicity; Asian and African American males are less likely to have *ERG* rearrangements than Caucasian men. All ethnic groups do, however, have *PTEN* loss, especially in more late stages of the disease (Al Bashir *et al.*, 2024; Bismar *et al.*, 2012). Therapeutically, *ERG*-driven tumours respond well to AR-targeted treatments including abiraterone, enzalutamide, and androgen deprivation therapy (ADT). However, through the activation of alternate signalling pathways, *PTEN* depletion frequently confers resistance (Graff *et al.*, 2015; Yoshimoto *et al.,* 2007). Inhibitors that target the PI3K/AKT/mTOR pathway are being researched to address this, and when combined with AR-targeted treatments, they show promising results (Cham *et al.,* 2021; Graff *et al.*, 2015; Tortorella *et al*., 2023). Additionally, immunosuppressive properties such as decreased CD8+ T-cell

infiltration and enhanced recruitment of myeloid-derived suppressor cells are commonly seen in *ERG+/PTEN*- tumours (Esteban-Villarrubia *et al.,* 2024; Vidotto *et al*., 2020). The effectiveness of immune checkpoint inhibitors (ICIs), such as anti-PD-1/PD-L1 or anti-CTLA-4 antibodies, is restricted by these attributes (Vidotto *et al*., 2020). Some subtypes, however, may benefit from combination treatments that concurrently target the PI3K/AKT pathway and boost immune responses (Esteban-Villarrubia *et al*., 2024; Vidotto *et al.,* 2020).

2.14 ERG and PTEN Tumor Molecular subtypes and their association with PSA in prostate cancer

Several earlier studies studied the relationship between PSA levels at diagnosis and tumour molecular subtypes based on *ERG* and *PTEN* expression to understand the heterogeneity in prostate cancer. These molecular subtypes have been demonstrated to display unique biological and clinical traits that may affect PSA levels. The presence or absence of *PTEN* loss and *ERG* overexpression characterizes them (Birol *et al.*, 2021). Since ERG overexpression has been connected to PSA levels in multiple studies, ERG-positive cancers often have higher PSA levels, which may reflect a rising tumour burden or altered androgen receptor signalling associated with this molecular subtype (Rostad *et al.,* 2009). However, other studies have found no significant differences in PSA levels between *ERG*-positive and *ERG*-negative tumours, suggesting differences in outcomes and the potential influence of additional factors like cancer stage and grade, which necessitates further research (Birol *et al.*, 2021; Mitala *et al.,* 2024).

Similarly, *PTEN* depletion is linked to faster tumour growth, but it is unclear how it relates to PSA levels. Previous research indicates that whereas *PTEN*-deficient tumours are frequently more aggressive, they may not necessarily show

correspondingly higher PSA levels (Ahearn *et al.*, 2016b). However, *PTEN* loss results in changed tumour biology, which may impact PSA release (Krohn *et al.*, 2012; Lotan *et al.*, 2017). This finding implies that the traditional correlation between PSA production and tumour volume may be upset by *PTEN* loss (Ahearn *et al.*, 2016b). The combined study of *ERG* and *PTEN* expression has allowed us to better understand the relationship between these molecular markers and PSA levels. Studies show that aggressive features are more common in tumours that are *PTEN*-negative and ERG-positive (Voulgari *et al*., 2023). PSA levels differ among the molecular subtypes that are identified by their ERG and *PTEN* status. Although these tumours are usually associated with advanced disease, additional clinicopathological factors, including tumour differentiation and androgen receptor signalling, may influence the PSA levels of *ERG*-positive/*PTEN*-negative malignancies at diagnosis (Lotan, Gupta, *et al.*, 2011).

Studies are still being conducted to determine whether PSA levels at diagnosis correlate with molecular subtypes of prostate cancer based on *ERG* and *PTEN* expression. Even though several patterns have emerged, including the link between increased PSA levels and ERG overexpression and the complex relationship between *PTEN* loss and these patterns, the results are conflicting (Birol *et al.*, 2021) underscoring the need for more research to clarify these relationships and their clinical significance.

2.15 Conceptual Framework

The conceptual framework for this study examines the relationship between prostate cancer risk factors, genetic mutations, molecular subtypes, and clinicopathological characteristics. The independent variables consist of modifiable and non-modifiable

risk factors, including sociodemographic factors (age, race, ethnicity), family history of prostate cancer, and lifestyle influences (diet, obesity, smoking, and physical inactivity). These factors act as triggers that may increase susceptibility to prostate cancer by inducing genetic alterations. The dependent variables are clinicopathological outcomes, specifically the Gleason score, a histological measure of tumour aggressiveness and preoperative prostate-specific antigen (PSA) levels, which serve as biomarkers for disease progression. The relationship between risk factors and clinical outcomes is moderated by key gene mutations, particularly in DNA repair pathways such as homologous recombination (e.g., *ATM* mutations) and mismatch repair (e.g., *PMS2* mutations). These mutations disrupt normal cellular repair mechanisms, increasing genomic instability and promoting tumour development. Additionally, the framework incorporates modulating variables in the form of ERG/PTEN tumour molecular subtypes, which further influence disease aggressiveness. For instance, *ERG* gene fusions and *PTEN* loss are associated with more aggressive prostate cancer phenotypes. The central hypothesis is that prostate cancer risk factors contribute to adverse clinicopathological characteristics by inducing genetic mutations that, in turn, shape molecular tumour subtypes. By investigating these pathways, this study aims to elucidate whether genetic and molecular mechanisms mediate the impact of risk factors on disease severity, potentially enabling better risk stratification and personalised therapeutic approaches (Figure 2.0).

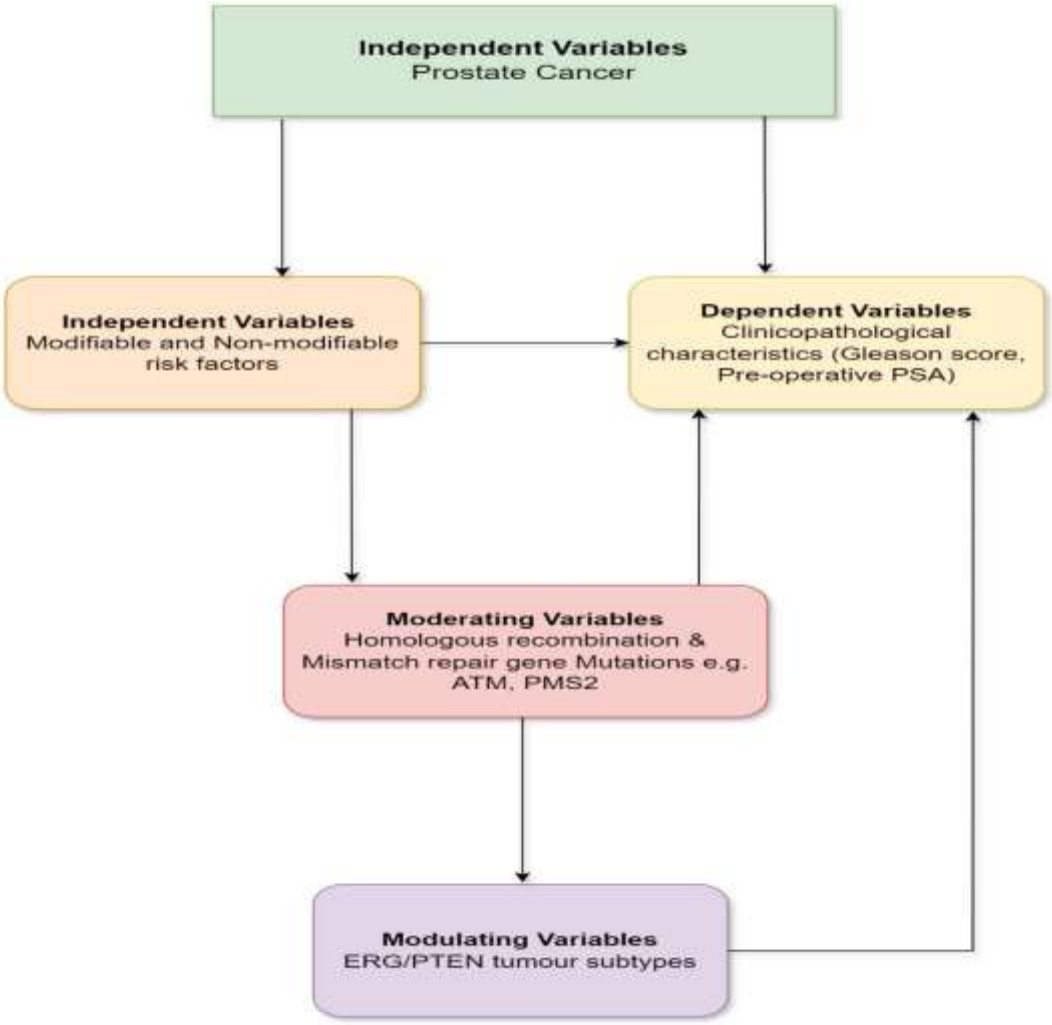


Figure 2.1 Conceptual framework

CHAPTER THREE

3.0 STUDY METHODOLOGY

3.1 Study sites

The study was carried out at the Ocean Road Cancer Institute (ORCI) and Muhimbili National Hospital (MNH), two renowned medical facilities in Dar es Salaam, Tanzania. Muhimbili National Hospital is the nation's largest public hospital, providing a broad range of medical services. It is situated at 6.8231° S latitude and 39.2666° E longitude. It is one of the main referral hospitals for patients from Tanzania and the surrounding nations. The Ocean Road Cancer Institute is a specialised facility in cancer treatment and research, located at 6.7924° S latitude and 39.2682° E longitude. ORCI is situated very near the Indian Ocean coast (Figure 3.1). These publicly owned hospitals are situated in the centre of Dar es Salaam, making them perfect study sites and important centres for National healthcare.

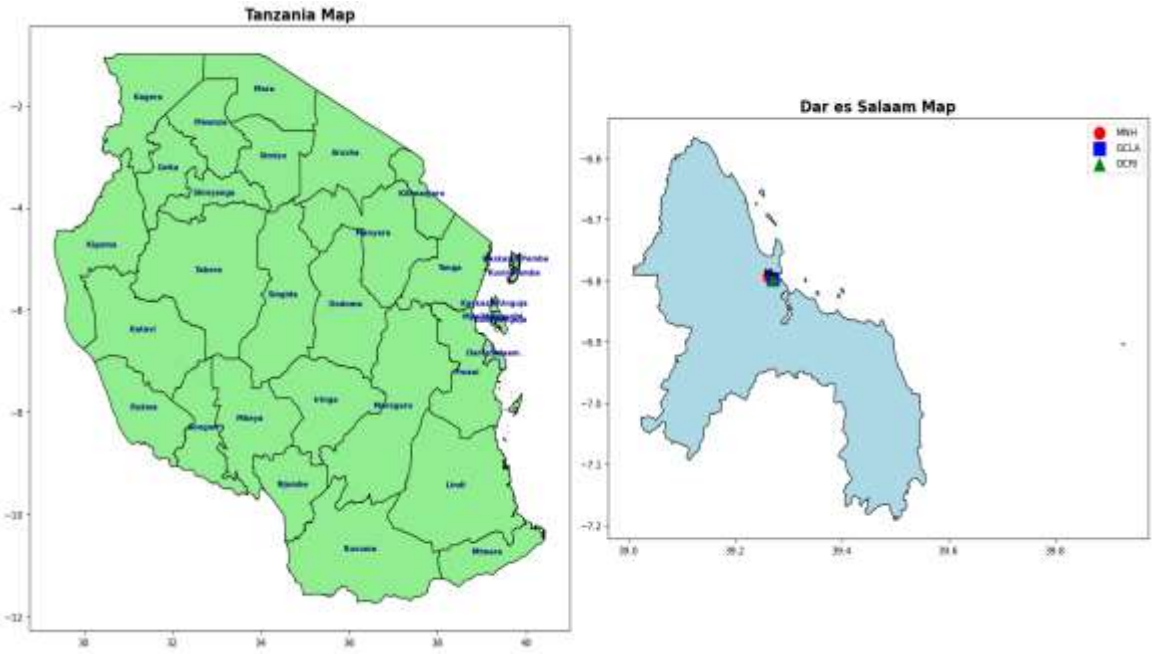


Figure 3.1.The Dar es Salaam Map showing study sites: MNH, GCLA and ORCI

3.2 Study Population

The study population was Tanzanian prostate cancer Patients as cases and the control group who were diagnosed and received their medical treatment at Ocean Road Cancer Institute and Muhimbili National Hospital. Those participants met the enrollment criteria set.

3.3 Study design

A combination of unmatched case-control and cross-sectional study designs was used. The case-control component identified prostate cancer risk factors by comparing men with and without PCa, while the cross-sectional analysis characterised tumour subtypes using *ERG* and *PTEN* markers and gene mutation spectrum. This integrated approach provided comprehensive insights into genetic and environmental contributors to prostate cancer in the Tanzanian population.

3.4 Sampling and Participant Enrolment

Purposive sampling was employed to recruit 166 participants who provided consent from the Ocean Road Cancer Institute (ORCI) and Muhimbili National Hospital (MNH). Recruitment was guided by predefined inclusion and exclusion criteria, whereby 83 cases and 83 controls were enrolled. To ensure comparability and minimise potential confounding effects, both groups were matched in a 1:1 ratio

3.4.1 Selection of Case and Control

The cases were selected from prostate cancer patients with a histologically confirmed prostate cancer diagnosis as evidenced by their histopathology reports. The cancer patients were recruited from both Ocean Road Cancer Institute (ORCI) and Muhimbili National Hospital (MNH), where they received treatment. The control group was composed of patients who attended either ORCI or MNH for urology clinic medical checkups or treatment of conditions other than prostate cancer. The records for their pre-operative PSA level and biopsy histopathological examination were

accessed before selection, and those who met the eligibility criteria were enrolled as the control group after obtaining informed consent

3.5 Inclusion Criteria

3.5.1 Case Group

The case group included participants who met the following criteria:

i. Prostate-Specific Antigen (PSA) Levels:

Men with pre-operative PSA levels exceeding 4 ng/mL.

ii. Prostate Cancer Confirmation:

A positive prostate cancer diagnosis was confirmed through either digital rectal examination (DRE) or histopathology.

iii. Accessibility of Medical Records:

Men whose histopathological and pre-operative PSA results are retrievable from the Ocean Road Cancer Institute (ORCI) or Muhimbili National Hospital (MNH).

iv. Availability of archived tissue samples:

Participants whose archived histopathological tissue samples were accessible at ORCI or MNH.

v. Consent:

Men who provided informed consent to participate in the study.

3.5.2 Control Group

The control group included participants who met the following criteria:

i. Prostate-Specific Antigen (PSA) Levels:

Men with pre-operative PSA levels below 4 ng/mL.

ii. Prostate Cancer Confirmation:

Negative prostate cancer diagnosis confirmed through either digital rectal examination (DRE) or histopathology.

iii. Accessibility of Medical Records:

Men whose histopathological and pre-operative PSA results were retrievable from ORCI or MNH.

iv. Consent:

Men who provided informed consent to participate in the study.

3.6 Exclusion Criteria

3.6.1 Case Group

Participants who met the following criteria were excluded from the case group:

i. Prostate-Specific Antigen (PSA) Levels

Men with pre-operative PSA levels ≤4 ng/mL.

ii. Prostate Cancer Status

Negative prostate cancer diagnosis confirmed via digital rectal examination (DRE) or histopathology.

iii. Inaccessible Medical Records

Men whose histopathological and pre-operative PSA results are unavailable at the Ocean Road Cancer Institute (ORCI) or Muhimbili National Hospital (MNH).

iv. Unavailability of Archived Tissue Samples

Participants whose archived histopathological tissue samples are inaccessible at ORCI or MNH.

v. Lack of Consent

Men who have not provided informed consent to participate in the study.

3.6.2 Control Group

Participants who met the following criteria were excluded from the control group:

i. Prostate-specific antigen (PSA) Levels or Cancer Status

Men with pre-operative PSA levels >4 ng/mL or a positive prostate cancer diagnosis confirmed via DRE or histopathology.

ii. Inaccessible Medical Records

Men whose histopathological and pre-operative PSA results are unavailable at ORCI or MNH.

iii. Lack of Consent

Men who have not provided informed consent to participate in the study.

3.6.3 Criteria for germline mutation testing:

Selected participants in the case group underwent targeted next-generation sequencing for germline mutations in *BRCA1/2, ATM, TP53, PALB2, CHEK2, MLH1, MSH2, MSH6,* and *PMS2* if they met any of the following NCC guidelines criteria:

i. **Family History of Cancer**

A family history of prostate, ovarian, breast, colorectal, endometrial, or pancreatic cancer in ≥1 first- or second-degree relative(s), particularly if diagnosed at an early age (e.g., breast or colorectal cancer diagnosed before age 50) or if multiple relatives are affected (NCCN, 2021, 2023)

ii. **Metastatic Prostate Cancer**

A diagnosis of metastatic prostate cancer at any age, regardless of family history (NCCN, 2023).

iii. **High-Risk Localized Prostate Cancer with Family History**

A Gleason score ≥7 and ≥1 close blood relative(s) (first- or second-degree) with ovarian, pancreatic, metastatic prostate cancer, or breast cancer diagnosed before age 50 (NCCN, 2023).

iv. **Very High-Risk Prostate Cancer**

A Gleason score ≥8 at diagnosis, regardless of family history (NCCN, 2023).

v. **Additional Considerations**

Individuals with a personal or family history of multiple primary cancers (e.g., breast and ovarian cancer in the same individual or family) or Ashkenazi Jewish ancestry, given the higher prevalence of certain germline mutations (e.g., BRCA1/2) in this population (NCCN, 2023).

3.7 Sample size determination

3.7.1 For unmatched case control

For this study, unmatched case control study, the sample size estimate was determined using Kesley et al., 1996; Fleis et al., 1981).

Sample Size Formula (Unmatched Case-Control):

$$n = \frac{(Z_{\alpha/2} + Z_{\beta})^2 \times [p_1(1-p_1) + p_0(1-p_0)]}{(p_1 - p_0)^2}$$

Where:

- n = sample size per group (cases or controls)
- $Z_{\alpha/2}$ = 1.96 (for 95% confidence)
- Z_{β} = 0.84 (for 80% power)
- p_0 = exposure prevalence in controls
- p_1 = exposure prevalence in cases, calculated by:

$$p_1 = \frac{OR \times p_0}{1 + p_0 \times (OR - 1)}$$

The sample size for this unmatched case-control study was estimated based on key epidemiological parameters to ensure adequate statistical power. Drawing from previous studies conducted in Sub-Saharan Africa that reported moderate associations between prostate cancer and various risk factors(Oczkowski et al., 2021; Samtal et al., 2022; Seraphin, Joko-Fru, et al., 2021). The current study selected an effect size represented by an odds ratio (OR) of 2.6. The exposure prevalence among controls (P_0) was set at 20% (0.20), a conservative estimate aligned with similar epidemiological studies and methodological recommendations when local data are unavailable (Hajian-Tilaki, 2013).

Using standard statistical parameters - 80% power (1-β) and a 0.05 significance level (α, two-tailed) - the calculation yielded a required sample size of 83 cases and 83 controls (total N=166). This sample size provides sufficient power to detect the specified effect size while accounting for potential confounding factors in the analysis of prostate cancer risk factors. The selected parameters and resulting sample size ensure the study's robustness in examining associations between exposures and clinical outcomes.

3.7.2 For a cross-sectional study

For a cross-sectional study design to determine ERG/PTEN tumour subtypes and gene mutation, the sample size was determined using Cochran`s formula for sample size determination. The sample size was calculated using Cochran`s formula, and adjustments were made using the finite correction factor for the finite population as follows;

$$n_0 = \frac{z^2 P(1-p)}{d^2}$$

where n_0 is sample size for the infinite population, z is the confidence interval at 95% taken at 1.96, and P is the prevalence of prostate cancer, taken, where Globocan

shows a global prevalence of 7.3% (Sung *et al.*, 2021), and **d** is the level of precision or statistical significance, which is set at $0.0365 = \frac{7.3\%}{2}$ to prevent the 95% CIs of the estimated prevalence with irrelevant negative lower-bound values or larger than 1 upper-bound values (Liu *et al.*, 2019). Therefore,

$$n_0 = 1.96^2 \frac{0.073 \times 0.927}{(0.0365)^2} = 195\ patients$$

Adjusting for the Infinite correction factor, where N is the number of patients who are eligible for mutation and enrollment **(N** = 120 patients)

$$n = \frac{n_0}{1 + \frac{n_0 - 1}{N}} = \frac{195}{1 + \frac{195 - 1}{120}} = 75\ Patients$$

Accounting for 10% Dropouts = $\frac{75}{1-0.1} = 83\ patients.$

For the determination of ERG/PTEN tumour molecular subtypes, 83 cases were analysed. From this initial cohort, a subset of 30 cases was selected for targeted next-generation sequencing (NGS) based on the National Comprehensive Cancer Network (NCCN) guideline criteria for high-risk or genetically predisposed prostate cancer. This selective approach ensured that the sequenced cases were clinically relevant and aligned with established prognostic markers.

3.7.3 Questionnaire Administration for Risk Factor Data Collection

A standardised questionnaire (Czorny *et al*., 2017; Mukherjee *et al.*, 2021) was used to collect data on known and unknown risk factors from 166 participants, comprising 83 cases and 83 controls. After obtaining informed consent (Appendix 7), the questionnaire was administered to participants in a private setting to ensure confidentiality and comfort. The questionnaire was pre-tested to validate its content and structure, focusing on relevant risk factors such as demographic characteristics, dietary and lifestyle behaviours, medical conditions, and environmental exposures

(Appendix 5). Trained interviewers administered the questionnaire uniformly to minimise bias and ensure consistency in data collection. Responses were recorded systematically, and any incomplete or unclear answers were clarified during the interview process. The collected data were then organised for subsequent analysis to compare risk factor prevalence between the case and control groups.

3.7.4 Clinical Data Collection

A clinical data collection tool (Appendix 6) was used to collect medical-related information from hospital records. The tool was developed to capture variables including medication history, comorbid medical conditions, prostate cancer screening/diagnosis documentation, pre-operative prostate-specific antigen (PSA) levels, and body mass index (BMI) at diagnosis, with pre-testing conducted to validate its alignment with clinical documentation practices and ensure clarity of variable definitions. Trained research assistant (data abstractor) underwent instruction in medical terminology, tool navigation, and record interpretation. Data collection prioritised primary sources such as admission/discharge summaries, pathology reports, and laboratory results, with ambiguous entries (e.g., incomplete PSA values) resolved through direct clinician verification. Quality assurance measures included random audits of abstracted data against source records. Ethical compliance was ensured through anonymisation of patient identifiers and adherence to institutional review board protocols, with access restricted to authorised personnel. This structured approach mitigated risks of incomplete documentation while maintaining fidelity to clinical realities, enabling robust analysis of risk factor associations.

3.8 Sample collection

3.8.1 Archived Tissue Sample Collection

Archived formalin-fixed, paraffin-embedded (FFPE) prostate tissue blocks were retrospectively collected from the histopathology archives of the Ocean Road Cancer Institute (ORCI) and Muhimbili National Hospital (MNH) for the determination of *ERG* protein expression and *PTEN* loss using immunohistochemistry (IHC). Only tissue samples from consented participants who met the inclusion criteria for the case group were included in the analysis. The FFPE blocks were handled with care to ensure antigen preservation; they were stored at room temperature in airtight containers to prevent antigen degradation until the IHC staining procedure was performed. This procedure ensured the integrity of the samples, enabling reliable immunohistochemical analysis to determine molecular tumour subtypes associated with prostate cancer.

3.8.2 Dried Blood Spot (DBS) Collection

Enabling high-throughput analysis of genes linked to oncogenic risk. Venous blood samples (50–100 µL) were collected via sterile venipuncture and applied to designated circles on Dried Blood Spot (DBS) cards, followed by air-drying at room temperature for 4 hours to ensure sample stability. DBS cards were subsequently stored in sealed plastic bags containing desiccant packs at room temperature until genomic DNA extraction. Quality control measures were implemented, with sample rejection for hemolysis or incomplete saturation to ensure optimal DNA integrity and sequencing accuracy. This procedure prioritised precision in sample handling and storage, critical for the reliable detection of low-frequency germline variants.

3.9 Ethical and Logistical Protocols

To ensure ethical compliance and logistical efficiency throughout the study. Anonymisation was achieved by labelling samples with unique alphanumeric codes, effectively unlinking personal identifiers from research data to protect participant confidentiality. For sample transportation, all collected FFPE blocks were transferred to the Muhimbili National Hospital (MNH) histopathology laboratory for *ERG* and *PTEN* immunohistochemistry analysis, while DBS cards were sent to the DNA laboratory at the Government Chemistry Laboratory Authority (GCLA) for DNA extraction and quantification before shipping extracts for NGS to the Inqaba laboratory. Study participants provided consent for sample shipment to Ingaba for next-generation sequencing (NGS). Additionally, authorisation for sample transfer was secured upon completion of both a Material Transfer Agreement (MTA) and a Data Transfer Agreement (DTA). To maintain sample integrity, all transfers were conducted using temperature-controlled containers, ensuring that the biological specimens remained stable during transportation.

3.10 Research study workflow

Following institutional ethical approval and authorisation, a research assistant was trained on standardised data collection tools, study objectives, and ethical compliance protocols to ensure adherence to methodological rigour and participant rights. Participants were recruited after providing informed consent, with cases and controls were administered a standardised questionnaire to systematically collect risk factor data, forming the first objective. Concurrently, clinical data collection from hospital records captured medically relevant variables, providing complementary insights into risk associations. For consented cases, archived tissue blocks were retrieved and subjected to *ERG/PTEN* IHC analysis to determine *ERG/PTEN* tumour molecular

subtypes, addressing the second objective. Cases meeting National Comprehensive Cancer Network (NCCN) guidelines for germline genetic testing underwent DBS sampling, followed by NGS to characterise mutation spectra, fulfilling the third objective. This sequential workflow (Figure 3.2) was a key to ensuring the study was conducted to achieve all research objectives while maintaining methodological integrity. Each phase's necessity stemmed from its role in addressing specific research gaps: the questionnaire and clinical data provided information related to Prostate cancer risk, IHC-identified *ERG/PTEN* tumour subtypes, and NGS-elucidated genetic predisposition mechanisms. By integrating these approaches, the study achieved comprehensive data, enhancing the validity of findings and aligning with research objectives.

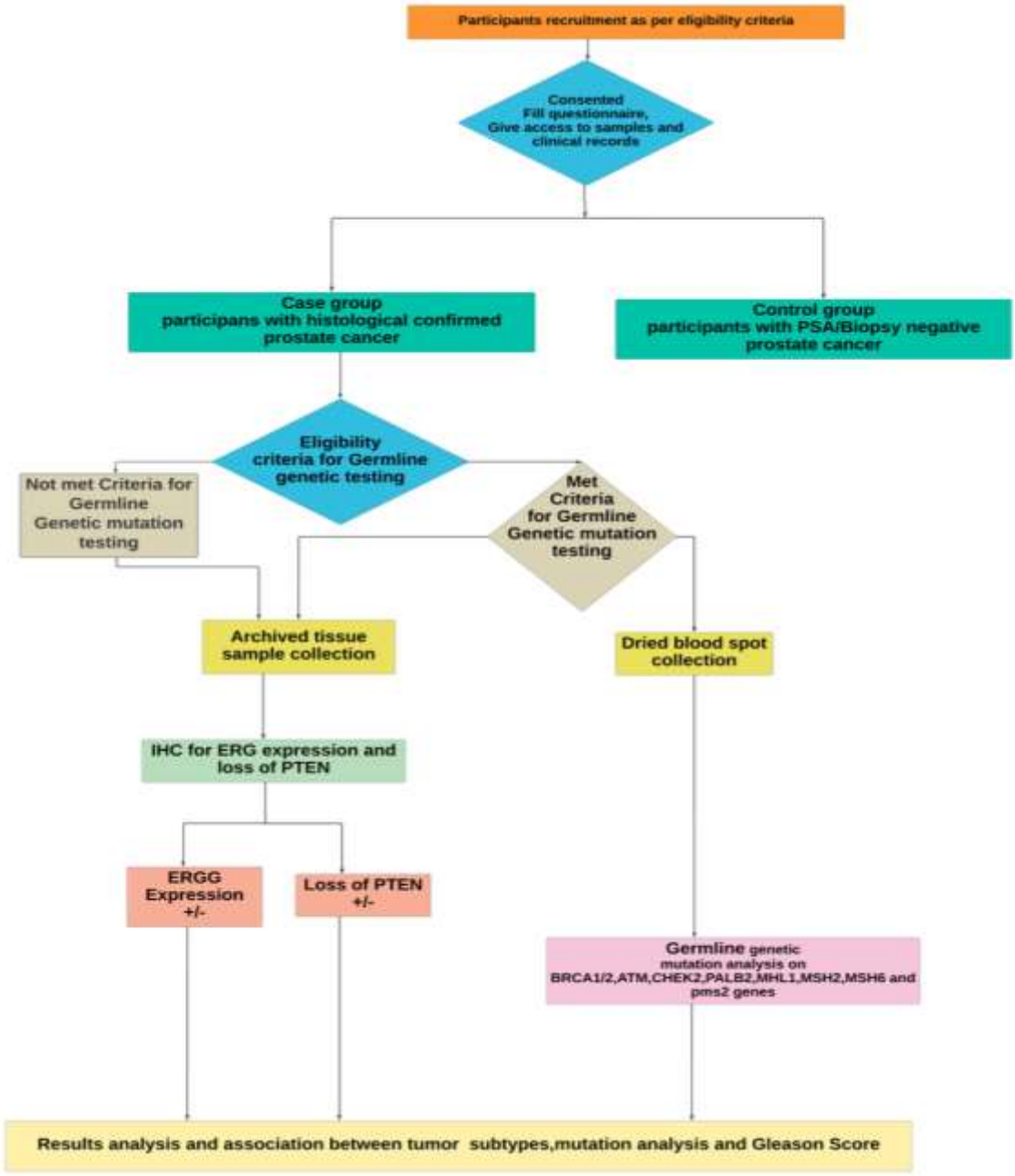


Figure 3.2: Research study workflow procedures

3.11Laboratory Methods

3.11.1 DNA Extraction and Quantification

Genomic DNA was extracted from dried blood spot (DBS) samples using the QIAamp DNA Mini Kit (QIAGEN, 2016) following the manufacturer's instructions. Briefly, six punched-out circles from each DBS were placed into a 1.5 mL microcentrifuge tube, and 180 µL of Buffer ATL was added, followed by incubation at 85°C for 10 minutes. After a brief centrifugation to remove residual droplets from the lid, 20 µL of Proteinase K was added, mixed by vortexing, and incubated at 56°C for one hour. Subsequently, 200 µL of Buffer AL was added, mixed thoroughly, and incubated at 70°C for 10 minutes, followed by another brief centrifugation.

Ethanol (200 µL, 96–100%) was then added to the lysate, and the mixture was vortexed thoroughly. The solution was carefully transferred to a QIAamp Mini spin column (without wetting the rim) and centrifuged at 6,000 × g for 1 minute. The spin column was then transferred to a fresh 2 mL collection tube, and the filtrate was discarded. Next, 500 µL of Buffer AW1 was added to the column, centrifuged at 6,000 × g for 1 minute, and the filtrate was discarded. This was repeated with 500 µL of Buffer AW2, followed by centrifugation at 20,000 × g for 3 minutes to ensure complete drying of the membrane. To eliminate potential buffer carryover, the column was placed in a new 2 mL collection tube and centrifuged at full speed for an additional minute.

Finally, the spin column was transferred to a clean 1.5 mL microcentrifuge tube, and 150 µL of Buffer AE (or nuclease-free water) was added. After a 1-minute incubation at room temperature, the column was centrifuged at 6,000 × g for 1 minute to elute the DNA. The extracted genomic DNA was quantified using a Nanodrop™ 2000 Spectrophotometer (Thermo Scientific, 2000), and stored at –20°C until shipment to

Ingaba Biotechnical Industries (Pty) Ltd, South Africa, for downstream next-generation sequencing (NGS) analysis.

3.11.2 Next-Generation Sequencing and Bioinformatics Analysis

3.11.2.1 Library Preparation

Library preparation was performed using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina (NEB, 2024). This kit is designed for PCR-free workflows and offers reliable fragmentation and library preparation without amplification bias, making it suitable for a variety of DNA samples, including those with high GC content or FFPE DNA. DNA was fragmented enzymatically to produce fragments of 270–300 bp, ensuring compatibility with sequencing platforms (NEB, 2024). Adapter sequences were dA-tailing ligated to the ends of the fragments to facilitate binding to the sequencing platform. This step is streamlined to combine fragmentation, end repair, and dA-tailing without intermediate clean-up steps, minimising sample loss and reducing hands-on time (NEB, 2024). Size selection was conducted using bead-based methods to ensure uniformity and minimise unwanted artefacts. The prepared libraries were quantified using Qubit and diluted to the target concentration range of 8 -10 pM , before undergoing paired-end sequencing by synthesis using the MiSeq sequencer (NEB, 2024)(Illumina, 2019)

3.11.2.2 Targeted Next-Generation Sequencing

Targeted next-generation sequencing (NGS) was employed to analyse specific genomic regions of interest. Targeted amplification of the coding regions and splicing sites of *BRCA1/2, ATM, PALB2, TP53, CHEK2, MLH1*, *MSH2, MSH6*, and *PMS2* genes was performed using the designed Onco Risk panel for Illumina (Illumina, 2019). This process involved enriching these regions through hybridisation probe techniques, ensuring that only the regions of interest were sequenced. The enriched

libraries were sequenced on an Illumina platform, producing raw sequence reads in FASTQ format (Bentley *et al*., 2008; Illumina, 2019)

3.11.3 Quality assessment

Quality control assessments of the raw reads were conducted using FastQC to evaluate key read quality metrics, including Phred scores, GC content, and adapter contamination (Andrews, 2024). Phred scores, which measure the accuracy of base calls, were assessed to ensure that the majority of reads had high-quality scores, typically above 30. GC content analysis helped identify any biases in sequencing that might affect downstream analyses. Additionally, FastQC checked for adapter contamination. low-quality reads were trimmed using the Trimmomatic tool (Bolger *et al*., 2014).

3.11.4 Sequence Alignment and Data Annotation

The MiSeq sequencer's local run manager analysed the base quality and amplicon coverage of the raw reads (Illumina, 2019). Reads passing a Phred quality score threshold of 30 were aligned to the human reference genome hg19/GRCh37 using BWA-MEM version 0.7.9a-i-sis-1.0.2 (H. Li & Durbin, 2009). Alignment files were stored in BAM format for further processing. Variants such as single-nucleotide polymorphisms (SNPs) and insertions/deletions (indels) were identified using Pisces Variant Caller version 5.2.9.23 (Ahmed *et al.,* 2024; Tariq *et al.,* 2021). Data visualisation tools, Integrative Genomics Viewer (IGV), were employed for manual inspection of aligned reads and detected variants. The identified variants were annotated with functional information using the curated databases ClinVar and dbSNP to link genetic changes to potential phenotypic effects or disease associations (DePristo *et al.,* 2011).

3.11.5 Sequence Data Interpretation

The interpretation of genetic variants was performed using curated databases and bioinformatics tools. Variants were categorised as pathogenic, likely pathogenic, or variants of uncertain significance (VUS) based on ClinVar annotations and cancer risk analysis conducted with the Annotated Cancer Risk Variant Analysis Tool (CRAVAT). Sorting Intolerant From Tolerant (SIFT) was used to assess amino acid substitutions, classifying them as damaging or tolerated based on a threshold score of 0.05 (Sim *et al., 2012)* Additionally, Variant Effect Predictor (VEP) provided functional predictions for gene and protein alterations (McLaren *et al.*, 2016; Sim *et al.*, 2012). The interpretation process adhered to guidelines established by the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (Richards *et al.*, 2015). This comprehensive approach enabled the identification of clinically significant genetic variations and provided insights into their implications for disease diagnosis and treatment.

3.11.6 Immunohistochemistry Assay for *ERG* and *PTEN*

Immunohistochemistry procedures were performed on the Ventana Benchmark GX automated slide stainer Machine using Rabbit anti-*ERG* (MD 188R) and Mouse anti-*PTEN* (6H2.1) antibodies (Maiques *et al.*, 2014). Formalin-fixed paraffin-embedded tissue blocks were sectioned at 3 µm thickness and mounted on positively charged slides (Dako Flex IHC Microscopic Slide, code K8020), which were then heated at 55°C in a hot air oven for 2 hours before staining. For each case, two slides were sectioned and labelled *ERG* and *PTEN* (Maiques *et al.*, 2014).

Deparaffinization, rehydration, and antigen retrieval were carried out on the Ventana Benchmark GX automated slide stainer (VSS v12.5.4 software) using EZ Prep (Ventana 950-102), reaction buffer (Ventana 950-300), and Cell Conditioning 1

(CC1) solutions (Ventana 950-124) (Atlas Antibodies, 2017; Ilie *et al.*, 2017). Antigen retrieval was performed using a heat-induced epitope retrieval technique for 32 minutes at 100°C for the *ERG* antibody and 40 minutes at 100°C for the *PTEN* antibody. During this process, the CC1 buffer was heated to 100°C to break protein cross-links formed during fixation, allowing the antibodies better access to the antigenic sites and improving staining quality and specificity.

Endogenous peroxidase activity was blocked by incubating the slides with Optiview Peroxidase Inhibitor (Ventana 253-4578) for 4 minutes at 37°C (Maiques *et al.*, 2014). The slides were then incubated with Rabbit anti-ERG primary antibody (Medaysis, Clone MD188R) at a dilution of 1:100 for 40 minutes, and Mouse anti-PTEN primary antibody (Medaysis, Clone 6H2.1) at a dilution of 1:50 for 60 minutes. Both antibodies were diluted with Ventana antibody diluent (251-018, Ventana Medical Systems, Inc., Tucson, Arizona) (Leng *et al*., 2019; Rizzardi *et al.*, 2014).

Visualisation was performed using the Ventana Optiview DAB IHC detection kit (760-700, Ventana Medical Systems, Inc., Tucson, Arizona), followed by counterstaining with Ventana Hematoxylin II reagent (Ventana 790-2208) for 8 minutes and bluing in bluing reagent (Ventana 760-2037) for 4 minutes. The slides were then dehydrated in ethanol, cleared in xylene, and covers lipped using a Sakura SC 200 cover slipper (Maiques *et al.*, 2014).

Each IHC run included a positive control (known positive *ERG* and *PTEN* prostate tissue section on slide) and a negative control slide, where the primary antibody was substituted with a buffer solution.

3.11.7 Microscopic Slide Evaluation for *ERG* and *PTEN* Expression

ERG nuclear staining and *PTEN* nuclear and cytoplasmic staining were analysed objectively and qualitatively. While the percentage of positively stained cells scored as a continuous variable, the intensity of staining was graded as follows: no staining (0), weak (1 +), intermediate (2 +), and strong (3 +). The proportion of tumour cells and the staining intensity were graded on a scale of 0 to 3 and were also used to assess whether the results were positive or negative. *ERG* or *PTEN* expression was graded as either negative (no staining), weak (1+), only visible at high magnification, intermediate (2 +), noticeable at low magnification, or strong (3 +), exceptional at low magnification (Donahoe, 2012; Hashmi *et al*., 2019). Two independent expert pathologists who were blinded to the clinical characteristics of the patients examined both the histological and IHC-stained tissue slides. The stained slides were assessed independently by two skilled pathologists to reduce inter-observer variability. To maintain scoring consistency, disagreements over interpretation were settled through collaborative review. Pre-operative PSA levels and clinicopathological factors, including Gleason scores retrieved from clinical records, were combined with the IHC analysis data for statistical analysis.

3.12 Data Management and Analysis

3.12.1 Data Management Plan

Histological and clinical data of consented patients were acquired from patient's records at ORCI/MNH while a standardized questionnaire was used to collect information related to sociodemographic characteristics, dietary frequency intake, lifestyle behaviour, family history with prostate cancer, exposure to diseases (Czorny *et al*., 2017; Mukherjee *et al.*, 2021). The written questionnaire was then entered into the Redcap Database for validation checks, data quality checks, accuracy,

completeness of data, and storage. Source documents were used to clean erroneous and missing data. All hard copies were kept in locked cabinets at all times to ensure the confidentiality and integrity of study data. In ensuring data quality, proactively identifying potential challenges, and facilitating resolutions, a data review meeting was held every two weeks to assess the recruitment rate, accuracy of the data, and overall data management process. The meeting involved the principal investigator, supervisors, and the data management team. All study data were backed up on a study hard drive every week. De-identified final datasets were used for analysis by the principal investigator and exported to R statistical software for analysis.

3.12.2 Data Analysis Plan.

3.12.2.1 Descriptive Statistics.

The data was checked for missing values and organised into tables. It was then summarised using frequency tables and histograms, allowing for both a numerical and visual assessment of the data distribution. Continuous variables were tested for normality using the Shapiro-Wilk test to determine if they followed a Gaussian distribution. Variables that met this criterion were summarised using the mean and standard deviation. Binary and categorical variables were analysed by calculating frequency counts and percentages.

Tumour molecular subtypes, based on ERG and PTEN IHC expression, were also summarised using descriptive statistics. The distribution of these subtypes was presented in a frequency table and histogram, offering a clear overview of their prevalence within the dataset. The results from the descriptive statistics were interpreted within the context of the research question.

3.12.2.2 Inferential Statistics.

Inferential statistics were conducted based on the specific objective of the study and outcome of interest as described below;

a) To determine the associated risk factors, a Generalised Linear Model (GLM) with a binomial error structure and a logit link function was used to evaluate Prostate cancer incidence as a function of several risk factors. The response variable was dichotomous, i.e. present (1) and absent (0). Second, we performed multivariate GLM for variables in each variable group by undertaking analyses on all permutations of all possible covariate combinations, and the best model was selected based on Akaike information criteria (AIC) using the MuMIn R package (K, 2023). For the best model in each covariate group, the area under the curve (AUC) of the receiver operating characteristic (ROC) curve was calculated, as it provides an overall measure of model fit. ROC analysis is a powerful tool for assessing the predictive performance of risk factors. We also determined several pseudo-R^2 for the fitted model to assess fit, including McFadden's R^2, Maximum likelihood pseudo-R^2, Nagelkerke's $R^{2,}$ and the Coefficient of Discrimination D. For example, McFadden R^2 of 0.2–0.4 is considered an excellent fit (Hensher & Stopher, 2021). Several measures of model fit and model selection, including AIC, AUC, and Pseudo R^2 statistics, were used to rank the importance of the different risk factor groups for prostate cancer in a Tanzanian male patient population (Team, 2023). Odd ratios were presented at 95% CI (p-value, 0.05) after adjusting for confounding variables.

b) Chi-square analysis was used to determine the association between ERG expression, gene Mutation and Gleason Score.

3.12.2.3 Bioinformatics sequence data Analysis

a) Variant Annotation and Functional Prediction

Bioinformatics tools were employed to annotate genetic variants and predict their functional impact using specialised algorithms and databases. The Cancer-Related Analysis of Variants Toolkit (CRAVAT) was used to prioritise cancer-associated mutations by integrating genomic data with clinical evidence, including tumour suppressor genes and oncogenes. This approach enabled the identification of mutations linked to cancer risk and therapeutic targets.

The Ensembl Variant Effect Predictor (VEP) provided comprehensive functional predictions by annotating variants with molecular consequences (e.g., splice site disruptions, missense mutations) and cross-referencing data from ClinVar, gnomAD, and pathogenicity prediction tools. VEP categorised variants based on their potential to alter gene expression or protein structure, facilitating the prioritisation of clinically relevant variants.

b) Pathogenicity Prediction Using SIFT

The Sorting Intolerant From Tolerant (SIFT) algorithm evaluated amino acid substitutions by analysing evolutionary conservation and physicochemical properties. Substitutions were classified as damaging (score ≤ 0.05) or tolerated (score > 0.05), reflecting their likelihood to disrupt protein function. This threshold-based approach ensured the systematic identification of likely benign, benign, and likely pathogenic variants, particularly in conserved protein domains, thereby enhancing the reliability of pathogenicity predictions. These tools collectively enabled the identification of high-impact variants by integrating genomic, structural, and clinical data.

3.12.3 Statistical Software

All the analyses were conducted using the R statistical software version 4.4.2 (Team, 2023). R scripts are also attached as an appendix for the reproducibility of the results.

3.13 Ethical Consideration

Ethical approval for the research work was obtained from both the Institutional Research and Ethical Committee (IREC) of MTRH/MOI University (Appendix 8) as well as from the National Health Research Ethics Sub Committee of the National Institute of Medical Research (NIMR), Institutional Review Board of Ocean Road Cancer Institute and Muhimbili National Hospital Tanzania (Appendix 9). All information provided orally by participants or, information received from hospital records was treated with confidentiality. There was no coercion or inducements used to attract participants. Patients were asked to sign a consent form (Appendix 7), and those who opted not to participate were not affected by how they were being treated.

CHAPTER FOUR

4.0 RESULTS

4.1 Participants' socio-demographic characteristics

In the current study, among all 166 enrolled participants, the mean Age at diagnosis for the control group was 68.87 years (SD ±7.20), and the case group was slightly older at 71.77 years (SD ±8.53). Monthly income, measured in Tanzanian Shillings (TZS), highlights notable disparities between the groups. While most participants in both groups earn up to 300,000 TZS per month, higher incomes are more prevalent in the control group. Education levels also vary significantly, with participants categorised into the following groups: diploma, primary, secondary, university degree, or no education. The control group has a higher proportion of individuals with lower education levels(having no degree and diploma) compared to the case group. Marital status also differs, with 40.65% of controls being married compared to 59.35% in the case group (Table 4.1).

Table 4.1: Participants' socio-demographic and biological characteristics

Variable	**Unit of measurement**	**Control Group (Mean± SD)**	**Case Group (Mean± SD)**	
Age	(Years)	68.87±7.20	71.77±8.53	
Height	Meters (M)	1.68±0.07	1.66±0.07	
Weight	Kilogram (Kg)	68.0±6.64	69.19±9.60	
Number of Children		4 ±2	5±2	
Variable	**Status**	**Control Group n(%)**	**Case Group n (%)**	**Total N(%)**
Marital status (groups collapsed)	Married	50 (40.65)	73 (59.35)	123 (74.56)
	Other	33 (76.74)	10 (23.26)	43 (25.44)
Education level (Grouped)	Higher	20 (48.78)	21 (51.22)	41 (26.04)
	Lower	63 (50.40)	62 (49.60)	125 (73.96)
Occupation	Agriculture	13 (50.00)	13 (50.00)	26 (15.98)
	Business entrepreneur	16 (45.71)	19 (54.29)	35 (21.30)
	Employed	12 (60.00)	8 (40.00)	20 (12.27)
	Peasant	16 (55.17)	13 (44.83)	29 (17.84)
	Retiree	26 (46.43)	30 (53.57)	56 (34.57)
Monthly Income in 1000s of Tanzanian Shillings	Up to 300	39 (42.86)	52 (57.14)	91 (54.49)
	301 - 800	24 (47.06)	27 (52.94)	51 (30.54)
	801 - 1,500	13 (81.25)	3 (18.75)	16 (9.58)
	1, 500 - 2,000	5 (100.00)	0 (0.00)	5 (2.99)
	2, 001 and greater	2 (66.67)	1 (33.33)	3 (1.80)
Children	Absent	34 (41.98)	47 (58.02)	81 (48.52)
	Present	49 (57.65)	36 (42.35)	85 (51.48)

4.1.1 Risk factors associated with prostate cancer among cancer patients

The sociodemographic factors of Marital status, income, age, and number of children were statistically significant in bivariate GLM analysis as factors influencing the incidence of prostate cancer. Prostate cancer incidence was found to be reduced with increased income, and older patients were found to have an elevated risk of developing prostate cancer compared to younger ones (COR = 1.05), while the incidence of prostate cancer increased with the number of children (COR = 1.17) (Table 4.2). In addition, the level of education, occupation, and body mass index were not found to be predictors for prostate cancer in bivariate analyses.

In a multivariate marital status, income, age, and level of education were found to predict the incidence of prostate cancer (AUC = 0.745, McFadden's R^2 =0.134, Maximum likelihood pseudo R^2 = 0.169, Nagelkerke's R^2 = 0.226, Coefficient of Discrimination D = 0.173. The incidence of Prostate cancer presented a substantial reduction with increasing monthly income (AOR = 0.51, 95% CI: 0.305–0.800). Moreover, older patients had an elevated risk of developing Prostate cancer compared to their younger counterparts (AOR = 1.05, 95% CI: 1.006–1.097) (Table 4.2). Furthermore, the level of education was an important risk factor for prostate cancer. A higher incidence of diseases was found among individuals with a higher education level (a diploma and higher) compared to those who attained a lower education level (Secondary education and below) (AOR = 0.45,95% CI: 0.175- 1.032) (Table 4.2).

In addition, individuals who are not married are found with a reduced risk of developing prostate cancer compared with individuals who are married (Divorced, widowed, single) (AOR=0.23). The summary marginal effects of socio-demographic variables such as age, marital status, educational attainment, and income on the incidence of Prostate cancer among patients are presented in Figure 4.1.

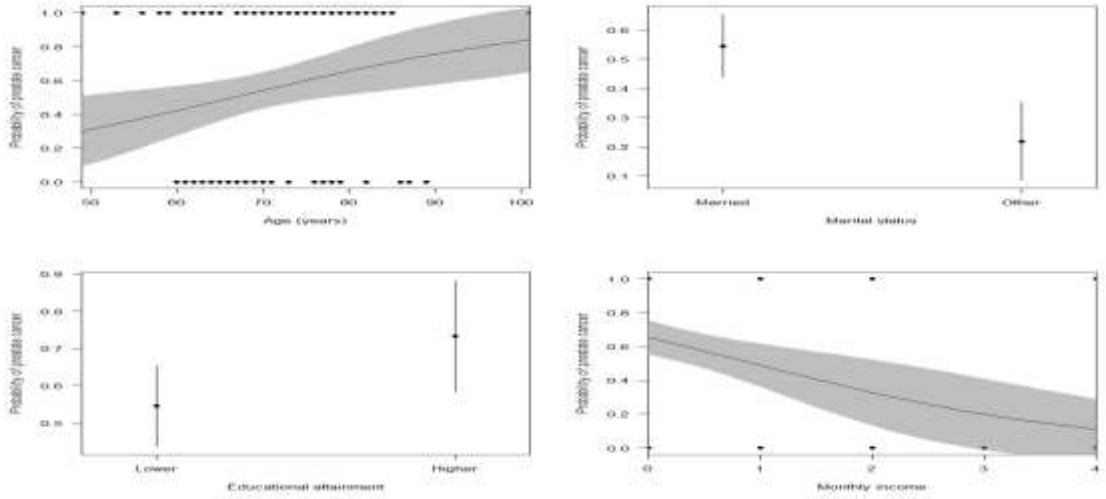


Figure 4.1: The marginal effects of socio-demographic variables

Table 4.2. Results of Bivariate and multivariate analysis for sociodemographic variables

	Bivariate analysis					Multivariate analysis				
Variables	**Std. Error**	**z value**	**COR**	**P-value**	**95%CI**	**Std. Error**	**Z statistic**	**AOR**	**P-value**	**95%CI**
Other cf. Married	0.394	-3.770	0.23	0.0002	0.01-0.05	0.418	-3.510	0.23	0.0005	0.10-0.52
Monthly income	0.196	-2.960	0.56	0.0030	0.04-0.08	0.243	-2.710	0.52	0.0067	0.32-0.84
Age (years)	0.020	2.440	1.05	0.0150	11.03-11.93	0.021	2.300	1.05	0.0213	1.01-1.09
Number of children	0.063	2.530	1.17	0.0120	11.10-14.20					
Children present, compared with.					0.78-9.86					
Children absent	0.647	1.020	1.94	0.3100						
Body Mass Index	0.027	-0.820	0.98	0.4100	0.42-0.46					
Lower education compared with Higher					0.45-1.76					
Education	0.351	-0.120	0.96	0.9000		0.450	-1.770	0.45	0.0764	0.19-1.09

4.1.2 Lifestyle risk factors

Among recruited respondents from both MNH and ORCI, the high frequency of cases (88.24%) reported drinking alcohol 3-4 times a week before prostate cancer diagnosis, while the majority (66.34%) of the respondents' control group reported never taking alcohol. Similarly, the (39.47%) of respondents' cases were characterised by high tobacco smoking behaviour, and 60.53%) nonsmokers represented control respondents (Table 4.3). Most of the recruited cases (84.0%) never engaged in physical exercise. However, the majority (85.0%) of respondents from the control group reported being engaged in physical activity 3-4 times a week (Table 4.3).

Table 4.3: Prostate cancer incidence across persons with different lifestyle choices

Variable	Variable Status	PCa Control Group n (%)	PCa Case Group n (%)	Total N (%)
Alcohol consumption (times/week)	Never	67 (66.34)	34 (33.66)	101 (60.00)
	Rarely	11 (55.00)	9 (45.00)	20 (11.88)
	1–2	3 (16.67)	15 (83.33)	18 (10.59)
	3–4	2 (11.76)	15 (88.24)	17 (10.00)
	5–7	0 (0.00)	10 (100.00)	10 (5.88)
Physical activity (times/week)	Never	8 (16.00)	42 (84.00)	50 (29.41)
	Rarely	21 (60.00)	14 (40.00)	35 (20.59)
	1–2	36 (65.45)	19 (34.55)	55 (32.35)
	3–4	17 (85.00)	3 (15.00)	20 (11.76)
	5–7	1 (16.67)	5 (83.33)	6 (3.53)
Tobacco smoking	Highly smoker	69 (60.53)	45 (39.47)	114 (67.06)
	Moderate smoker	7 (33.33)	14 (66.67)	21 (12.35)
	Rarely smoker	3 (16.67)	15 (83.33)	18 (10.59)
	Non-smoker	4 (30.77)	9 (69.23)	13 (7.65)
Bicycle riding (km/week)	Never	28 (35.90)	50 (64.10)	78 (45.88)
	Rarely	15 (65.22)	8 (34.78)	23 (13.53)
	1–50	31 (70.45)	13 (29.55)	44 (25.88)
	51–100	5 (35.71)	9 (64.29)	14 (8.24)
	101–150	4 (66.67)	2 (33.33)	6 (3.53)
	Above 150	0 (0.00)	1 (100.00)	1 (0.59)
Motorbike riding (km/week)	Never	71 (49.31)	73 (50.69)	144 (84.71)
	Rarely	2 (33.33)	4 (66.67)	6 (3.53)
	1–100	5 (62.50)	3 (37.50)	8 (4.71)
	101–200	1 (50.00)	1 (50.00)	2 (1.18)
	201–300	2 (50.00)	2 (50.00)	4 (2.35)
	Above 300	2 (100.00)	0 (0.00)	2 (1.18)

Bivariate models for the incidence of prostate cancer among urology patients seen at MNH and ORCI revealed that the frequency of alcohol consumption (COR = 2.75) and tobacco smoking (COR = 1.36) increased the incidence of cancer (Table 4.4). These two factors were statistically significant in bivariate models as factors influencing the incidence of prostate cancer. Patients with a higher frequency of physical activity, track driving, and bicycle riding had a reduced incidence of prostate cancer. Also, the frequency of sexual activity and motorbike riding had no influence on the incidence of cancer among urology patients from a bivariate model context (Table 6). Additionally, multivariable analyses found the most parsimonious model clarifying the incidence of Prostate cancer among case participants was cigarette smoking, alcohol consumption, and physical activity (AUC = 0.846, McFadden's R^2 = 0.292, Maximum likelihood pseudo R^2 = 0.333, Nagelkerke's R^2 = 0.444, Coefficient of Discrimination D = 0.365. Alcohol consumption was observed to be significantly linked with an elevated likelihood of incidence of Prostate cancer (AOR = 2.75, 95% CI: 1.87 - 4.390), while physical activity was associated with a reduction in the incidence of prostate cancer (AOR = 0.462, 95% CI: 0.315 - 0.656 as presented in Table 4.4, and marginal effects of lifestyle factors on the incidence of Prostate cancer is summarised in figure 4.2

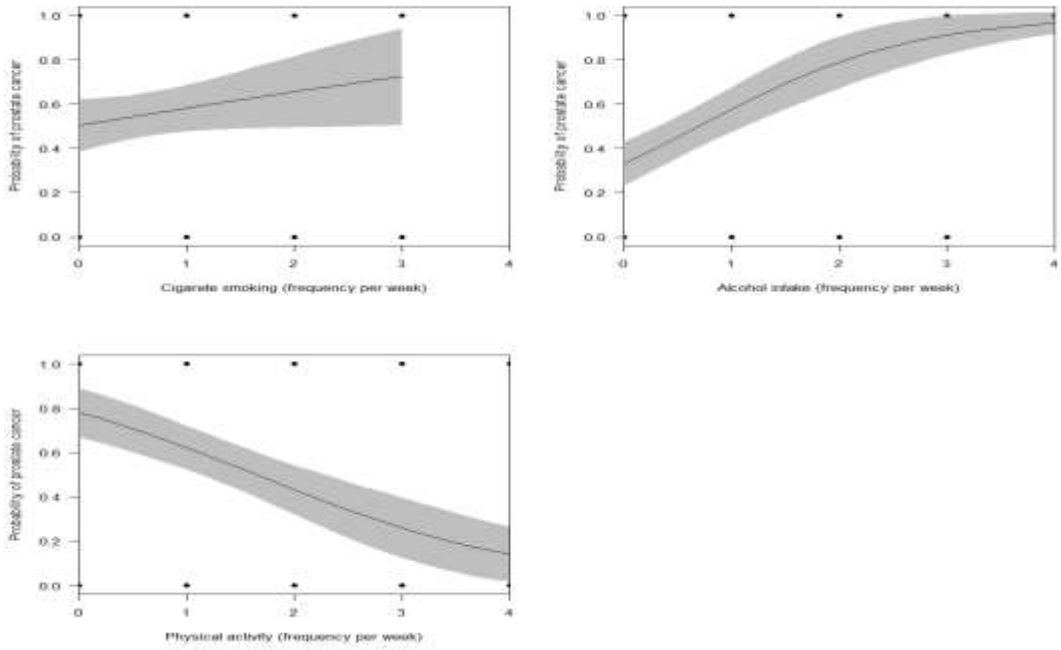


Figure 4.2: Marginal effects of lifestyle factors on the incidence of Prostate cancer

Table 4.4: Results of Bivariate and Multivariate analysis for lifestyle factors

	Bivariate Analysis					Multivariate analysis				
	Std. Error	**z value**	**P-value**	**COR**	**95%CI**	**Std. Error**	**z value**	**P-value**	**AOR**	**95%CI**
Alcohol	0.192	5.27	0.000	2.75	1.89, 4.01	0.215	4.7	0.000	2.75	1.80-4.19
Physical activities	0.154	-4.29	0.000	0.52	0.38, 0.70	0.186	-4.15	0.000	0.46	0.32-0.66
Tobacco smoking	0.191	3.65	0.000	2.01	1.38, 2.92	0.217	1.46	0.140	1.37	0.90-2.10
Track driving	0.600	-2.09	0.036	0.29	0.09, 0.92					
Bicycle riding	0.130	-2.02	0.043	0.77	0.60, 0.99					
Sexual activity	0.145	-1.75	0.081	0.78	0.58, 1.03					
Motor bike riding	0.1547	-0.49	0.620	0.93	0.68, 1.26					

4.1.3 Dietary risk factors

Among recruited participants, the high frequency of control group participants (93.75%) reported never consuming red meat, while the majority (84.38%) of the recruited cases reported consuming red meat 3-4 times a week before prostate cancer diagnosis. Similarly, the majority (66.67%) of control participants were characterised by high Soya consumption (3 -4 times a week), as compared with 87.50% of cases who rarely consume soya (Table 4.5). Most of the recruited cases (86.8%) rarely consume tomatoes. However, the majority (88.64%) of control group participants reported with high rate of tomato consumption 3-4 times a week (Table 4.5). Parallel to this, the present study reported high coffee consumption among control group participants compared to cases. The majority (51.85%) of Cases rarely consume coffee as compared to control participants (82.05%) who consume coffee 3- 4 times a week (Table 4.5).

Table 4.5: Prostate cancer incidence across persons with different dietary patterns

Variable	Variable Status	PCa Control group n (%)	PCa Case group n (%)	N (%)
Red meat	Never	30 (93.75%)	2 (6.25%)	32 (19.41%)
	Rarely	40 (70.18%)	17 (29.82%)	57 (34.12%)
	1-2 times/week	8 (22.86%)	27 (77.14%)	35 (21.18%)
	3-4 times/week	5 (15.63%)	27 (84.38%)	32 (19.41%)
	5-7 times/week	0 (0.00%)	10 (100.00%)	10 (5.88%)
Soy	Never	1 (16.67%)	5 (83.33%)	6 (4.12%)
	Rarely	3 (12.50%)	21 (87.50%)	24 (14.12%)
	1-2 times/week	33 (47.83%)	36 (52.17%)	69 (41.18%)
	3-4 times/week	40 (66.67%)	20 (33.33%)	60 (35.88%)
	5-7 times/week	6 (60.00%)	4 (40.00%)	10 (5.88%)
Tomato	Never	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Rarely	11 (13.92%)	68 (86.08%)	79 (47.65%)
	1-2 times/week	20 (71.43%)	8 (28.57%)	28 (17.06%)
	3-4 times/week	39 (88.64%)	5 (11.36%)	44 (26.47%)
	5-7 times/week	13 (86.67%)	2 (13.33%)	15 (8.82%)
Coffee	Never	8 (16.00%)	42 (84.00%)	50 (30.00%)
	Rarely	13 (48.15%)	14 (51.85%)	27 (15.88%)
	1-2 times/week	27 (67.50%)	13 (32.50%)	40 (23.53%)
	3-4 times/week	32 (82.05%)	7 (17.95%)	39 (23.53%)
	5-7 times/week	3 (30.00%)	7 (70.00%)	10 (5.88%)

Vegetables	Never	1 (25.00%)	3 (75.00%)	4 (2.35%)
	Rarely	3 (25.00%)	9 (75.00%)	12 (7.06%)
	1-2 times/week	19 (55.88%)	15 (44.12%)	34 (20.00%)
	3-4 times/week	27 (50.94%)	26 (49.06%)	53 (32.35%)
	5-7 times/week	33 (52.38%)	30 (47.62%)	63 (38.24%)
Dairy food	Never	9 (60.00%)	6 (40.00%)	15 (8.82%)
	Rarely	28 (50.91%)	27 (49.09%)	55 (33.53%)
	1-2 times/week	31 (54.39%)	26 (45.61%)	57 (34.71%)
	3-4 times/week	12 (41.38%)	17 (58.62%)	29 (17.06%)
	5-7 times/week	3 (30.00%)	7 (70.00%)	10 (5.88%)
Fish	Never	0 (0.00%)	4 (100.00%)	4 (2.35%)
	Rarely	18 (43.90%)	23 (56.10%)	41 (24.71%)
	1-2 times/week	36 (57.14%)	27 (42.86%)	63 (38.24%)
	3-4 times/week	13 (40.63%)	19 (59.38%)	32 (19.41%)
	5-7 times/week	16 (61.54%)	10 (38.46%)	26 (15.29%)
Green or black tea	Never	0 (0.00%)	2 (100.00%)	2 (1.18%)
	Rarely	1 (12.50%)	7 (87.50%)	8 (5.29%)
	1-2 times/week	17 (54.84%)	14 (45.16%)	31 (18.82%)
	3-4 times/week	53 (64.63%)	29 (35.37%)	82 (48.82%)
	5-7 times/week	12 (27.91%)	31 (72.09%)	43 (25.29%)
Legumes	Never	2 (100.00%)	0 (0.00%)	2 (1.18%)
	Rarely	7 (33.33%)	14 (66.67%)	21 (12.94%)
	1-2 times/week	10 (37.04%)	17 (62.96%)	27 (16.47%)
	3-4 times/week	61 (59.80%)	41 (40.20%)	102 (61.18%)
	5-7 times/week	3 (21.43%)	11 (78.57%)	14 (9.41%)
Grains	Never	0 (0.00%)	2 (100.00%)	2 (1.18%)
	Rarely	6 (50.00%)	6 (50.00%)	12 (7.65%)
	1-2 times/week	49 (75.38%)	16 (24.62%)	65 (38.82%)
	3-4 times/week	28 (32.18%)	59 (67.82%)	87 (52.35%)
Food containing fats	Never	38 (63.33%)	22 (36.67%)	60 (35.88%)
	Rarely	33 (60.00%)	22 (40.00%)	55 (32.94%)
	1-2 times/week	11 (30.56%)	25 (69.44%)	36 (21.76%)
	3-4 times/week	1 (6.67%)	14 (93.33%)	15 (9.41%)
Vitamin-mineral	Never	80 (50.31%)	79 (49.69%)	159 (94.12%)
	Rarely	3 (60.00%)	2 (40.00%)	5 (3.53%)
	1-2 times/week	0 (0.00%)	1 (100.00%)	1 (1.18%)
	3-4 times/week	0 (0.00%)	1 (100.00%)	1 (1.18%)

Furthermore, the bivariate GLM revealed that a high frequency of red meat consumption, consumption of fatty food, and consumption of grains were associated with a higher incidence of prostate cancer among patients. However, a higher frequency of tomato consumption, coffee, and soy consumption was associated with a reduced incidence of prostate cancer among patients (Table 4.6). The frequency of

vitamin-mineral supplementation, fish consumption, dairy food consumption, legume consumption, vegetable consumption, and green or black tea consumption did not influence the incidence of prostate cancer among patients in bivariate models (Table 4.6). In multivariate analysis red meat consumption, vitamin-mineral supplementation, soya, coffee, and tomato intake were found to be the best dietary patterns explanatory variables for the incidence of Prostate cancer (AUC = 0.962, McFadden's R^2 = 0.642, Maximum likelihood pseudo R^2 = 0.589, Nagelkerke's R^2 = 0.785, Coefficient of Discrimination D = 0.696. The best-selected multivariate model has shown that the frequency of red meat consumption (AOR = 7.884, 95% C.I.: 3.954 - 19.150) and vitamin-mineral supplementation (AOR = 3.179, 95% C.I.: 0.832 - 174.895) were positively associated with increased risk of prostate cancer. In contrast, the frequency of coffee (OR = 0.571, 95% CI: 0.360 - 0.874), soya (AOR = 0.285, 95% CI: 0.132 - 0.552), and tomato intake (AOR = 0.174, 95% CI: 0.079 - 0.330) was inversely associated with the increased risk of prostate cancer among patients as summarised in Table 4.6.

Table 4.6: Results of Bivariate and Multivariate analysis for dietary factors

	Bivariate analysis					Multivariate analysis				
Variable	**Std. Error**	**z value**	**P-value**	**COR**	**95%CI**	**Std. Error**	**z value**	**P-value**	**AOR**	**95% CI**
Red meat	0.230	6.56	0.0000	4.53	2.89 - 7.11	0.397	5.2	0.0000	7.88	3.62 - 17.16
Tomatoes	0.224	-6.87	0.0000	0.21	0.14 - 0.33	0.359	-4.87	0.0000	0.17	0.08 - 0.34
Coffee	0.142	-5.23	0.0000	0.48	0.36 - 0.63	0.223	-2.51	0.0121	0.57	0.37 - 0.88
Food containing fats	0.183	4.33	0.0000	2.21	1.54 - 3.16					
Soya	0.202	-4.02	0.0001	0.44	0.30 - 0.65	0.360	-3.48	0.0005	0.29	0.14 - 0.59
grains	0.244	2.87	0.0041	2.01	1.25 - 3.24					
Vitamin-mineral supplements	0.517	1.48	0.1400	2.16	0.78 - 5.95	1.455	0.8	0.4266	3.18	0.18 - 55.07
Fish	0.148	-1.44	0.1500	0.81	0.61 - 1.08					
Dairy food	0.153	1.41	0.1600	1.24	0.92 - 1.67					
Vegetables	0.152	-1.68	0.0930	0.77	0.57 - 1.04					
Legumes	0.199	-0.53	0.6000	0.90	0.61 - 1.33					
Green and black tea	0.171	-0.14	0.8900	0.98	0.70 - 1.37					

The summary of marginal effects of effects of food and dietary factors in prostate cancer shown in figure 4.3.

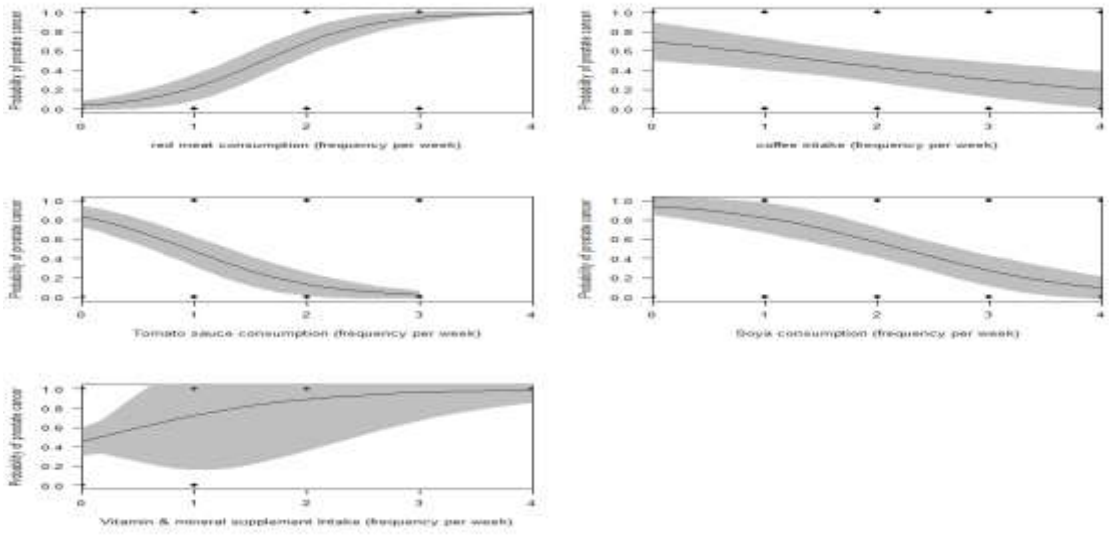


Figure 4.3: Marginal effects of food and dietary factors

4.1.4 Diseases

This study presents the disease factors linked to prostate cancer. Among all involved participants, it revealed a high frequency (68.75%) of the control group participants were diabetic, while a few (31.25%) of the diabetic participants were suffering from prostate cancer (Table 4.7). Similarly, among participants who reported a history of gonorrhoea infections, 97.59% were case participants and 2.41% were in the control group (Table 4.7).

Table 4.7: Prostate cancer incidence across participants with different disease statuses

Variable	Variable Status	Control Group n (%)	Case Group n (%)	N (%)
Diabetes	Negative	61 (45.52%)	73 (54.48%)	134 (80.0%)
	Positive	22 (68.75%)	10 (31.25%)	32 (20.0%)
Gonorrhea	Negative	81 (97.59%)	2 (2.41%)	83(49.70%)
	Positive	2 (2.41%)	81 (97.59%)	83 (50.30%)
Syphilis	Negative	81 (51.59%)	76 (48.41%)	157 (94.71%)
	Positive	2 (22.22%)	7 (77.78%)	9 (5.29%)
Other STD	Negative	83 (50.30%)	82 (49.70%)	165 (99.41%)
	Positive	0 (0.00%)	1 (100.00%)	1 (0.59%)

The bivariate modelling analysis revealed that a high frequency of diabetic participants was associated with reduced cancer incidence among participants. In contrast, the case participants with a high frequency of gonorrhoea were associated with an increased incidence of prostate cancer. However, syphilis and erectile dysfunction were not found to have an influence on prostate cancer among the recruited participants (Table 4.8). In line with this, the best multivariate analysis model included diabetes and gonorrhoea as the best disease-explanatory variables for the incidence of Prostate cancer in Tanzanian males (AUC = 0.614, McFadden's R^2 = 0.053, Maximum likelihood pseudo R^2 = 0.071, Nagelkerke's R^2 = 0.095, Coefficient of Discrimination D = 0.068. Exposure to gonorrhoea infections (AOR = 6.517, 95% CI: 1.710 - 42.749) was positively associated with an increased risk of prostate cancer (Table 4.8).

Table 4.8: Results of Bivariate and Multivariate analysis for disease factors

Variable	Bivariate analysis					Multivariate analysis				
	Std. Error	z value	P-valiue	Odds_Ratio	95% CI	Std. Error	z value	P-value	AOR	95%CI
Gonohrea	0.7764	2.53	0.011	7.11486	1.55- 32.58	0.783	2.49	0.013	7.045001	1.52- 32.54
Diabetic	0.398	-2.04	0.041	3.54375	0.20 - 0.97	0.407	-1.7	0.09	0.501455	0.22, 1.12
Syphylis	0.8171	1.55	0.12	3.54375	0.71- 17.56					
Erectil.Dys	0.432	1.32	0.19	1.772857	0.76 - 4.14	0.442	1.47	0.142	1.913899	0.80 - 4.58

Similarly, the Marginal effects of the influence of disease exposure on prostate cancer positivity are presented in Figure 6.

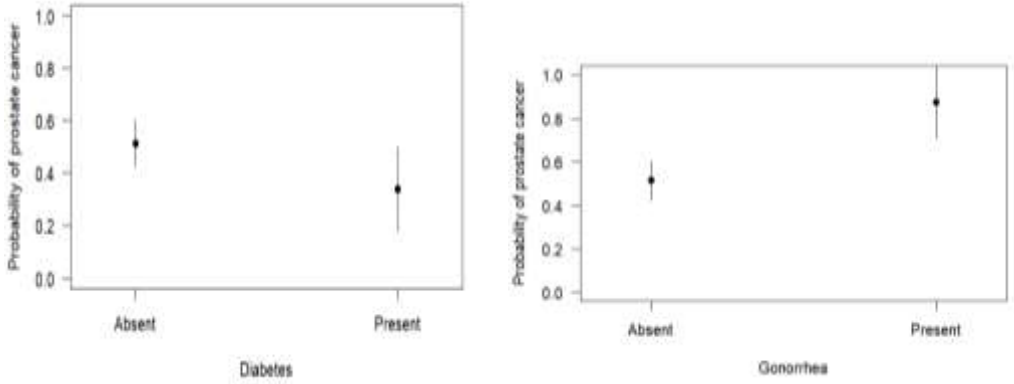


Figure 4.4: Marginal effects for the influence of disease exposure on prostate cancer

4.1.5 Family Background with Cancer

In assessing the aetiology of prostate cancer, the role of familial cancer history warrants a thorough investigation, as it provides critical insights into genetic predispositions and shared etiological factors. This study presents the association between a family history of various malignancies and the risk of developing prostate cancer using recruited participants from MNH and ORCI. Among recruited participants, the majority had a family history of prostate cancer. For case group participants, 76.19% had a family history of prostate cancer, and those cases without a family background with Prostate cancer were 23.17%. However, 76.83% of the control group participants had no family history of prostate cancer. (Table 4.9). Among all participants with a Family history of either Breast, Ovarian, Colorectal, or pancreatic Cancer,9.52% were not diagnosed with prostate cancer as compared with 90.48% with prostate cancer. However, case participants with a family history of any other type of cancer apart from either Prostate, Breast, Ovarian, Colorectal, or pancreatic were 86.9% of all recruited case group participants (Table 4.9).

Table 4.9: Prostate cancer incidence across participants with different family history of Cancer

Variable	Variable Status	PCa control group n (%)	PCa case group n (%)	N(%)
Family history of PCa	No	63 (76.83)	19 (23.17)	82(49.40)
	Yes	20 (23.81)	64 (76.19)	84 (50.60)
Family history of any cancer	No	4(4.88)	78 (95.12)	82(49.40)
	Yes	11 (13.1)	73 (86.9)	84 (50.60)
Family history of breast, ovarian, colorectal, and pancreatic cancer	No	0 (0.0)	82 (100)	82(49.40)
	Yes	8.0 (9.52)	76 (90.48)	84 (50.60)
Variable	**Variable status**	**Control Group n(%)**	**Case Group n(%)**	**Total (N)**
Family History with PCa				
	No	78(95.12)	4(4.88)	82
	Yes	63(74.12)	22(25.88)	85
Family History with any type of Ca				
	No	78(95.12)	4(4.88)	82
	Yes	73(85.88)	12(14.12)	85
Family history with Breast, Ovarian, Colorectal and pancreatic cancer				
	No	82(100)	0(0.00)	82
	Yes	77(90.59)	8(9.41)	85

The Bivariate GCLM model for the incidence of prostate cancer among urology patients seen at MNH and ORCI revealed that the family history of prostate cancer (OR = 6.81) increased the incidence of cancer (Table 4.10). However, other cancer types, including Breast, Ovarian, Colorectal, or pancreatic, had no influence on the incidence of prostate cancer among urology patients seen at MNH and ORCI based on our bivariate analysis model (Table 4.10).In addition, the multivariable model selection using Akaike Information Criteria (AIC) reported that the most parsimonious model clarifying the incidence of prostate cancer among recruited cases was family history of prostate cancer and family history for either breast, ovarian, colorectal, endometrial, and pancreatic cancers among recruited participants (AUC = 0.637, McFadden's R^2 = 0.106, Maximum likelihood pseudo R^2 = 0.137, Nagelkerke's R^2 = 0.183, Coefficient of Discrimination D = 0.124). The presence in a family with a history of prostate cancer (AOR = 6.388, 95% CI: 2.259 - 22.932) was found to increase the risk of developing prostate cancer among patients, as shown in Table 4.10.

Table 4.10: Results of Bivariate and Multivariate analysis for family history with cancer

	Bivariate analysis					Multivariate analysis				
	Std. Error	**z value**	**P-value**	**COR**	**95%CI**	**Std. Error**	**z value**	**P-value**	**AOR**	**95%CI**
Family history of prostate Ca	0.569	3.37	0.00075	6.81	0.63 -5.85	0.577	3.22	0.0013	6.39	0.6-5.74
Family history of any type of cancer	0.5999	1.94	0.052	3.21	0.36 -3.78	1.267942	0.01	0.9891	3.21	1.44- 20.6

Similarly, the marginal effects of cancer family history of prostate cancer are summarised in Figure 4.5.

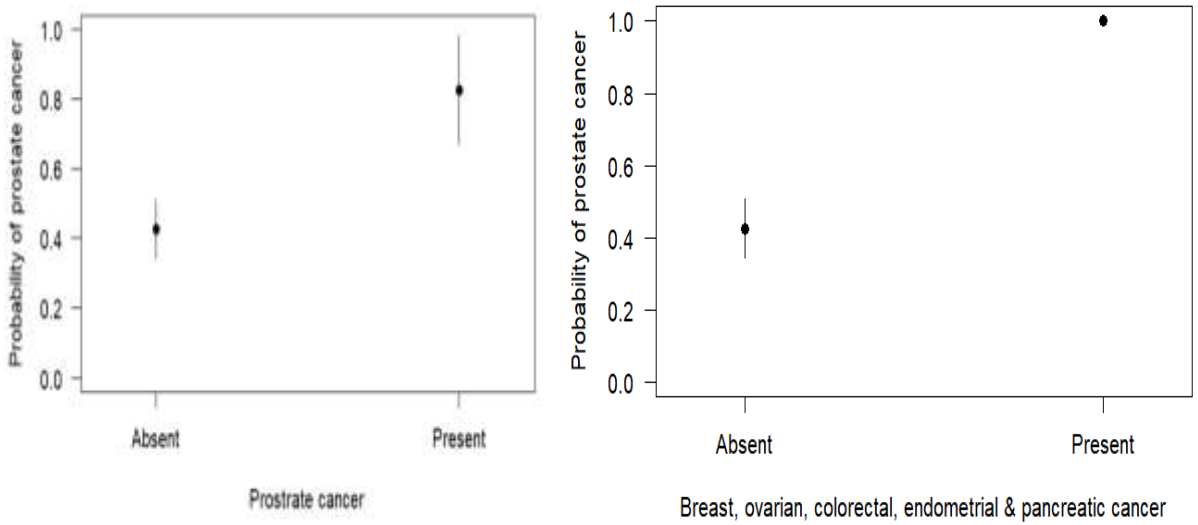


Figure 4.5: The marginal effects of cancer family history of prostate cancer

4.2 ERG and PTEN immunohistochemistry results

4.2.1 Prevalence of ERG Expression in Prostate Tumours

ERG expression was found in 25 out of 83 prostate archived tissue samples (30.1%), while the remaining 58 tumours (69.9%) were *ERG*-negative. The prevalence of *ERG* expression exhibited significant variation across Gleason score categories. The highest frequency of *ERG* positivity was observed in tumours with a Gleason score of ≤7, where 44.0% (11/25) of cases demonstrated *ERG* expression. In contrast, *ERG* positivity was less frequent in tumours with Gleason scores of 4+4 (16.0%, 4/25) and 4+5 (32.0%, 8/25). The lowest prevalence of *ERG* expression was noted in tumours with a Gleason score of >9, where only 8.0% (2/25) of cases were ERG-positive (Table 4.11).

4.2.2 Prevalence of PTEN Loss in Prostate Tumours

PTEN loss was identified in 98.8% (82/83) of the tumour samples, with only one case (1.2%) retaining *PTEN* expression. The frequency of *PTEN* loss is connected with increasing tumour grade. Specifically, 75.6% (62/82) of tumours with a Gleason score of ≤4+4 exhibited *PTEN* loss. In tumours with a Gleason score of 4+4, *PTEN* loss was observed in 26.8% (22/82) of cases. The highest frequency of *PTEN* loss, 28.0% (23/82), was observed in tumours with a Gleason score of 4+5. Among tumours with a Gleason score of >9, 21.9% (18/82) demonstrated PTEN loss (Table 4.11).

Table 4.11: ERG and PTEN expression and Tumour aggressiveness

	ERG/PTEN Immuno expression			
Gleason Score	ERG-	PTEN+	ERG+	PTEN-
≤7	10	1	11	20
4+4	18	0	4	22
4+5	15	0	8	23
5+4 and 5+5	16	0	2	18
Total	**58(69.9%)**	**1(1.2%)**	**25(30.1%)**	**82(98.80%)**

In addition, the immunohistochemical staining patterns of *ERG* and *PTEN* expression in prostate tumour tissues are illustrated in Figure 8

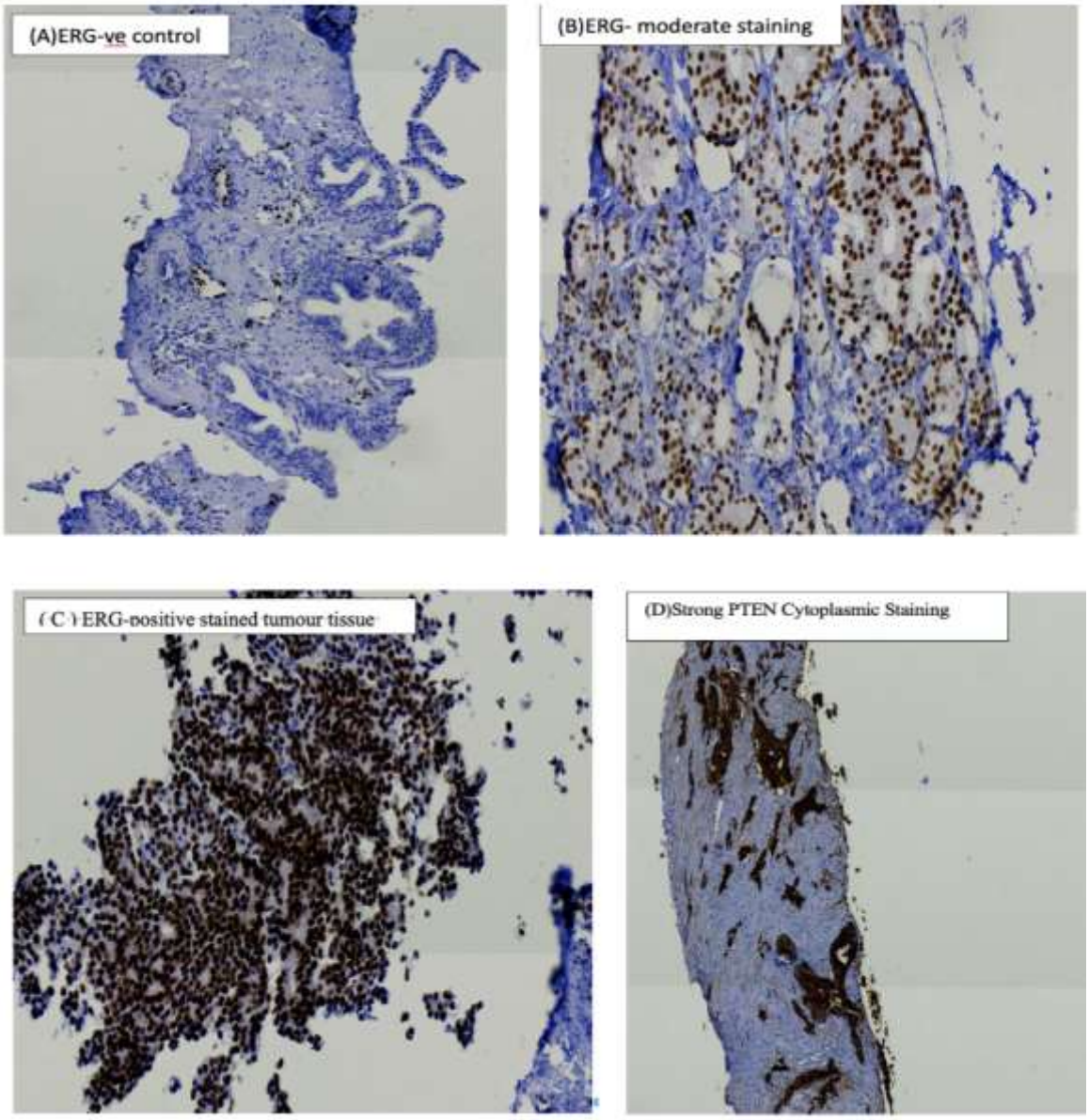


Figure 4.6: Immunohistochemical (IHC) staining patterns of *ERG* and *PTEN* expression in prostate tumour tissues. (A) Negative ERG staining; (B) Moderate ERG positivity; (C) Strong ERG positivity; (D) Strong cytoplasmic PTEN staining

4.2.3 ERG and PTEN Tumour Molecular Subtypes

Immunohistochemistry (IHC) analysis of 83 prostate cancer tumours demonstrated distinct molecular subtypes based on *ERG* and *PTEN* expression patterns. The majority of tumours (68.68%) were classified as *ERG-/PTEN-*,

while 30.12% exhibited the *ERG+/PTEN-* molecular subtype. Only one tumour (1.2%) was classified as *ERG-/PTEN+*. Notably, no tumours in this study demonstrated both *ERG+* and *PTEN+* expression. Also, when stratified by Gleason scores, the distribution of molecular subtypes varied significantly. Among tumours with a Gleason score of <7, nine tumours (10.84%) were classified as *ERG-/PTEN-*, 11 tumours (13.25%) demonstrated the *ERG+/PTEN-* subtype, and one tumour (1.2%) was *ERG-/PTEN+*. For tumours with a Gleason score of 4+4, 17 (20.48%) were classified as *ERG-/PTEN-*, while four (4.82%) demonstrated the *ERG+/PTEN-* molecular subtype. Among tumours with a Gleason score of 4+5, 15 (18.07%) were *ERG-/PTEN-*, and eight (9.64%) were classified as *ERG+/PTEN-*. Again, no tumours expressed both ERG+ and *PTEN+*. In tumours with Gleason scores >9, 16 tumours (19.28%)were *ERG-/PTEN-*, and two tumours (2.41%) exhibited *ERG+/PTEN-*. No tumours demonstrated both *ERG+* and *PTEN+* expression (Table 4.12).

Table 4.12: *ERG* and *PTEN* expression and Tumor aggressiveness

	Tumor Subtypes				
Gleason Score	ERG-/PTEN-	ERG-/PTEN+	ERG+/PTEN-	ERG+/PTEN+	**Total**
<7	9	1	11	0	21
4+4	17	0	4	0	21
4+5	15	0	8	0	23
5+4 and 5+5	16	0	2	0	18
Total	**57 (68.68%)**	**1(1.20%)**	**25(30.12%)**	**0(0.0%)**	**83**

4.3 The Mutation spectrum of BRCA1, BRCA2, ATM, PALB2, ATM, TP53, CHEK2, MLH1, MSH2, MSH6, and PMS2 gene among cancer patients

The Next Generation sequencing revealed that 246 mutation events were identified through the analysis of DNA damage and mismatch repair genes in blood samples taken from 30 participants with prostate cancer (Appendix table 2). These were classified using the SIFT (Sorting Intolerant from Tolerant) tool and curated categorisation from the ClinVar database according to their expected functional impact. The variations were further classified as pathogenic, likely pathogenic, variants of uncertain significance (VUS), and likely Benign using the ClinVar database, which added another level of functionality and clinical insight to the mutation data. The mutations were categorised into six main groups: Tolerated and Likely Benign, Tolerated and Likely Pathogenic, Tolerated and Variant of uncertain significance, Damaging and Variants of Uncertain Significance (VUS), Damaging and Likely Benign, and Damaging and Likely Pathogenic.

There were 28 mutations, *MLH1* (6 events, 2.44%), *ATM* (7 events, 2.85%), *BRCA1* (5 events, 2.03%), *BRCA2* (7 events, 2.85%), *MSH6* (1 event, 0.41%), and *TP53* (2 events, 0.81%) found in the Damaging and Likely Benign category, which accounted for 11.38% of all mutation events (Appendix table 2). Although ClinVar considered these mutations likely benign due to the lack of evidence connecting them to pathogenicity, the SIFT prediction identified them as harmful. Additionally, four mutations were found in the Damaging and Likely Pathogenic category, which accounted for 1.63% of all mutation events. These mutations were found in *PMS2* (1 event, 0.41%), *ATM* (1 event, 0.41%), and *MSH2* (2 events, 0.81%) (Table 19). SIFT categorised them as harmful, and ClinVar determined that they were likely pathogenic. Current evidence from ClinVar has connected these variations to an increased likelihood of contributing to prostate cancer. Similarly, four mutations

(1.63% of total) were found in the Damaging and VUS category; these were found in *ATM* (1 event, 0.41%), *BRCA1* (2 events, 0.81%), and *MSH2* (1 event, 0.41%) (Table 22). Although SIFT predicted that these mutations would be harmful, there was insufficient data in ClinVar to establish whether they were benign or pathogenic. Thus, they were categorised as variations of unknown significance (VUS). Across all the mutation occurrences, 210 (85.37%) were in the Tolerated and Likely Benign category, which had the highest percentage of mutations. ClinVar determined that these mutations, which SIFT predicted would be tolerated, were likely benign, meaning they are unlikely to impact protein function or play a role in the genesis of cancer. This group comprised mutations in *TP53* (18 events, 7.32%), *ATM* (23 events, 9.35%), *BRCA1* (29 events, 11.79%), *BRCA2* (52 events, 21.14%), *MLH1* (7 events, 2.85%), *MSH6* (1 event, 0.41%), *PALB2* (23 events, 9.35%), and *PMS2* (49 events, 19.92%). One mutation event was found in *ATM*, and 0.41% of all mutation events were categorised as Tolerable and Likely Pathogenic. This mutation was predicted to be tolerated by SIFT but was classified as likely pathogenic in ClinVar**,** indicating a potential role in prostate cancer development (Appendix table 2). Furthermore, The Tolerated VUS mutations account for 2.85% of the total mutation, including *ATM* (3 occurrences, 1.22%), *CHEK2* (2 events, 0.81%), and *TP53* (2 events, 0.81%). The precise relevance of these mutations in the development of cancer is still unknown. However, SIFT predicted that they would be tolerated. ClinVar classed them as variants of uncertain significance (VUS). In this study, the tolerated mutations made up the majority of the total, with 210 occurrences (85.37%), whereas damaging mutations accounted for 36 events (14.63%). The dataset had 246 mutation events in all. The potential pathogenicity of these genomic changes in prostate cancer was

better understood by combining SIFT predictions with ClinVar classifications (Table 4.14 & 4.15).

Table 4.13: Summary of Mutation Spectrum of Homologous Recombination and Mismatch Repair Genes

Mutation Type	Gene	Total Mutation Event	Percentage (%)
Damaging and Likely Benign	*ATM*	7	2.85
	BRCA1	5	2.03
	BRCA2	7	2.85
	MLH1	6	2.44
	MSH6	1	0.41
	TP53	2	0.81
Damaging and Likely Pathogenic	*PMS2*	1	0.41
	ATM	1	0.41
	MSH2	2	0.81
Damaging and VUS	*ATM*	1	0.41
	BRCA1	2	0.81
	MSH2	1	0.41
Tolerated and Likely Benign	*ATM*	23	9.35
	BRCA1	29	11.79
	BRCA2	52	21.14
	MLH1	7	2.85
	MSH6	1	0.41
	PALB2	23	9.35
	PMS2	49	19.92
	TP53	18	7.32
Tolerated Likely Pathogenic	*ATM*	1	0.41
Tolerated VUS	*ATM*	3	1.22
	CHEK2	2	0.81
	TP53	2	0.81
Total Damaging		**36**	**14.63**
Total Tolerated		**210**	**85.37**
Overall Total		**246**	**100.00**

Table 4.14: Homologous recombination and Mismatch repair gene mutation spectrum with Variant of uncertain significant and likely pathogenic results

Gene	Chromosome	Type	Impact	Prediction Tool (SIFT)	Nucleotide Change	Nucleotide Change Frequency	Protein Change	Clinical Significance
ATM	chr11	SNP	Missense variant	Damaging	c.2442C>A	1	p. Asp814Glu	VUS
BRCA1	chr17	SNP	Missense variant	Damaging	c.557C>A	1	p.Ser186Tyr	VUS
BRCA1	chr17	SNP	Missense variant	Damaging	c.1137T>G	1	p. Ile379Met	VUS
MSH2	chr2	SNP	Missense variant	Damaging	c.380A>G	1	p. Asn127Ser	VUS
ATM	chr11	SNP	Missense variant	Tolerated	c.1541G>A	3	p. Gly514Asp	VUS
CHEK2	chr22	SNP	Missense variant	Tolerated	c.1343T>G	2	p. Ile448Ser	VUS
TP53	Chr17	SNP	Missense variant	Tolerated	c.917G>A	2	p. Arg306Gln	VUS
ATM	chr11	SNP	Missense variant	Damaging	c.131A>G	1	p. Asp44Gly	Likely pathogenic
PMS2	chr7	SNP	Missense variant	Damaging	c.497T>C	1	p. Leu166Pro	Likely pathogenic
ATM	chr11	SNP	Missense variant	Tolerated	c.2289T>A	1	p. Phe763Leu	Likely pathogenic

4.3.1 Gene-specific Mutation spectrum of homologous recombination repair genes

4.3.1.1 BRCA1 Gene Mutation Spectrum

Among all 30 samples analysed, this study reported 36 mutation events, of which eight (8) were different mutation variants in chromosome 17. Two nucleotide missense variants c.1137T>G and c.557C>A, were found to be variants with uncertain significance, while other remaining six missense mutation variants c.1427A>G, c.2458A>G, c.2612C>T, c.3113A>G, c.4600G>A, c.4837A>G and c.557C>A were likely benign. (Table 4.15).

Table 4.15: Results of *BRCA1* gene Mutation spectrum

Gene	Ref allele	Alt allele	Impact	Nucleotide Change	Nucleotide Change Frequency	Protein Change	Clinical Significance
BRCA1	A	C	missense_variant	c.1137T>G	1	p.Ile379Met	VUS
BRCA1	T	C	missense_variant	c.1427A>G	1	p.His476Arg	likely_benign
BRCA1	T	C	missense_variant	c.2458A>G	1	p.Lys820Glu	likely_benign
BRCA1	G	A	missense_variant	c.2612C>T	17	p.Pro871Leu	likely_benign
BRCA1	T	C	missense_variant	c.3113A>G	9	p.Glu1038Gly	likely_benign
BRCA1	C	T	missense_variant	c.4600G>A	1	p.Val1534Met	likely_benign
BRCA1	T	C	missense_variant	c.4837A>G	5	p.Ser1613Gly	likely_benign
BRCA1	G	T	missense_variant	c.557C>A	1	p.Ser186Tyr	VUS

4.3.1.2 BRCA2 Gene Mutation Spectrum

For all analyzed and annotated 30 Vcf files, the study analysis found 59 *BRCA2* nucleotide mutation variants in chromosome 13 whereby eighteen (18) nucleotide missense variants, c.10234A>G, c.1114A>C, c.2971A>G, c.4090A>C, c.4241C>T c.4681C>A,c.5414A>G,c.5640T>G,c.5704G>A,c.6412G>T,c.7017G>C,c.7150C>A, c.7319A>G,c.7397T>C,c.7712A>G,c.8830A>T,c.9730G>A, c.9875C>T were found to be different. All these reported *BRCA2* mutation variants are classified as likely benign (Table 4.16).

Table 4.16: Results of *BRCA2* Gene Mutation spectrum

Gene	Ref allele	Alt allele	Impact	Nucleotide Change	Nucleotide ChangeFrequency	Protein Change	Clinical Signi ficance
BRCA2	A	G	missense_variant	c.10234A>G	9	p.Ile3412Val	likely_benign
BRCA2	A	C	missense_variant	c.1114A>C	5	p.Asn372His	likely_benign
BRCA2	A	C	missense_variant	c.4090A>C	1	p.Ile1364Leu	likely_benign
BRCA2	C	T	missense_variant	c.4241C>T	2	p.Thr1414Met	likely_benign
BRCA2	C	A	missense_variant	c.4681C>A	1	p.His1561Asn	likely_benign
BRCA2	A	G	missense_variant	c.5414A>G	1	p.Asn1805Ser	likely_benign
BRCA2	T	G	missense_variant	c.5640T>G	1	p.Asn1880Lys	likely_benign
BRCA2	G	A	missense_variant	c.5704G>A	2	p.Asp1902Asn	likely_benign
BRCA2	G	T	missense_variant	c.6412G>T	2	p.Val2138Phe	likely_benign
BRCA2	G	C	missense_variant	c.7017G>C	1	p.Lys2339Asn	likely_benign
BRCA2	C	A	missense_variant	c.7150C>A	1	p.Gln2384Lys	likely_benign
BRCA2	A	G	missense_variant	c.7319A>G	1	p.His2440Arg	likely_benign
BRCA2	T	C	missense_variant	c.7397T>C	29	p.Val2466Ala	likely_benign
BRCA2	A	G	missense_variant	c.7712A>G	1	p.Glu2571Gly	likely_benign
BRCA2	A	T	missense_variant	c.8830A>T	3	p.Ile2944Phe	likely_benign
BRCA2	G	A	missense_variant	c.9730G>A	1	p.Val3244Ile	likely_benign
BRCA2	C	T	missense_variant	c.9875C>T	1	p.Pro3292Leu	likely_benign

4.3.1.3 ATM Gene Mutation Spectrum

For all analyzed and annotated 30 Vcf files, the study analysis found 36 *ATM* nucleotide mutation variants in chromosome 13 whereby eleven (11) nucleotide missense variants, c.131A>G,c.1541G>A,c.1636C>G,c.2442C>A,c.2614C>T,c.3383A>G,c.378T>A,c.4138C>T,c.4792C>A,c.6176C>T,c.6995T>C were found to be different. Out of those reported 11 missense variants, c.131A>G is classified as likely pathogenic, while c.1541G>A and c.2442C>A are classified as variants of uncertain significance, and the remaining variants were classified as likely benign (Table 4.17).

Table 4.17: Results of ATM Gene Mutation Spectrum

Gene	Ref allele	Alt allele	Impact	Prediction Tool (SIFT)	Nucleotide Change	Number of Nucleotide Change	Protein Change	Clinical Significance
ATM	A	G	Missense variant	Damaging	c.131A>G	1	p.Asp44Gly	likely_pathogenic
ATM	G	A	Missense variant	Tolerated	c.1541G>A	3	p.Gly514Asp	VUS
ATM	C	T	Missense variant	Tolerated	c.1810C>T	2	p.Pro604Ser	likely_benign
ATM	T	A	Missense variant	Tolerated	c.2289T>A	1	p.Phe763Leu	likely_pathogenic
ATM	C	A	Missense variant	Damaging	c.2442C>A	1	p.Asp814Glu	VUS
ATM	C	T	Missense variant	Tolerated	c.2614C>T	2	p.Pro872Ser	likely_benign
ATM	A	G	Missense variant	Damaging	c.3383A>G	3	p.Gln1128Arg	likely_benign
ATM	T	A	Missense variant	Tolerated	c.378T>A	18	p.Asp126Glu	likely_benign
ATM	C	T	Missense variant	Damaging	c.4138C>T	1	p.His1380Tyr	likely_benign
ATM	T	A	Missense variant	Damaging	c.4633T>A	1	p.Leu1545Ile	likely_benign
ATM	C	A	Missense variant	Damaging	c.4792C>A	1	p.Leu1598Ile	likely_benign
ATM	C	T	Missense variant	Damaging	c.6176C>T	1	p.Thr2059Ile	likely_benign
ATM	T	C	Missense variant	Tolerated	c.6995T>C	1	p.Leu2332Pro	likely_benign

4.3.1.4 *CHEK2* Gene Mutation Spectrum

For all analysed and annotated 30 Vcf files, the study analysis found two (2) *CHEK2* nucleotide mutation variants in chromosome 2, whereby all reported nucleotide missense variants, have the same c.1343T>G mutation, which is classified with ambiguous clinical significance classification. This point mutation, c.1343T>G results in protein change p. Ile448Ser, where the amino acid isoleucine (Ile) at position 448 is replaced by serine (Ser) (Table 4.17).

4.3.1.5 *PALB2* Gene Mutation Sspectrum

The present study reveals 23 *PALB2* nucleotide mutation variants in chromosome 6. Among all reported 23 variants, only three-point mutation variants c.1676A>G, c.629C>T, c.925A>G, were found to be different. The mutation variant c.1676A>G results in protein change p. Gln559Arg where the amino acid glutamine (Gln) at position 559 is replaced by arginine (Arg). Also c.629C>T results in protein change p. Pro210Leu where the amino acid proline (Pro) at position 210 is replaced by leucine (Leu). Similarly, the reported nucleotide variant c.925A>G results in p. Ile309Val protein change where the amino acid isoleucine (Ile) at position 309 is replaced by valine (Val). However, all reported *PALB2* point mutation variants are clinically likely benign (Table 4.17).

4.3.1.6 *TP53* Gene Mutation Spectrum

For the complete set of 30 VCF files that were subjected to analysis and annotation, the study analysis found 22 *TP53* nucleotide mutation variants in chromosome 17. Among all reported variants, only three-point mutation variants c.139C>T, c.215C>G, and c.917G>A were found to be different. The mutation variant c.139C>T, results in protein change p. Pro47Ser where the amino acid proline (Pro) at position 47 is replaced by serine (Ser), The mutation variant c.215C>G, results in protein change p. Pro72Arg where the amino acid proline (Pro) at position 72 is replaced by arginine

(Arg). These two reported point mutation variants are clinically likely benign but the remaining c.917G>A mutation variant is classified as clinically ambiguous in significance. This variant causes protein change p.Arg306Gln where the amino acid arginine (Arg) at position 306 is replaced by glutamine (Gln).(Table 4.18).

Table 4.18: Results of TP53,PALB2 and CHEK2 Gene Mutation spectrum

Gene	Ref allele	Alt allele	Impact	Prediction Tool(SIFT)	Nucleotide Change	Nucleotide Change Frequency	Protein Change	Clinical Significance
TP53	G	A	missense_variant	Damaging	c.139C>T	2	p.Pro47Ser	likely_benign
TP53	G	C	missense_variant	Tolerated	c.215C>G	18	p.Pro72Arg	likely_benign
TP53	C	T	missense_variant	Tolerated	c.917G>A	2	p.Arg306Gln	VUS
PALB2	T	C	missense_variant	Tolerated	c.1676A>G	18	p.Gln559Arg	likely_benign
PALB2	G	A	missense_variant	Tolerated	c.629C>T	2	p.Pro210Leu	likely_benign
PALB2	T	C	missense_variant	Tolerated	c.925A>G	3	p.Ile309Val	likely_benign
CHEK2	A	C	Missensevariant	Tolerated	c.1343T>G	2	p.Ile448Ser	VUS

4.3.2 Gene-specific Mutation spectrum of Mismatch repair gene mutation

The present study also leverages targeted next-generation sequence technology to conduct a thorough analysis of Mismatch repair gene variants within prostate cancer samples, aiming to identify and annotate mutation spectrum that may impact *PMS2, MSH2, MSH6, MLH1* functionality and influence disease outcomes. The presented results provide insights into the specific Mismatch repair gene variants present in our cohort, their potential functional consequences, and their relevance to prostate cancer pathology and treatment. The mutation spectrum of selected Mismatch repair genes is presented below.

4.3.2.1 PMS2 Gene Mutation spectrum

For all analyzed and annotated 30 Vcf files, the study analysis found 50 *PMS2* nucleotide mutation variants in chromosome 7 whereby eight (8) nucleotide missense variants. Out of those reported 8 missense variants, c.497T>C is classified as likely pathogenic classification, and the remaining variants were classified as likely benign. This point mutation, c.497T>C results in protein change p. Leu166Pro where the amino acid leucine (Leu) at position 166 is replaced by proline (Pro). (Table 4.19)

Table 4.19: Results of *PMS2* Gene Mutation spectrum

Gene	Ref allele	Alt allele	Impact	Nucleotide Change	Prediction Tool (SIFT)	Nucleotide Change Frequency	Protein Change	Clinical Significance
PMS2	G	A	Missense variant	c.1408C>T	Tolerated	2	p.Pro470Ser	likely benign
PMS2	G	A	Missense variant	c.1532C>T	Tolerated	6	p.Thr511Met	likely benign
PMS2	C	T	Missense variant	c.1609G>A	Tolerated	1	p.Glu537Lys	likely benign
PMS2	T	C	Missense variant	c.1621A>G	Tolerated	28	p.Lys541Glu	likely benign
PMS2	C	A	Missense variant	c.1688G>T	Tolerated	1	p.Arg563Leu	likely benign
PMS2	A	G	Missense variant	c.497T>C	Tolerated	1	p.Leu166Pro	Likely pathogenic
PMS2	C	T	Missense variant	c.59G>A	Tolerated	3	p.Arg20Gln	likely benign
PMS2	C	G	Missense variant	c.924G>C	Tolerated	8	p.Glu308Asp	likely benign

4.3.1.3 *MSH2* Gene Mutation Spectrum

For the complete set of 30 VCF files that were subjected to analysis and annotation, the study analysis found only one, c.380A>G nucleotide mutation variant in chromosome 2. The reported mutation variant *MSH2* c.380A>G, results in protein change p.Asn127Ser where the amino acid asparagine (Asn) at position 127 is replaced by serine (Ser). The replacement of asparagine with serine may affect the protein's structure and function due to the differing chemical properties of their side chains. Asparagine's amide group is involved in hydrogen bonding, whereas serine's hydroxyl group could alter these interactions, potentially impacting protein folding and molecular interactions. However, the reported point mutation variant is clinically classified as a variant of uncertain significance due to current limitations to support its pathogenicity impacts. (Table 4.19).

4.3.2.2 MSH6 Gene Mutation spectrum

Similarly,the study analysis found two, c.155A>C, and c.15C>A nucleotide mutation variants in chromosome 2. The reported mutation variant c.155A>C, results in protein change p. Glu52Ala where the amino acid glutamic acid (Glu) at position 52 is replaced by alanine (Ala). The impact of the p.Glu52Ala mutation depends on the specific role of position 52 in the protein. If this position is crucial for the protein's function or interaction with other molecules, the mutation could lead to functional changes. Also, c.15C>A nucleotide mutation variant results in protein change p.Ser5Arg where the amino acid serine (Ser) at position 5 is replaced by arginine (Arg). Similarly, replacing serine with arginine presents an important change in the chemical characteristics of the side chain. Arginine's positive charge and larger side chain may disrupt local interactions and alter the protein's structure and function, compared to the smaller, neutral serine. The *MSH6* point mutation variants revealed in this study are clinically classed as ambiguous, nonetheless, because there is currently insufficient evidence to substantiate their pathogenicity implications. (Table 4.19).

4.3.2.3 MLH1 Gene Mutation spectrum

For the complete set of 30 VCF files that were subjected to analysis and annotation, the study analysis found thirteen (13), nucleotide mutation variants in chromosome 3. Out of those variants, three variants c.1963A>G, c.2152C>T, and c.655A>G were found to be different. The reported mutation variant c.1963A>G results in protein change p.Ile655Val where the amino acid isoleucine (Ile) at position 655 is replaced by valine (Val). The switch of isoleucine with valine at position 655 could affect the protein's stability and interactions due to the understated differences in their side chains. This change may affect the protein's hydrophobic core, potentially influencing its overall structure or function. Also, the reported mutation variant c.2152C>T, results in protein

change p.His718Tyr where the amino acid histidine (His) at position 718 is replaced by tyrosine (Tyr)**.** Tyrosine in place of histidine could have a substantial effect on the structure and functionality of the protein. Local interactions, protein stability, or catalytic activity may change when the positively charged imidazole side chain is replaced with a phenolic side chain that has different electronic characteristics.

Likewise, c.655A>G missense mutation variant results in protein change p.Ile219Val where the amino acid isoleucine (Ile) at position 219 is substituted by valine (Val). Because of the variations in side chain lengths and branching, the replacement of valine with isoleucine at position 219 may have an impact on the structural integrity and stability of the protein. Even though both amino acids are hydrophobic, valine's smaller size and branching may cause minute modifications to the way proteins fold or interact. However, due to available information, the reported *MLH1* point mutation variants reported in the present study are clinically classified as likely benign, and thus they are not expected to cause disease (Table 4.20).

Table 4.20: Results of *MLH1,MSH6,* and *MSH2* Gene Mutation spectrum

Gene	Ref allele	Alt allele	Impact	Prediction Tool (SIFT)	Nucleotide Change	Nucleotide Change Frequency	Protein Change	Clinical Significance
MLH1	A	G	missense_variant	Tolerated	c.1963A>G	2	p.Ile655Val	likely_benign
MLH1	C	T	missense_variant	Damaging	c.2152C>T	6	p.His718Tyr	likely_benign
MLH1	A	G	missense_variant	Tolerated	c.655A>G	5	p.Ile219Val	likely_benign
MSH6	A	C	missense_variant	Tolerated	c.155A>C	1	p.Glu52Ala	likely_benign
MSH6	C	A	missense_variant	Damaging	c.15C>A	1	p.Ser5Arg	likely_benign
MSH2	A	G	missense_variant	Damaging	c.380A>G	1	p.Asn127Ser	VUS

4.4 The results of the association between Gene mutation, tumour subtypes, Age at diagnosis, family history of cancer, and Gleason score

This study reveals the interplay patterns between age at diagnosis, family history of cancer, tumour subtypes, Gleason score, and likely pathogenic mutations in prostate cancer. Younger patients (<60 years) with a family history of prostate cancer often showed aggressive *ERG+/PTEN*- subtypes, higher Gleason scores, and likely pathogenic mutations (*ATM* and *PMS2*), suggesting a strong genetic link to early-onset aggressive disease. For example, Patient ID 44 (age 53) and ID 16 (age 59) both had *ERG+/PTEN*- tumours with Gleason scores ≥4+4, aligning with early-onset, high-risk phenotypes tied to genetic predisposition (Table 4.20).

Older patients (>70 years) with non-prostate familial cancers (e.g., breast or ovarian) more often had *ERG-/PTEN*- PTEN-subtypes and intermediate Gleason scores. For instance, Patient ID 25 (age 74) with ovarian cancer history showed an *ERG-/PTEN*- tumour (Gleason 4+4) and an *ATM* VUS, while Patients ID 3 (71) and ID 6 (73) with breast cancer history had *ERG-/PTEN*- tumours (Gleason 4+3 and 4+4) and damaging *BRCA1* VUS. These findings suggest less aggressive prostate tumours in older patients with non-prostate familial cancers. Patients aged 60–70 years showed mixed profiles based on family history. For example, Patient ID 31 (age 62) with a prostate cancer history had an aggressive *ERG+/PTEN*- tumour (Gleason 4+5) and an *ATM* VUS, while Patient ID 57 (age 74) with a breast cancer history presented an *ERG-/PTEN*- tumour (Gleason 4+3) and a *CHEK2* VUS. (Table 4.20).

Overall, the study highlights distinct age-associated patterns in gene mutation, tumour subtypes and Gleason score in prostate cancer. Younger patients with familial prostate cancer are more likely to harbour aggressive *ERG+/PTEN*- tumours linked to likely pathogenic mutations, *ATM* and *PMS2*. In contrast, older patients with non-prostate familial cancers predominantly exhibit less aggressive ERG-/PTEN- tumours associated with VUS in genes like *ATM*, *BRCA1*, or *CHEK2*(Table 4.21).

Table 4.21: The Association between Gene mutation, tumour subtypes, Age at diagnosis, family history with cancer, and Gleason score

Sample ID	Gene	Chromosome	Impact	Prediction tool(SIFT)	Nucleotide change	Variant ID	Protein change	Clinical Significance	Gleason Score	Pre-operative PSA	History with Cancer	ERG/PTEN Subtypes	Age (Years)
25	***ATM***	chr11	Missense variant	Damaging	c.2442C>A	rs3218695	p.Asp814Glu	VUS	**4+4**	**23.3**	Oca	***ERG-/PTEN-***	**74**
3	***BRCA1***	chr17	Missense variant	Damaging	c.557C>A	rs55688530	p.Ser186Tyr	VUS	**4+3**	**20.3**	Brca	***ERG-/PTEN-***	**71**
6	***BRCA1***	chr17	Missense variant	Damaging	c.1137T>G	rs56128296	p.Ile379Met	VUS	**4+4**	**20.2**	Brca	***ERG-/PTEN-***	**73**
17	***MSH2***	chr2	Missense variant	Damaging	c.380A>G	rs17217772	p.Asn127Ser	VUS	**3+3**	**9.2**	Pca	***ERG-/PTEN-***	**68**
1	***ATM***	chr11	Missense variant	Tolerated	c.1541G>A	rs2235000	p.Gly514Asp	VUS	**4+4**	**15.2**	CRca	***ERG+/PTEN-***	**74**
13	***ATM***	chr11	Missense variant	Tolerated	c.1541G>A	rs2235000	p.Gly514Asp	VUS	**4+3**	**25.4**	Oca	***ERG-/PTEN-***	**73**
31	***ATM***	chr11	Missense variant	Tolerated	c.1541G>A	rs2235000	p.Gly514Asp	VUS	**4+5**	**38.3**	Pca	***ERG+/PTEN-***	**62**
42	***CHEK2***	chr22	Missense variant	Tolerated	c.1343T>G	rs17886163	p.Ile448Ser	VUS	**5+5**	**7.5**	Pca	***ERG+/PTEN-***	**63**
57	***CHEK2***	chr22	Missense variant	Tolerated	c.1343T>G	rs17886163	p.Ile448Ser	VUS	**4+3**	**19.7**	Brca	***ERG-/PTEN-***	**74**
44	***ATM***	chr11	Missense variant	Damaging	c.131A>G	rs150143957	p.Asp44Gly	Likely pathogenic	**5+5**	**85.3**	Pca	***ERG+/PTEN-***	**53**
16	***PMS2***	chr7	Missense variant	Damaging	c.497T>C	rs116349687	p.Leu166Pro	Likely pathogenic	**4+4**	**21.5**	Pca	***ERG+/PTEN-***	**59**
15	***ATM***	chr11	Missense variant	Tolerated	c.2289T>A	rs34231402	p.Phe763Leu	Likely pathogenic	**4+4**	**18.6**	Pca	***ERG+/PTEN-***	**74**

4.4.1 The association between *ERG* expression and Gleason score (in class categories)

A significant relationship was found by chi-square analysis between *ERG* expression (positive or negative) and Gleason class (low, intermediate, and high) ($\chi^2 = 7.5721$, df = 2, p = 0.02269). In particular, different Gleason classes have different *ERG* expression distributions. The high Gleason class category included the majority of patients (60%) with negative *ERG* expression, suggesting an association between negative *ERG* expression and more aggressive tumour characteristics. On the other hand, a high Gleason score was found for only 16% of individuals with positive *ERG* expression. These results imply a relationship between *ERG* expression status and Gleason classification, with patients with higher tumour grades more likely to have negative *ERG* expression.

4.4.2 The results of the association between the Gleason Group and Pre-operative PSA

A linear regression analysis was used to evaluate the association between the Gleason group and pre-operative PSA levels in patients with prostate cancer. The findings showed a substantial, statistically significant correlation (p-value = 0.000) between the Gleason group and pre-operative PSA levels. These results imply a close association between rising pre-operative PSA levels and higher Gleason groups, which indicate enhanced tumour aggressiveness. This suggests that PSA may be useful in predicting tumour grade and supports its function as an indicator of prostate cancer severity. Additional research may shed more light on the clinical significance of this link in directing prostate cancer patients' prognosis and their subsequent treatment. (Table 4.22). The summary results of the Gleason score and pre-operative PSA of the case participants are presented in Appendix Table 1

Table 4.22: Results showing the association between Gleason Group and Pre-operative PSA

	Estimate	Std. Error	t value	P-value
Intercept	3.00	0.17	17.55	< 2e-16
PSA_level	0.03	0.00	7.21	0.00000

4.4.3 The results of the association between Gene mutations and pre-operative PSA among Prostate cancer patients

The relationship between pre-operative PSA levels and gene alterations in patients with prostate cancer was examined. Out of all the mutations evaluated, the *ATM* and *PMS2* genes were shown to have significant associations with pre-operative PSA levels; both variants have been classified as harmful and most certainly pathogenic. Both genes had p-values of 0.036, indicating that the associations were significant. The *MLH1* gene mutation, which is considered benign and tolerable, also showed a significant correlation with preoperative PSA levels (p-value = 0.0178) (Table 4.23). The results show that *ATM* and *PMS2* mutations, both of which are regarded as likely pathogenic, may have a significant impact on PSA levels in patients with prostate cancer, suggesting that they may be biomarkers for the progression or severity of the disease. Despite the *MLH1* mutation's classification as benign, its association with PSA levels raises the possibility that its function in the biology of prostate cancer warrants more research. These findings highlight the value of using genetic mutation analysis to understand the molecular causes and clinical manifestations of prostate cancer.

Table 4.23: Linear regression coefficients for the effect of mutation on pre-operative PSA in prostate cancer patients

Models	Covariates	Estimate	Std. Error	t value	P-value
ATM_Dpa	ATM_Dpa	54.33	24.68	2.20	0.0358
PMS2_Dpa	PMS2_Dpa	54.23	24.69	2.20	0.0362
MLH1_Tbe	MLH1_Tbe	21.92	8.73	2.51	0.0178

CHAPTER FIVE

5.0 DISCUSSION

5.1 Non-modifiable risk factors

5.1.1 Age at diagnosis

Prostate cancer is among the quickest-growing non-communicable diseases in low and middle-income countries, including Tanzania. In the current study, the mean age at diagnosis was 71.77±8.53 years. By using a multivariate statistical analysis model, the current study has demonstrated that older men in Tanzania experience a significantly higher risk of developing prostate cancer compared to younger men (OR = 1.05, 95% CI: 1.006–1.097) Specifically, the risk increases by 5% with each additional year of age. The absence of 1.0 within the confidence interval indicates a statistically significant correlation between age and prostate cancer risk at the 5% significance level ($p < 0.05$). Furthermore, the narrow confidence interval underlines the precision of this finding. These results align with existing research, which identifies ageing as a well-established risk factor for prostate cancer, likely due to hormonal changes, age-related physiological decline, and the accumulation of genetic mutations over time. This evidence highlights the critical role of age in prostate cancer risk and underscores the need for targeted health interventions for older populations. (Cannarella *et al*., 2021; Zhang *et al*., 2023). These results highlight the significance of early identification and focused screening for older men, especially in Tanzania, where the prevalence of prostate cancer is on the rise (Zhang *et al.*, 2023). Furthermore, similar findings were reported in similar studies conducted in Namibia, Uganda and South Africa, with the mean age at diagnosis 70.5, 70.6, and 71 Years for South African men, respectively (Tindall *et al*., 2014). Also, this study's findings corroborate other studies and confirm that prostate cancer is more common among men aged at diagnosis of > 65. Similar findings were reported in many African,

American, European and multi-ethnic Asian men (Katongole *et al.*, 2020; Lim *et al.*, 2021; Rawla, 2019).

However, the present studies reported a younger mean age compared to 74.2 and 72.6 mean ages at diagnosis reported in Eldoret, Kenya and central Sudanese men, respectively (Seraphin, Joko-Fru, Manraj, *et al.*, 2021; Taha *et al.*, 2020). The reported mean age variation across the study population can also be explained by various factors, including inclusion criteria and population demographics of study participants (Rawla, 2019).

5.1.2 Family Background with Prostate Cancer

In Sub-Saharan Africa, several research studies have explored the link between family history and prostate cancer risk, highlighting the genetic and hereditary aspects. Studies have shown that men who have a family history of prostate cancer are at a higher risk. The strength of correlation varies due to differences in lifestyle choices and external environmental factors among populations (Bruner *et al.*, 2003; Johns & Houlston, 2003; Kiciński *et al.*, 2011; Powell *et al.*, 2010). Additionally, data from the African Cancer Registry Network shows that the incidence of prostate cancer is on the rise and that a strong familial link is a significant risk factor (Seraphin, Joko-Fru, Kamaté, *et al.*, 2021). Despite the scarcity of data on the East African population, this study is the first to demonstrate a significant connection between prostate cancer risk and a family history of prostate cancer. Tanzanian men who have first-degree relatives who have prostate cancer are more likely to develop prostate cancer themselves (AOR=6.388,95%CI:2.259-22.932). These findings indicate that Tanzanian men who have a first-degree relative with prostate cancer have a far higher chance of getting the disease than men who don't have such a family history. In particular, the risk of prostate cancer is approximately six times higher for men with

family history of prostate cancer. The confidence interval indicates that, at the 5% level ($p < 0.05$), there is a statistically significant correlation between the increased risk of prostate cancer and having a first-degree relative with the condition. Given the possible genetic or inherited risk factors linked to prostate cancer, this study underscores the significance of family history as a critical factor in early detection and prevention methods.

Furthermore, our findings collaborate with previous studies conducted in different ethnic groups, which also reported a significant association between family history and increased risk of developing prostate cancer. For example, the studies involving black, white, and Asian-American populations reveal their likelihood of developing prostate cancer increased with a family history of a first-degree relative with prostate cancer (AOR= 2.5, 95% CI:1.9–3.3) (Whittemore *et al.,* 1995). Also, studies conducted in other developing countries reported similar findings (Brandt *et al.,* 2012; Bruner *et al.,* 2003; Johns & Houlston, 2003; Kiciński *et al.,* 2011). These findings are in disagreement with other studies conducted, which found no significant association between family background and risk of developing prostate cancer. For example, a study conducted by Morrison & Gordon and a similar study conducted by Saarimäki found no association between a family history of prostate cancer and an increased risk of developing prostate cancer in the Jamaican population and the Finnish Population, respectively (Morrison & Gordon, 2019; Saarimäki *et al.,* 2015). This variation can also be attributed to variations in study design and participants' demographic variation (Rawla, 2019). Prostate cancer research is scarce in many sub-Saharan African countries due to a lack of awareness and financing, conflicting health priorities, and inadequate diagnostic facilities. (Jalloh *et al.,* 2024; Lombe et al., 2024) This study closes these gaps by offering regional data on Tanzanian men and

illuminating regional risk factors. The results highlight the need for improved screening, awareness, and healthcare infrastructure in the region to enhance early detection and treatment (Barber *et al.*, 2018).

5.2 Modifiable risk factors

5.2.1 Social Demographic Risk Factors

The findings of the present study provide significant new insight into the modifiable factors influencing Tanzanian men's prostate cancer risk. A multivariate GLM statistical model was used to find several sociodemographic characteristics that were significant predictors.

5.2.2 Marital status

Previous studies have investigated the association between marital status and the incidence of prostate cancer. The available evidence has shown that being in a committed relationship is linked to reduced risk and better prognosis of diseases. The social support that comes with marriage may encourage people to lead healthier lives and seek medical care more frequently. Due to psychological and social support, married men have a reduced risk of developing prostate cancer compared to their unmarried counterparts in different populations (Chen *et al.,* 2024; Salmon *et al*., 2021). Though the information is limited in the African population, the available data, including those representing American and Canadian populations, found that unmarried men have an elevated risk of developing prostate cancer compared to married men (OR 1.40; CI 1.35-1.44) and (OR 1.80; 95% CI 1.29–2.51) respectively (fgnfg. Like many African countries with high incidence and mortality rates of prostate cancer, the association between marital status and prostate cancer incidence in Tanzania is little understood. Using a multivariate model with potential confounders adjusted, we examined the relationship between marital status and

prostate cancer risk. The current results reveal that Tanzanian men who are single, widowed, or divorced have a lower risk of prostate cancer than married males (AOR=0.23,95%CI;0.103-O.553). These findings are not in line with previous research, and the inconsistency can be explained by spousal support in married men who might be more health-conscious and more likely to undertake routine medical check-ups, leading to detection bias due to earlier detection of Prostate cancer among married (Blumberg *et al*., 2014). This detection bias and increased risk of prostate cancer among men has also been widely reported in previous studies (Blumberg *et al*., 2014; Q. Chen *et al*., 2024).

5.2.3 Monthly income

Generally, health and monthly income or socioeconomic status are connected. Individuals with lower socioeconomic status have limited access to medical care and a high incidence of Prostate cancer and mortality (Saydah *et al*., 2013). Still, previous authors reported inconsistent results in various populations, including Canadians and the USA (Mackillop *et al.*, 2000; Williams & Horm, 1977). Parallel to this, it is not clear how monthly income and Prostate cancer incidence are related in the Tanzania population and many other African populations. In contrast, others indicated that the incidence of Prostate cancer rose with reduced monthly income and dropped as social and economic status or monthly income increased (Coughlin, 2020). The majority of these studies have been carried out in Western populations, with very few being carried out in Tanzania or nearby East African populations, where prostate cancer has lately increased in incidence. Using multivariate analysis modelling, this study indicated that lower monthly income among Tanzanian men increased their likelihood of developing prostate cancer (AOR= 0.51,95%CI:0.305-0.800). The findings suggest that Tanzanian men with lower incomes may have more difficulty getting medical

care, similar to previous research findings. Based on current findings, Tanzanian males with low monthly incomes have an elevated risk of prostate cancer due to continued exposure to environmental or occupational risk factors, limited access to medical care, and delays in diagnosis (Bosland, 2024; Rock *et al.*, 2023).

5.2.4 Education level

The relationship between education level and increased risk of prostate cancer remains debatable. While several previous studies have reported a significant association between education level and prostate cancer, others reported mixed findings (Hodge *et al.*, 2023).

Using a multivariate model, we found that Tanzanian men with lower education levels have a reduced risk of developing prostate cancer (AOR=0.44,95%CI:0.175-1.032). Though there is limited data from African populations, Similar findings were also reported in the American and Norwegian populations, where men with higher education levels were found to have an increased risk of developing prostate cancer (Al-Rammahy *et al.*, 2024; Mouw *et al.*, 2008; Nilsen *et al.*, 2000; Steenland *et al.*, 2004). This may appear to be at odds with overall health trends at first, but this is because of the "surveillance effect." Higher-skilled people are typically more health-conscious, get regular PSA testing, and are diagnosed with prostate cancer earlier (Bugoye *et al.*, 2019; Steenland *et al.*, 2004). The increased knowledge and availability of healthcare among well-educated people could result in a higher prevalence of prostate cancer cases among the case participants we selected. Additionally, the association between higher education levels and a greater risk of prostate cancer appears to be due to improved screening methods and early diagnosis rather than a higher biological risk (Bugoye *et al.*, 2019). These results highlight the need for focused interventions to increase access to cancer screening and preventive

healthcare for a variety of demographic groups, especially those with lower socioeconomic levels (Bugoye *et al*., 2019).

5.2.5 Lifestyle Risk Factors

Through bivariate logistic regression statistical modelling, the results of the research showed that while physical exercise seemed to be a protective factor, alcohol use and tobacco use were significant risk factors for prostate cancer. After adjusting for confounding variables in the multivariate model, physical activity remained protective, but alcohol use and tobacco smoking lost importance as predictors. It is thought that exercise lowers the risk of prostate cancer in several ways. Maintaining a healthy body weight lowers levels of circulating insulin and inflammation markers, both of which have been connected to cancer development. Exercise helps to achieve this. In addition, regular exercise strengthens the immune system and lowers oxidative stress, two things that help stop cellular damage that may otherwise encourage the development and spread of cancer (Benke *et al*., 2018; Friedenreich *et al*., 2010).

The preventive effects of exercise, however, are less consistent in some Asian research (Lee, 2003). This could be because of genetic changes altering the physiologic response to physical activity or variations in lifestyle factors. Although population variations point to the necessity for specific setting preventative strategies, in general, the findings underscore the significance of lifestyle factors like alcohol consumption and exercise to prostate cancer risk. In contrast to other demographics, research conducted in African communities has yielded inconsistent conclusions regarding the influence of alcohol and tobacco use on the incidence of prostate cancer. For example, Huncharek and colleagues indicated that smoking had a lesser association with risk than alcohol intake did (Huncharek *et al.,* 2010). This study is

not in agreement with other studies that found that alcohol is a significant predictor, and the current study indicates that variations in alcohol use patterns among cultures may have an impact on risk. Similar results were observed in studies conducted in Western populations (Platz *et al.*, 2004), which showed that moderate alcohol use was associated with decreased risks, while severe consumption of alcohol increased the risk of cancer (Platz *et al.*, 2004). On the other hand, varied genetic and environmental combinations may have contributed to the inconsistent associations between alcohol consumption and prostate cancer as previously reported in the Asian population (Lin *et al.*, 2021).

5.2.6 Dietary risk factors

Based on a complex interaction between many dietary components and prostate cancer risk, the current study findings offer insights into the dietary influencing factors connected to prostate cancer incidence.

5.2.6.1 Red meat consumption

According to previous studies, red meat has been linked to an increased risk of prostate cancer due to its ability to affect inflammation and the mechanisms underlying carcinogenic processes (Marshall, 2012). For instance, several studies have found a strong correlation between eating red meat and African men's advanced prostate cancer, underscoring the significance of nutrition as a modifiable risk factor for prostate cancer (Major *et al.*, 2011). Red meat is commonly consumed in Tanzania, and like many other African countries, dietary changes that increase red meat intake have been connected to an increasing incidence of prostate cancer (Nouri-Majd *et al.*, 2022). Epidemiologic data linking red meat consumption to prostate cancer risk in East Africa were found to be scant and inadequate to conclude the role of red meat in the current increase in prostate cancer incidence among Tanzanian

males. In the present study, it is demonstrated that consuming red meat frequently was linked to a higher risk of prostate cancer (AOR=7.884,95%CI:3.954-19.150). The results are in agreement with previous findings reported using African descendants. Similarly, in a study conducted using the American population, black Americans were found to have an increased risk of developing prostate cancer with increasing red meat intake (AOR= 2.0; 95% CI, 1.0-4.2) compared to those with less intake of red meat (Rodriguez *et al.*, 2006). The magnitude of these correlations, however, differs among populations and geographical areas. This variation highlights the importance of considering regional and cultural dietary customs when determining risk factors for prostate cancer.

In line with this, previous studies reported inconsistent results on associations between the red meat diet and the incidence of prostate cancer in various populations (Bylsma & Alexander, 2015). For instance, a comparative study that looked at the impact of red meat consumption in North America and other populations found a link between prostate cancer and consuming a lot of processed red meat, while others, revealed contradictory findings with various populations (Wu *et al.*, 2016). The findings in West Africa using a Nigerian population have reported a significant association between high red meat consumption and increased risk of developing prostate cancer (AOR= 1.74,95%, CI:0.59–5.17) (Ukoli *et al*., 2009). Similar results have been reported in the Asian population due to dietary shifts in Asia (Kimura & Egawa, 2018). In the last four decades, the intake of meat and animal fat has also increased in Chinese, Korean, and Japanese populations. These lifestyle changes could be the reason behind the recent rise in prostate cancer in the Asian population (Kimura & Egawa, 2018). Furthermore, studies on diet and cancer by the American Institute of Cancer Research found restricted evidence for a link between different

meats and the risk of prostate cancer. Discrepancy in results has also been noted in various populations (Punnen *et al.,* 2011). Differences in genetic susceptibility, food preparation methods, and preventive nutritional components such as antioxidants can all contribute to the variability in outcomes among cultures. Methodological inconsistencies in sample sizes, study designs, and confounding factor control further complicate comparisons and highlight the need for more comprehensive, region-specific research (Punnen *et al*., 2011).

5.2.6.2 Coffee intake

Similarly, a Multivariate logistic regression was used to control for potential confounding variables as it explored the relationship between the consumption of coffee and prostate cancer risk in Tanzanian men. Coffee consumption and the risk of prostate cancer were found to be statistically inversely related (AOR=0.57, 95% CI: 0.360- 0.874). This result is consistent with epidemiological research that indicates coffee drinking may have protective benefits against prostate cancer (Chen *et al.,* 2021; Wilson *et al.,* 2011). Caffeine, diterpenes, and polyphenols are bioactive substances in coffee that have anti-inflammatory, anti-carcinogenic, and antioxidant qualities that may be essential for the unfavourable correlation reported in this study (Nigra *et al.,* 2021).

These compounds are thought to affect important molecular processes that contribute to carcinogenesis, such as DNA repair mechanisms and apoptosis (Chen *et al.*, 2021). Also, considering the known connections between metabolic dysfunction and the risk of prostate cancer, coffee's possible ability to lower insulin resistance may be significant (Wilson *et al.,* 2020). It is necessary to consider Tanzanian population-specific genetic characteristics and cultural dietary practices when interpreting these data, even in light of the protective relationship. This study offers current

perspectives on the association between coffee consumption and prostate cancer in an African population. although research in the African population is limited, European Western and Asian populations have produced conflicting results in several studies (Imatoh *et al.*, 2022; Schmit *et al.*, 2020; Taylor *et al.*, 2017). Diversity in risk reduction between populations may be explained in part by differences in lifestyle factors, inadequate adjustment for covariates and genetic predispositions (Li *et al.*, 2013; L.-G. Zhao *et al.*, 2020)(Li *et al.*, 2013; Zhao *et al.*, 2020)

5.2.6.3 Tomato consumption

Prostate cancer has generated much interest in tomatoes and tomato-based goods like ketchup and purees. Lycopene, one of the primary carotenoids ingested in the Western diet, gives tomatoes their colour (Xu et *al.*, 2016). In addition to its potent antioxidant qualities, it might also contain extra DNA-repairing capabilities that could enhance its chemo preventive effects. Numerous investigations have revealed that lycopene has a preventive effect against prostate cancer (Chen *et al.*, 2021; Giovannucci, 2005; Wang *et al.*, 2024; Wilson *et al.*, 2011). Thus, in light of the dearth of research and the high cancer incidence burden in Tanzania, we aimed to conduct a case-control study to find out the correlation between lycopene and tomato consumption and prostate cancer risk.

The present study demonstrated a significant inverse association between a higher tomato intake and reduced prostate cancer risk (AOR=0.174, 95% CI: 0.079- 0.330). The results reveal an inverse relationship, meaning that people who eat tomatoes have a lower risk of developing prostate cancer than individuals who do not. Thus, this study supports previous research findings (Wang *et al.*, 2024). The influences of tomato on reducing risk of prostate cancer have yielded inconsistent results in other populations (Luo *et al.*, 2021). Although there is little data linking tomato

consumption to an increased risk of prostate cancer in the African population, the subgroup analysis by geographic region showed that tomatoes may prevent prostate cancer in Asians (RR 0.43, 95% CI 0.22–0.85, P = 0.015) and Oceania (RR 0.81, 95% CI 0.67–0.99, P = 0.035), but not in other populations. Consuming tomatoes may, in general, protect against prostate cancer. The study design, participant selection, and extreme heterogeneity all contribute to the reported conflicting outcomes (Luo *et al.*, 2021).

5.2.6.4 Soya Consumption

The current study used multivariate analysis to adjust for possible confounding variables and evaluated the association between Tanzanian male' consumption of soy and their risk of prostate cancer. With odds (AOR=0.285,95%CI: 0.132-0.552), the results indicated a strong negative correlation between soy consumption and the risk of prostate cancer. This conclusion supports an earlier study on the possible anticancer effects of soy-based foods (Applegate *et al*., 2018; C. Wang *et al.*, 2024) and implies that increased soy consumption may be preventive against the development of prostate cancer.

The preventive effect of soy is often attributed to its high isoflavone content, which includes phytoestrogens like genistein and daidzein (He *et al.*, 2015). These compounds can attach to estrogen receptors and provide estrogen-like effects, which may alter the processes linked to the emergence of prostate cancer (Pejčić *et al.*, 2023). Isoflavones have been demonstrated to have anti-inflammatory, apoptosis-promoting, and cell-proliferation-inhibiting activities, all of which may help explain their potential to prevent cancer (Pejčić *et al.*, 2023). The current research supports these concepts and adds to the body of evidence supporting soy's protective effects against prostate cancer. Also, it is worth noting that the observed inverse correlation

maintained well even after controlling for other dietary factors. This indicates the potential of dietary changes as a prostate cancer preventative measure and implies that the protective effect of soy is independent of other dietary factors (Applegate *et al*., 2018).

However, despite the strong evidence in this study, it is important to take into account the dietary and cultural customs that are specific to Tanzanians. Tanzanians' frequency and type of soy consumption may be very different across the population the majority of previous studies have been carried out (Applegate *et al.*, 2018).

Although the present study provides convincing proof, previous studies on the association between soy diet and prostate cancer risk are still inconclusive across several populations, including those in East Africa. Additionally, soy has been demonstrated in many studies to have preventive effects, especially in Asian populations where it is a staple food. The evidence from African groups, including those in East Africa, is inconclusive and limited (Wang *et al.*, 2024). Soya is a less common part of the customary diet in East Africa, where dietary patterns differ greatly from those in Asia (He & Chen, 2013). Furthermore, other dietary and lifestyle characteristics that are common in the area, like consuming more red meat and fewer fruits and vegetables, may make the links between soy consumption and Prostate cancer risk more difficult to interpret (Yan & Spitznagel, 2009).

The impact of soy consumption on the incidence of prostate cancer may be influenced by cultural and socioeconomic factors, as well as genetic variability among people, making generalisations from different countries more difficult (Schulman & Zlotta, 2003). This study additionally underscores the importance of conducting research

specific to a specific area to develop culturally relevant dietary recommendations and preventative measures.

5.2.7 Diseases

5.2.7.1 Gonorrhoea

This study explored the strong association between a history of gonorrhoea and an elevated risk of prostate cancer in Tanzanian males (AOR=6.52,95%CI:1.71-42.75). This finding supports the theory that chronic inflammation driven by infections may play a role in the development of prostate cancer by highlighting a possible connection between STIs and the disease (Caini *et al.,* 2014; Vázquez-Salas *et al.,* 2016) The results support the concept that chronic inflammation in the prostate might lead to genetic changes in and promote an environment conducive to tumour development (Archer *et al.,* 2020; Stark *et al.,* 2015). Inflammation of the urogenital tract is recognized to be a biological effect of gonorrhoea, which is caused by Neisseria gonorrhoeae. Repeated or uncontrolled infections can cause the prostate to experience protracted inflammatory reactions, which raises the possibility of DNA damage and encourages the development of cancer (Archer *et al.,* 2020; Stark *et al.,* 2015). Additionally, the function of cytokines and other inflammatory mediators can accelerate cellular alterations that promote the growth of prostate cancer (Stark *et al.,* 2015). The considerable odds ratio in this study highlights how significant these effects could be among Tanzanian men. Although there is a paucity of data from African populations, the majority of comparable studies have been documented in developing countries. There are discrepancies in the intensity of the link between present findings and those from other populations. Though the OR of 6.517 is currently high, studies of Asian and Western populations have found lower odds ratios, usually in the range of 1.5 to 3.0 (Lian *et al.,* 2015; Stark *et al.,* 2015).

Variations in gonorrhoea prevalence, genetic background, and access to healthcare may be the cause of these discrepancies. For example, more severe or frequent infections may be experienced by African populations, which might exacerbate inflammatory processes and raise the risk of cancer (Lian *et al*., 2015). In contrast, weaker relationships are frequently observed in populations with more effective STI prevention and treatment efforts (Lian *et al*., 2015; Vázquez-Salas *et al.,* 2016).

There is still conflicting evidence in the literature about the relationship between STIs like gonorrhoea and prostate. These disparities imply that lifestyle, environmental, and genetic factors may have varying effects on prostate cancer risk in different populations (Lian *et al., 2015).* Additionally, differences in study design, sample size, and STI exposure assessment accuracy may be factors in the discrepancies seen in the literature. Accordingly, the current study highlights the important role of region-specific research to iron out these discrepancies and gain a better understanding of how gonorrhoea affects the risk of prostate cancer in African contexts.

5.3 ERG and PTEN tumour molecular subtypes

5.3.1 Prevalence of ERG and PTEN Immunoexpression and Tumour Characteristics

The expression patterns of *ERG* and *PTEN* in the Tanzanian prostate cancer patients' study population were examined to gain a better understanding of their roles in the progression of prostate cancer. *PTEN* loss was found in 98.8% of tumours, whereas *ERG* expression was observed in 30.1% of cases. The relevance of *PTEN* loss in prostate cancer biology is shown by the noteworthy fact that just 1.2% of tumours maintained the expression of *PTEN* in the absence of *ERG*. *ERG*-positive was more prevalent in lower-grade tumours, while *PTEN* loss rose as Gleason scores rose, indicating that *PTEN* is linked to more aggressive illness. These findings align with trends observed in other African populations but differ significantly from those reported in non-African studies, highlighting potential geographic and genetic variations in prostate cancer molecular profiles. For example, studies in African populations have reported varying rates of *ERG* expression. In South Africa, RNA sequencing revealed an *ERG* expression rate of 13% among Black men, while RT-PCR studies reported rates of 25% in African ancestry populations and 18% in Ghana (Blackburn *et al.*, 2019). However, an immunohistochemistry (IHC) study conducted in Uganda revealed a significantly increased *ERG* expression rate of 75.4%, which may indicate variations in cancer biology, detection sensitivity, or study demographics Mitala *et al.,* 2024).

Outside of Africa, fluorescence in situ hybridisation (FISH) investigations in Canada found *ERG* expression in 41.5% of cases, while IHC reports of *ERG* positive in Brazil and Switzerland indicate that *ERG* is present in 39.6% of cases and 35.5% of cases, respectively (Rupp *et al.*, 2024; Yorioka *et al.*, 2024). These differences could be caused by variations in genetic predispositions, environmental variables, and

detecting techniques. Many African populations have comparatively lower *ERG* expression, which may be due to differences in androgen receptor signalling pathways and a lower frequency of *TMPRSS2-ERG* gene fusions, which are the main cause of *ERG* overexpression (Bismar *et al., 2012)*. In this study, *PTEN* loss was the most prevalent molecular change, occurring in 98.8% of patients and rising dramatically with tumour grade. This result is consistent with other studies conducted in the Jewish Hospital in Montreal, Canada, where *PTEN* deletion was identified by FISH in 63.6% (Bismar *et al.,* 2012). Other research, however, has used IHC to reveal lower percentages of *PTEN* deletion, such as 12.6% and 25% (Krohn *et al.,* 2012). In comparison, the Asian population (20–50%) and European populations (40–60%) are generally less likely to experience *PTEN* loss. One possible explanation for the more aggressive clinical presentation of prostate cancer frequently seen in African cultures is the high prevalence of *PTEN* loss in these populations, resistance to treatment and high proliferation (Hashemi *et al.,* 2023). These findings underscore the potential importance of targeting the PI3K/AKT pathway in prostate cancer treatment, particularly in African patients, given *PTEN*'s critical role in tumour proliferation and therapy resistance (Hashemi *et al.,* 2023).

Different molecular subtypes of prostate cancer in Tanzanian patients have been identified by the high frequency of *PTEN* loss in high-grade tumours and the reported decrease in ERG positivity with increasing Gleason score (McMenamin *et al.,* 1999; Trock *et al*., 2016). Through its negative regulation of the PI3K pathway, *PTEN* suppresses tumours; its loss frequently results in the overexpression of downstream targets, including Akt and mTOR. In primary prostate tumours, increased phosphorylation levels of Akt and S6 ribosomal protein (S6RP), a downstream target of mTOR, have been found, suggesting that *PTEN*-deficient malignancies activate

both pathways (Phin *et al*., 2013; W. Zhang *et al.,* 2009). Thus These results demonstrate the possible therapeutic advantages of Akt and mTOR targeting in Tanzanian prostate cancer patients (Phin *et al.*, 2013). Clinical studies have shown that mTOR inhibitors, such as rapamycin analogues (e.g., ridaforolimus and temsirolimus), exhibit antitumour effects in prostate cancer (Phin *et al.*, 2013; Zhang *et al*., 2012).

5.3.2 ERG and PTEN Tumour Molecular Subtypes

The present study addresses knowledge gaps in the literature by providing the first research data on tumour molecular subtypes of prostate cancer in the Tanzanian population. The current findings reveal that 68.68% of tumours were classified as *ERG*-negative/*PTEN*-negative molecular subtypes, while 30.12% demonstrated the *ERG*-positive/*PTEN*-negative tumour subtype. Only one case (1.2%) demonstrated *ERG*-negative/*PTEN*-positive immunostaining, and no tumours were identified as *ERG*-positive/*PTEN*-positive. The distribution of *ERG*-negative/*PTEN*-negative and *ERG*-positive/*PTEN*-negative subtypes was notably balanced in tumours with lower Gleason scores (≤7). Higher Gleason scores, on the other hand, were primarily linked to the *ERG*-negative/*PTEN*-negative profile (4+4, 4+5, 5+4 and >9), highlighting a clear correlation between this subtype and more aggressive tumours. These findings are consistent with earlier research conducted on the Greek population (Lahdensuo *et al*., 2016), which provides more evidence that aggressive disease is caused by *ERG*-negative/*PTEN*-negative tumours. The significance of these findings is further highlighted when compared to data from other regions, which reveal substantial variability in tumour molecular subtypes across populations. For example, a UK study using a similar immunohistochemical (IHC) technique revealed that *ERG*-negative/*PTEN*-positive tumours accounted for 54.22%

of all tumours, with less common subtypes being *ERG*-positive/*PTEN*-positive (27.59%), *ERG*-positive/*PTEN*-negative (12.01%), and *ERG*-negative/*PTEN*-negative (6.16%) (Reid *et al.*, 2010). This distinct molecular subtype suggests that different oncogenic pathways may dominate in the Tanzanian population, potentially leading to unique clinical behaviours compared to other populations, such as those reported in the UK (Reid *et al.*, 2010). Brazilian research, on the other hand, that used fluorescence in situ hybridisation (FISH) to determine *PTEN* and *ERG* expression found different molecular patterns. In one Brazilian study, *ERG*-positive/*PTEN*-positive cancers comprised 26% of all tumours, followed by *ERG*-negative/*PTEN*-negative tumours at 17%, ERG-positive/*PTEN*-negative tumours at 23%, and *ERG*-negative/*PTEN*-positive tumours at 29% (Yoshimoto *et al.*, 2008). Similarly, 21.8% of tumours were found to be *ERG*-positive/*PTEN*-negative in a Canadian study using the FISH method (Yoshimoto *et al.*, 2008). In another Brazilian study using immunohistochemistry (IHC), the *ERG*-positive/*PTEN*-negative subgroup was present in 33% of the subjects. These differences not only highlight the impact of different methods of detection (IHC versus FISH), but they also suggest that genetic, ethnic, and environmental factors may play a part in determining the biology of tumours.

In the Tanzanian population, the *ERG*-negative/*PTEN*-negative subtype was more common, especially among high-grade tumours. This shows that the concurrent loss of *PTEN* and *ERG* may be a key factor in the development of aggressive prostate cancer in the Tanzania population. This finding is consistent with increasing evidence demonstrating that molecular changes have a major impact on tumour behaviours and prognosis (Spinos *et al.*, 2023). In populations with a higher prevalence of other molecular subtypes, as in the UK, different oncogenic processes may control the

course of the disease, resulting in different clinical results (Adamo & Ladomery, 2016; Voulgari *et al.*, 2023) . These results highlight the significance of taking population- and region-specific tumour molecular subtypes into consideration when establishing prostate cancer treatment and diagnostic approaches.

5.4 Mutation Spectrum of Homologous recombination and Mismatch Repair Genes

5.4.1 Homologous recombination Repair Gene Mutation

i. *ATM* Mutation

Prostate cancer and other hereditary cancers have been linked to the *ATM* gene, which is an essential part of homologous recombination repair pathways (Karlsson *et al.*, 2021). The current study found several missense variants of the *ATM* gene, including two variants of uncertain significance *ATM* c.2442C>A, (p.Asp814Glu), and *ATM* c.1541G>A, (p.Gly514Asp) and two likely pathogenic variants *ATM* c.131A>G, (p.Asp44Gly) and *ATM* c.2289T>A, (p.Phe763Leu), while others reported missesnce variants were classified as likely benign. Notably, among 30 Tanzanian prostate cancer patients involved in germline next-generation sequencing, the likely pathogenic variants *ATM* c.131A>G and c.2289T>A each accounted for 3.33% of all reported mutations. These results are in line with earlier studies conducted in Uganda, where individuals with prostate cancer had a similarly low frequency (3.6%) of the c.131A>G variant (Gheybi *et al.*, 2023). In addition, this study reports *ATM* c.2442C>A and *ATM* c.1541G>A as variants of uncertain significance, while the previous study using different ethnic groups has classified these variants as likely benign in cases of hereditary breast and ovarian cancer (da Costa e Silva Carvalho *et al.*, 2020). These likely pathogenic mutations found in this study, *ATM* c.131A>G and c.2289T>A, have been reported to have effects beyond prostate cancer. For instance, the *ATM* c.2289T>A variant has been identified as a

variant of uncertain significance in African American patients with haematological and breast cancer (Bishop *et al.*, 2020; Paglia *et al.*, 2010). While *ATM* mutations are generally more common in African communities than in Western populations, there is growing evidence that genetic variation may have an impact on the occurrence of mutations and clinical effects (Mahal *et al.*, 2020). Genomic instability is frequently observed in tumours deficient in *ATM* gene function, which raises neoantigen burdens and may increase immunogenicity. This could potentially render a patient more vulnerable to immune checkpoint inhibitors (Bugoye *et al.*, 2023; Gulliver *et al.*, 2022; Hu *et al.*, 2021).

Along with immunotherapy, targeted treatments such as PARP inhibitors (e.g., rucaparib and olaparib) have demonstrated potential in the treatment of prostate cancers with *ATM* gene mutations and other homologous recombination repair deficits (de Bono *et al.*, 2020; Neeb *et al.*, 2021). However, due to likely different DNA repair pathways, *ATM*-mutated tumours had poorer response rates to PARP inhibitors than *BRCA2*-mutated malignancies (Tomasik *et al.*, 2021). Alternative strategies, like ATR and DNA-PK inhibitors, have been studied in ongoing studies to take advantage of weaknesses unique to tumours with *ATM* deficiency (Qian *et al.*, 2024). Also, in cases of aggressive or advanced prostate cancer with *ATM* mutations, combination therapy offers a possible path towards better results. For example, like many other solid tumours, including prostate cancer, combining PARP inhibitors with immune checkpoint inhibitors may synergistically enhance treatment efficacy by targeting both DNA repair deficiencies and immune evasion pathways (Mateo *et al.*, 2020; L. Zhou *et al.*, 2024)

This study underscores the importance of characterising *ATM* mutations across diverse populations. While the prevalence and impact of these mutations align with global trends, their unique features in African populations remain underexplored (Bugoye *et al.,* 2023). Addressing this gap is important for developing therapeutic strategies tailored to genetic diversity. Finally, the current studywarrants further research on integrating genomic insights with targeted therapies as it holds potential for advancing personalised medicine and improving outcomes for patients with *ATM*-mutated prostate cancer in the Tanzanian population.

ii. *BRCA1* Mutation

In this study, two damaging *BRCA1* variants of uncertain significance, specifically *BRCA1* c.557 C>A and *BRCA1* c.1137T>G, have been identified. These variants offer valuable insights into the genetic spectrum and its potential therapeutic implications among Tanzanian prostate cancer patients. *BRCA1* plays a significant role in the homologous recombination repair pathway, and mutations in this gene may compromise DNA repair, driving tumorigenesis (Bugoye *et al.,* 2023). Different studies have reported varying clinical significance for these *BRCA1* variants. For example, *BRCA1* c.557C> A has been classified as benign in ovarian and breast cancer (Lindor *et al.,* 2012), while *BRCA1* c.1137T>G was reported as an unclassified variant in a multicenter study (Borg *et al.,* 2010). There is still limited information on how different *BRCA* variants influence responsiveness to PARP inhibitor therapy. Patients with these Variants of Uncertain Significance, *BRCA1* c.557 C>A and c.1137T>G, do not meet all the criteria for classification as pathogenic or likely pathogenic, but they may be reclassified over time. Therefore, they are currently not eligible for treatment based on *BRCA*-targeted therapies, and their family members do not qualify for genetic counselling (Zannini *et al.,* 2025).

However, if the identified variants are later reclassified as pathogenic or likely pathogenic, patient management will be adjusted accordingly, and *BRCA* testing for at-risk relatives is recommended to guide surveillance and preventive measures (Mersch *et al.*, 2018; Zannini *et al.*, 2025). Thus, the findings of this study warrant further research investigating how these identified variants of uncertain significance impact immune evasion mechanisms and could open new avenues for combining PARP inhibitors with immune checkpoint blockade therapies, offering a dual approach to treatment. (Borg *et al.*, 2010; Federici & Soddu, 2020; Hart *et al*., 2019).

iii. *CHEK2* Mutation

The current study discovered that two of the thirty Tanzanian prostate cancer patients involved in this study had the *CHEK2* c.1343T>G mutation. This discovery adds to the increasing amount of data pointing to a potential role of this variation in the increasing risk of prostate cancer. In this study, with currently available evidence, the reported variant was classified as a variant of uncertain significance, but it has been previously reported to occur more frequently and is linked to a threefold increased risk of prostate cancer in males of African origin and higher risk of breast cancer among European women (McCarthy-Leo *et al.*, 2024; Reap *et al.*, 2018; Southey, Goldgar, *et al.*, 2016). These results underline the importance of further studies on the prevalence and functional significance of the *CHEK2* c.1343T>G variation in Tanzanians. Considering that *CHEK2* plays a role in DNA damage repair (Stolarova *et al*., 2020). The variant *CHEK2* c.1343T>G (p.Ile448Ser) may impact genomic stability, resulting in altered immune recognition and a greater tumour mutational burden. Thus, targeted therapies may be improved by understanding their effect on therapy sensitivity or resistance, especially to immunotherapies and PARP inhibitors (Bugoye *et al.*, 2023; Southey, Goldgar, *et al*., 2016).

iv. ***TP53* Mutation**

The *TP53* gene functions as an important regulatory control over cell proliferation and homeostasis (Bugoye *et al.,* 2023). In line with this, previous research has indicated that *TP53* mutations are more common in advanced and aggressive prostate cancer and have variable frequencies in different populations (Maxwell *et al.,* 2022; W. Zhang *et al.,* 2022). In the current study, the *TP53* c.917G>A variant of uncertain significance mutation was reported. The reported variant has been reported in previous studies and linked to several types of cancer. In the United States, *TP53* c.917G>A mutations are non-functional in Li-Fraumeni Syndrome (LFS), a disorder that is frequently treated with radiation caution and surveillance methods (de Andrade *et al*., 2019). Though this study classifies as a variant of uncertain significance, it adds to the increasing amount of evidence which suggests the potential role of the variant in the increasing risk of cancer (Bugoye *et al.,* 2023; de Andrade *et al.*, 2019). Since *TP53* plays a role in both tumour suppression and genomic integrity, this *TP53* c.917G>A reported variant might accelerate the growth of prostate cancer, therefore, it warrants further functional analysis to determine its significance in Tanzanian prostate cancer patients and establish whether their tumours react differently to targeted treatments, such as immunotherapies and PARP inhibitors (Bugoye *et al*., 2023; Mirgayazova *et al*., 2024; Ofner *et al*., 2025).

5.4.2 Mismatch repair gene mutation

i. *PMS2* Mutation

In this study, the missense variant *PMS2* c.497T>C (p.Leu166Pro) variant has been found as a likely pathogenic mutation. The current finding highlights the important function that DNA mismatch repair genes play in prostate cancer risk. The reported likely pathogenic mutation accounts for 0.41% of the mutations observed in this study

and is consistent with *PMS2*'s known function in maintaining genomic integrity by fixing replication mistakes (Bugoye *et al*., 2023). The results are in agreement with other studies using African Americans that highlight the significance of *PMS2* mutations in African descendants with prostate cancer, where this variant was found significantly predictive of pathogenicity (Beebe-Dimmer *et al.*, 2018). In addition, the variant has also been identified as a variant of unknown significance with colorectal cancer in the Hispanic Black community (Yurgelun *et al.*, 2017). This suggests that the clinical significance of *PMS2* mutations may vary across different ethnic groups and cancer types. Though the frequency of specific mutations can differ due to genetic diversity and factors unique to certain populations (Bugoye *et al*., 2023; Kohaar *et al*., 2022), Asian populations have a lower prevalence of mismatch repair variants than African populations, where these mutations are less studied but increasingly identified for their role in aggressive disease phenotypes (Bugoye *et al*., 2023; Song & Byun, 2021). Furthermore, PMS*2* mutations are more frequently linked to Lynch syndrome-related cancers in both European and American populations; they have been shown to play a role in prostate cancer, especially when the disease is advanced and treatment is not working (Riaz *et al.*, 2024). For example, it has been previously reported that *PMS2* variations are a component of a larger mutational landscape that contributes to aggressive tumours and influences their sensitivity to targeted treatments (Riaz *et al*., 2024). Similarly, immune checkpoint inhibitors such as pembrolizumab may be used to treat tumours with *PMS2* mutations since these tumours frequently exhibit microsatellite instability (MSI), which increases the neoantigen burden and improves immunogenicity (Kohaar *et al*., 2022; Lenis *et al.*, 2024). The FDA has approved this drug for a variety of cancer types with tumours that have a high MSI or deficient mismatch repair. However, homologous recombination repair deficits in prostate

cancer may be treated using PARP inhibitors like olaparib and rucaparib in the treatment of prostate cancer. Ongoing trials are investigating the use of these drugs in mismatch repair-deficient tumours, such as those with *PMS2* mutations, even though their primary target is *BRCA1/2*-mutated cancers (Zacchi *et al.*, 2024).

Furthermore, the comparative analyses of *PMS2* mutations across global populations show both shared trends and unique characteristics, emphasizing the critical role of immunotherapies and diverse genomic studies in addressing inconsistencies in prostate cancer care (Kohaar *et al.*, 2022; Lenis *et al.*, 2024). The identification of the *PMS2* c.497T>C mutation in Tanzanian prostate cancer patients underscores the potential for personalised medicine through targeted therapies, such as immune checkpoint inhibitors. However, further research is essential to validate these findings through functional assays and to develop novel therapeutic strategies specifically designed for mismatch repair-deficient tumors.

ii. ***MSH2* mutation**

Out of the 30 prostate cancer samples analysed by targeted next-generation sequencing, the *MSH2* c.380A>G (p.Asn127Ser) mutation of unknown significance was reported in this study. The results point to a significant gap in clinical interpretation and genetic research among under-represented populations, including Tanzanians (Bugoye *et al.*, 2023). The results indicate the importance of population-specific research to elucidate this variant's function in the development of prostate cancer and its response to treatment. Similarly, this finding emphasises the need for functional analysis to understand the function of the reported variant and its effects on precision therapy, even though current clinical guidelines do not recommend targeted therapies based solely on variants of uncertain significance classifications, limiting treatment options for patients with these variants (Drost *et al.*, 2019; Mahdouani *et*

al., 2024). Although studies have found inconsistent classifications among ethnic groups, there is insufficient information on *MSH2* mutations in African populations (Bugoye *et al.*, 2023). *MSH2* c.380A>G in Caucasians has been seen as pathogenic and probably benign (Limburg *et al.*, 2010). This is backed by studies conducted in Hungary, Finland, Canada, and Denmark that have classified *MSH2* c.380A>G variations as either variants of uncertain significance or likely benign, demonstrating the influence of genetic background on variant interpretation (Christensen *et al.*, 2008; Samowitz *et al.*, 2001; Tanyi *et al.*, 2006). In line with this, a Finnish study found that the same variant significantly lowered DNA repair function when it co-occurred with another *MSH2* mutation, indicating that its effects might vary depending on the genetic environment (Kantelinen *et al.*, 2012). The clinical importance of *MSH2* c.380A>G in Tanzanian patients must be established through functional analysis, as these variations indicate (Drost *et al.*, 2019; Mahdouani *et al.*, 2024). Determining its role in deficiencies in DNA repair may facilitate its application as a biomarker for identifying patients who might profit from targeted therapies, including immune checkpoint inhibitors, which work well for tumours with impaired DNA repair systems (Bugoye *et al.*, 2023; Silva *et al.*, 2022). The dearth of African genetic information in global databases limits access to precision medicine and delays the reclassification of variants of uncertain significance. The need for population-specific research to improve cancer care in Tanzania is emphasised by this study.

5.5 The association between Risk factors, Gene mutation, subtypes and Gleason score

5.5.1 Association between Age, family history of prostate cancer and Gene mutation

This study's findings underscore the critical role of age and prostate cancer family history in determining prostate cancer characteristics, offering insights for risk stratification and therapeutic applications (Barber *et al.*, 2018; N. J. Graham *et al.*, 2025). The current study demonstrated that Patient ID 44 (age 53) and ID 16 (age 59) with a family history of prostate *cancer* both had *ERG+/PTEN*- tumours molecular subtypes with Gleason scores ≥4+4, aligning with early-onset, high-risk characteristics tied to genetic predisposition (Bugoye *et al.*, 2023). The observed link between younger age, familial prostate cancer history, and aggressive tumour molecular characteristics strongly supports the hypothesis that genetic predisposition drives early-onset prostate cancer (Barber *et al.*, 2018; Bleyer *et al.*, 2020; Bugoye *et al.*, 2025). For instance, the occurrence of likely pathogenic mutations *ATM* c.131A>G and PMS*2* c.497T>C in younger patients with prostate cancer family histories aligns with broader evidence linking germline mutation to high-risk prostate cancer (Bataba *et al.*, 2024; Bugoye *et al.*, 2023). These age-associated patterns reported in this study, align with global epidemiological trends, where African populations exhibit increased frequencies of aggressive and early-onset disease linked to *BRCA* mutations, while American and European studies highlight familial history with prostate cancer as a significant predictor, especially in cases with higher Gleason scores (Bugoye *et al.*, 2023; Hinata & Fujisawa, 2022; Lowder *et al.*, 2022). However, due to different study designs and recruitment criteria, other studies have reported inconsistent results between age at diagnosis and prostate cancer aggressiveness (Pettersson *et al.*, 2018).

This study also classified likely pathogenic *ATM* c.131A>G and *ATM* c.2289T>A mutations in patients with a family history of prostate cancer, aligning with findings in the European, Asian and across populations and highlighting shared genetic susceptibilities across diverse groups (Bugoye *et al.*, 2023; Karlsson *et al.*, 2021). Furthermore, age at diagnosis and family history provide insights for risk stratification; however, gaps remain in understanding how variants of uncertain significance affect outcomes, particularly in older patients (Chen *et al.*, 2023; Mighton *et al.*, 2021). These findings underscore the potential of gene mutation analysis in guiding personalised treatments, especially for younger patients with family histories of prostate cancer. The study's findings warrant further exploration in multi-ethnic studies to enhance our understanding of gene-environment interactions and variants of uncertain significance and validate results across diverse populations (Chen *et al.*, 2023; Mighton *et al.*, 2021). Investigating targeted therapies for *ATM* and *PMS2* mutations could also revolutionise care for high-risk prostate cancer patients, promoting the incorporation of genetic insights into clinical practice in Tanzania (Kaur *et al.*, 2020; Riaz *et al.*, 2024).

5.5.2 Association between Gene Mutation, Gleason Score and Tumour Subtypes

The current study reveals critical interdependencies between gene mutations, Gleason score, and tumour molecular subtypes in prostate cancer, with implications for precision oncology. The association between high Gleason scores (≥4+4) and likely pathogenic mutations in DNA damage repair genes *ATM* c.131A>G and *PMS2* c.497T>C aligns with an American study indicating that tumours with higher Gleason scores harbour significantly more mutations, particularly in advanced grade groups (Marshall *et al.*, 2019). Similarly, the current study highlights distinct prostate cancer subtypes associated with specific gene mutations. Patients with likely pathogenic

mutations in *ATM* c.131A>G (Gleason 5+5) and *PMS2* c.497T>C (Gleason 4+4) exhibited aggressive *ERG+/PTEN*- tumour subtypes; these results are consistent with previous studies suggesting a role of likely pathogenic gene mutations in driving aggressive disease (Bugoye *et al.*, 2023, 2025; Esteban-Villarrubia *et al.*, 2024; Lukashchuk *et al*., 2023). The current findings indicate that there is no direct link between *ATM* and *BRCA1* mutations classified as variants of uncertain significance and aggressive prostate cancer phenotypes, and these findings underscore the need for further studies to better understand the potential role of these mutations in prostate cancer progression. Improving the classification of variants of uncertain significance in *ATM* and *BRCA1* genes, as well as expanding research efforts, could provide more clarity regarding their impact (Boujemaa *et al.*, 2024).

5.5.3 The association between ERG expression and Gleason score

In the present study, the immunohistochemistry expression of *ERG*, its surrogate marker, demonstrated that the fusion gene using Tanzanian prostate cancer patients was found in 30.12% of the archived block tissue samples. These results are in line with studies from the American population that were obtained using comparable techniques and archival materials, which showed that 28.6% of prostate cancers had *ERG* expression. The Chinese and Indian populations showed similar results, with *ERG* expression rates of 33% and 27%, respectively. Studies in South Africa and Ghana found that the prevalence of the fusion gene was 13% and 18%, respectively, although the current study found a comparatively higher incidence than other African groups (Blackburn *et al.*, 2019; Zhou *et al.*, 2017). Many factors could account for the differences, but the most significant ones are genetic differences between West Africans, South Africans, and East Africans as well as variations in *ERG* detection techniques (Mitala *et al.*, 2024), For example, a study in South Africa used the real-

time polymerase chain reaction method (RT-PCR) rather than the immunoassay-based IHC that we reported in the current study (K. Park *et al.*, 2010).

Using chi-square analysis methods, the present study revealed a strong association between *ERG* expression and Gleason score (categorised in Classes) (P=0.02269), with prostate cancers that expressed *ERG* statistically substantially being linked to a lower Gleason score, whereas most tumours with a higher Gleason score did not express *ERG*. These results align with other research that used Ghanaian and Egyptian populations in Afro, America, and West and North Africa and revealed that *ERG* expression was strongly linked to lower Gleason scores (Abdel-Rahman & Habashy, 2020; Farrell *et al.*, 2014). The association between *ERG* expression and lower Gleason scores in prostate cancer reflects the unique molecular profile of *ERG*-positive tumours. *ERG* rearrangements, which are usually early oncogenic events, are associated with lower-grade patterns in well-differentiated tumours that have maintained glandular structures (Hughes *et al.*, 2005; Tomlins *et al.*, 2008). *ERG*-negative tumours, on the other hand, are frequently linked to other genetic changes, including *PTEN* deletion, which results in higher Gleason scores and more aggressive tendencies (Shah *et al.*, 2015). The association between *ERG*-positive tumours and less aggressive illness and better clinical outcomes is further influenced by population-specific and ethnic characteristics (Lowder *et al.*, 2022). In line with this, there have been conflicting findings in the European Afro-America community and the neighbouring country of Uganda, where there was no association between *ERG* expression and Gleason score. These discrepancies may be explained by variations in study design, and participant selection could influence this correlation (Farrell *et al.*, 2014). Additionally, according to the meta-analysis, there was no statistically significant association between the Gleason score and *ERG* expression claiming that

there was little proof that race affected these associations (Bhanushali *et al.,* 2018; Farrell *et al*., 2014). The association that has been found between Gleason classification and *ERG* expression highlights the possibility of using *ERG* as a biomarker for tumour aggression. High-grade tumours seem to be more likely to develop in patients with negative *ERG* expression, which suggests that *ERG* negativity may be a predictor of more aggressive illness. Furthermore, the variation in the prevalence of *ERG* expression and its correlation with cancer grade among groups emphasises how crucial it is to take regional and ethnic variations into account when developing diagnostic and treatment approaches that target the *ERG* pathway**.**

5.5.4 The association between the Gleason Group and Pre-operative PSA

Linear regression analysis in this study shows a significant association between pre-operative PSA levels and Gleason groups in patients with prostate cancer (p-value = 0.000). These results demonstrate that among Tanzanian prostate cancer patients, higher Gleason groups are strongly associated with rising pre-operative PSA values at diagnosis. In addition to being a diagnostic biomarker, this implies that pre-operative PSA offers important information about the aggressiveness and grade of the tumour.

The consistent findings in African contexts, further highlight PSA's potential as a predictive tool for determining the severity of prostate cancer and directing treatment plans in the Tanzanian population (Makau-Barasa *et al.,* 2022). Similar findings have been repeatedly documented by comparative studies conducted in various regions. For example, Okolo et al. (2008) found a significant association between advanced Gleason groups and raised pre-operative PSA levels in Nigeria, relating higher PSA levels to more aggressive tumour characteristics (Okolo *et al*., 2008). The link between PSA and advanced disease was further supported by Lojanapiwat et al.

(2014)(Lojanapiwat *et al.*, 2014), who found that patients in Thailand with Gleason scores ≥7 had noticeably higher PSA values. In North America, Pinsky et al. (2007) discovered that patients with higher Gleason groups had considerably higher pre-operative PSA levels, supporting PSA's potential to predict aggressive prostate cancer (Pinsky *et al.*, 2007). Additionally, Pierorazio *et al.* (2009) used Cox regression statistical models in a study conducted in Colombia to show a significant association between pre-operative PSA and Gleason score (Pierorazio *et al.*, 2009). There is not enough data to contradict this relationship, and more research is necessary to find any underlying causes or possible variability that would make it less strong. This information gap emphasises the necessity of more research that looks into how methodological, environmental, or genetic variations may affect the link that has been discovered.The findings highlight PSA's potential in estimating the severity of prostate cancer and directing therapeutic choices. While filling in the knowledge gaps about apparent inconsistencies, more research is advised to investigate the processes underlying this association and evaluate PSA's prognostic value in bigger, multi-regional cohorts.

5.5.5 The association between the Gene mutation and Pre-operative PSA

The significant association between mutations in the ATM and PMS2 genes and elevated pre-operative PSA levels in Tanzanian prostate cancer patients ($p = 0.036$) suggests that these genetic alterations may be markers of more aggressive disease. Similarly, research involving men of European and African ancestry, including Ugandan and African American cohorts, found that pathogenic variants in ATM significantly increase the risk of aggressive prostate cancer, suggesting these mutations contribute to more severe disease phenotypes that often present with elevated PSA levels (Matejcic et al., 2020)(Grochot et al., 2023). Similarly, PMS2

mutations have been implicated in genomic instability, elevated PSA levels and higher biochemical recurrence rates in prostate cancer (Wilczak et al., 2017). Although MLH1 mutations are generally classified as benign, our study uniquely identified a significant correlation between MLH1 and PSA levels ($p = 0.0178$). Though a previous study found MLH1's role is primarily through epigenetic modifications rather than germline mutations, which is not common in prostate cancer (Nourmohammadi Abadchi et al., 2023). The reported association between MLH1 and pre-operative PSA in Tanzanian prostate cancer patients underscores the need for further investigation into MLH1's functional role in prostate cancer within the Tanzanian population. Overall, these results highlight the potential of ATM and PMS2 mutations as important biomarkers for disease severity and progression, emphasising the value of integrating genetic testing into prostate cancer screening and management strategies in Tanzania

CHAPTER SIX

6.0 SUMMARY OF THE FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary findings

This study examined the risk factors linked to prostate cancer, tumour molecular subtypes and, gene mutations. The multivariate analysis found several significant risk factors, such as family history, lifestyle, dietary habits, sociodemographic, and disease-related factors. Being older was positively correlated with higher risk, whereas being unmarried and earning less was correlated with decreased risk. While exercise had a protective effect, consumption of red meat was linked to an increased risk. Though coffee, soy, and tomatoes showed protective effects, gonorrhoea infections were a major risk factor. The risk is significantly increased if there is a family history of prostate cancer. Prostate cancer risk is influenced by an intricate interaction of genetic, lifestyle, and sociodemographic factors; in contrast, a family history of other malignancies did not demonstrate a relevant association.

In addition, the study examined the molecular subtypes of prostate tumours based on the expression of *PTEN* and ERG. The most prevalent subtype was *ERG-/PTEN-*, observed in 68.68% of cases, followed by *ERG+/PTEN-* (30.12%). The rarest subtype was *ERG-/PTEN+* (1.18%), and no samples demonstrated the *ERG+/PTEN+* subtype. These results imply that the expression patterns of *ERG* and *PTEN* play an essential part in identifying tumour subtypes, influencing the behaviour of tumours, and potentially guiding therapeutic approaches.

Also, the current study investigated germline mutations in eleven important genes involved in Homologous recombinations (HRR) and mismatch repair(MMR) genes, detecting three potentially pathogenic mutations and several variants of unknown

significance. Mutations in Homologous recombinations repair and mismatch repair pathways are connected to significant mutations discovered in *ATM* (c.131A>G and c.2289T>A), *BRCA1* (c.557C>A and c.1137T>G), *MSH2* (c.380A>G), and *PMS2* (c.497T>C). The existence of likely pathogenic mutations in *PMS2* and *ATM* suggests that they may be contributing to the development of prostate cancer. To ascertain the clinical importance of numerous variants of uncertain significance, additional functional validation is necessary. Prostate cancer's genetic landscape has been revealed by these findings, which also emphasise the significance of further study into the disease's molecular mechanism and therapeutic options.

6.2 Conclusion

The current findings indicate the risk of prostate cancer among Tanzanian males is influenced by sociodemographic, lifestyle, nutritional, illness, and family history factors. Physical exercise and consumption of tomatoes, soy, and coffee showed protective effects, but older age, alcohol use, consumption of red meat, and a family history of prostate cancer were linked to higher risk of Prostate cancer. These results highlight the complex relationship between modifiable and non-modifiable elements in the aetiology of prostate cancer.

Also, the present study findings revealed that *ERG-/PTEN-*, the most prevalent subtype, was linked to more aggressive cancer behaviour. Immunohistochemistry analysis identified different tumour subtypes based on *ERG* and *PTEN* expression patterns. Furthermore, the present study explored the genetic mutations, tumour molecular subtypes, and multifactorial risk factors correlated with prostate cancer. The study found several mutations in the genes responsible for DNA repair and mismatch repair, such as *ATM, BRCA1, MSH2,* and *PMS2*. Some of these mutations were categorised as variants of unknown significance, while others were

likely pathogenic. Prostate cancer susceptibility is linked to this genetic mutation, especially in *ATM*, which emphasises the need for more study to fully understand their function in tumour growth. The results lay the groundwork for further research and therapeutic management approaches by advancing the current understanding of the genetic and molecular mechanisms of prostate cancer.

6.3 Recommendations for Future Research and Clinical Application

i. **Address Modifiable Risk Factors**: To focus on public health interventions that promote healthier lifestyle choices, such as reducing alcohol consumption, moderating red meat intake, and increasing physical activity, to lower the risk of prostate cancer. Additionally, prioritise early screening for individuals with a family history of prostate cancer to improve early detection and treatment outcomes.

ii. **Incorporate *ERG* and *PTEN* Profiling in Clinical Practice**: To integrate *ERG* and *PTEN* expression analysis into diagnostic protocols to better stratify prostate cancer risk. This will allow for the identification of high-risk patients and enable personalized treatment strategies tailored to tumour behaviour.

iii. **Focus on Functional Validation of Genetic Mutations** To uncover potential therapeutic targets and gain a better understanding of the role that variants of uncertain significance (VUS) play in the progression of prostate cancer, experimental research should be conducted to investigate the biological effects of VUS in important DNA repair and mismatch repair genes such as *BRCA1, ATM,* and *MSH2*.

iv. **Develop Comprehensive Genetic Panels for Prostate Cancer Risk**: Develop tailored multigene panels that include important mutations in genes linked to prostate cancer susceptibility. For those who are more susceptible to the illness, this may help with early detection and better risk assessment. Prostate cancer mortality could be decreased and clinical care could be greatly improved.

v. **Explore Targeted Therapies for Molecular Subtypes**: To examine the therapeutic possibilities of focussing on molecular pathways that are affected by *PTEN* loss and ERG alterations, especially in individuals with the aggressive *ERG-/PTEN*-phenotype. These efforts may result in more personalised, efficient prostate cancer treatments.

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APPENDICES

Appendix 1: Tumor characteristics, ERG expression, and loss of PTEN Immunohistochemistry results

Sample ID	Histological diagnosis	Gleason score	Grade group	PSA (ng/mL)	*ERG* Expression	Staining Intensity	Staining Pattern	% of Positive Cells	*PTEN* Expression	Staining Intensity	% of Positive Cells	Staining Pattern
PC01	Adenocarcinoma	4+4	4	15.2	negative	NA	NA	NA	Lost	NA	NA	NA
PC02	Adenocarcinoma	5+5	5	85.6	negative	NA	NA	NA	Lost	NA	NA	NA
PC03	Adenocarcinoma	4+3	3	20.3	negative	NA	NA	NA	Lost	NA	NA	NA
PC04	Adenocarcinoma	3+3	2	8.1	negative	NA	NA	NA	Lost	NA	NA	NA
PC05	Adenocarcinoma	4+4	4	18.4	negative	NA	NA	NA	Lost	NA	NA	NA
PC06	Adenocarcinoma	4+4	4	20.2	positive	Moderate	Nucleus	100%	Lost	NA	NA	NA
PC07	Adenocarcinoma	4+4	4	22.1	negative	NA	NA	NA	Lost	NA	NA	NA
PC08	Adenocarcinoma	4+5	5	35.7	positive	Strong	Nucleus	85%	Lost	NA	NA	NA
PC09	Adenocarcinoma	4+5	5	30.5	negative	NA	NA	NA	Lost	NA	NA	NA
PC10	Adenocarcinoma	4+5	5	40.8	positive	Strong	Nucleus	90%	Lost	NA	NA	NA
PC11	Adenocarcinoma	5+4	5	50.6	negative	NA	NA	NA	Lost	NA	NA	NA

PC 12	Adenocarcinoma	4+5	5	32.3	negative	NA	NA	NA	Lost	NA	NA	NA
PC 13	Adenocarcinoma	4+3	3	25.4	positive	Strong	Nucleus	60%	Lost	NA	NA	NA
PC 14	Adenocarcinoma	4+4	4	20.7	negative	NA	NA	NA	Lost	NA	NA	NA
PC 15	Adenocarcinoma	4+4	4	18.6	negative	NA	NA	NA	Lost	NA	NA	NA
PC16	Adenocarcinoma	4+4	4	21.5	negative	NA	NA	NA	Lost	NA	NA	NA
PC17	Adenocarcinoma	3+3	2	9.2	positive	NA	NA	NA	Lost	NA	NA	NA
PC18	Adenocarcinoma	4+5	5	37.9	negative	NA	NA	NA	**Intact**	NA	NA	NA
PC19	Adenocarcinoma	5+5	5	85.2	negative	NA	NA	NA	Lost	NA	NA	NA
PC20	Adenocarcinoma	5+5	5	80.3	negative	NA	NA	NA	Lost	NA	NA	NA
PC21	Adenocarcinoma	3+4	2	12.1	positive	Strong	Nucleus	65%	Lost	NA	NA	NA
PC22	Adenocarcinoma	4+4	4	18.5	negative	NA	NA	NA	Lost	NA	NA	NA
PC23	Adenocarcinoma	4+4	4	16.9	negative	NA	NA	NA	Lost	NA	NA	NA
PC24	Adenocarcinoma	5+4	5	50.4	positive	Strong	Nucleus	95%	Lost	NA	NA	NA
PC25	Adenocarcinoma	4+4	5	22.3	negative	NA	NA	NA	Lost	NA	NA	NA
PC26	Adenocarcinoma	4+4	4	17.8	negative	NA	NA	NA	Lost	NA	NA	NA
PC27	Adenocarcinoma	4+4	4	20.5	negative	NA	NA	NA	Lost	NA	NA	NA

PC28	Adenocarcinoma	4+3	3	23.8	positive	Strong	Nucleus	70%	Lost	NA	NA	NA
PC29	Adenocarcinoma	4+5	5	35.1	negative	NA	NA	NA	Lost	NA	NA	NA
PC30	Adenocarcinoma	5+4	5	45.2	negative	NA	NA	NA	Lost	NA	NA	NA
PC31	Adenocarcinoma	4+5	5	38.3	positive	Strong	Nucleus	80%	Lost	NA	NA	NA
PC32	Adenocarcinoma	4+5	5	34.2	negative	NA	NA	NA	Lost	NA	NA	NA
PC33	Adenocarcinoma	5+4	5	55.6	negative	NA	NA	NA	Lost	NA	NA	NA
PC34	Adenocarcinoma	4+5	5	36.7	negative	NA	NA	NA	Lost	NA	NA	NA
PC35	Adenocarcinoma	5+4	4	50.8	negative	NA	NA	NA	Lost	NA	NA	NA
PC36	Adenocarcinoma	3+4	2	14.2	negative	NA	NA	NA	Lost	NA	NA	NA
PC37	Adenocarcinoma	3+4	2	13.7	positive	Strong	Nucleus	45%	Lost	NA	NA	NA
PC38	Adenocarcinoma	4+4	4	20.3	negative	NA	NA	NA	Lost	NA	NA	NA
PC39	Adenocarcinoma	4+4	4	21.4	positive	Strong	Nucleus	90%	Lost	NA	NA	NA
PC40	Adenocarcinoma	4+3	3	18.2	negative	NA	NA	NA	Lost	NA	NA	NA
PC41	Adenocarcinoma	4+5	5	39.1	positive	Strong	Nucleus	80%	Lost	NA	NA	NA
PC42	Adenocarcinoma	3+3	2	7.5	negative	NA	NA	NA	Lost	NA	NA	NA
PC43	Adenocarcinoma	4+3	3	19.3	negative	NA	NA	NA	Lost	NA	NA	NA

PC44	Adenocarcinoma	5+5	5	85.3	negative	NA	NA	NA	Lost	NA	NA	NA
PC45	Adenocarcinoma	4+5	4	34.9	positive	Strong	Nucleus	80%	Lost	NA	NA	NA
PC46	Adenocarcinoma	4+3	3	22.5	positive	Strong	Nucleus	65%	Lost	NA	NA	NA
PC47	Adenocarcinoma	5+3	4	45.3	negative	NA	NA	NA	Lost	NA	NA	NA
PC48	Adenocarcinoma	4+4	4	19.2	negative	NA	NA	NA	Lost	NA	NA	NA
PC49	Adenocarcinoma	4+5	5	37.6	negative	NA	NA	NA	Lost	NA	NA	NA
PC50	Adenocarcinoma	4+4	4	20.4	negative	NA	NA	NA	Lost	NA	NA	NA
PC51	Adenocarcinoma	3+4	2	15.6	positive	Strong	Nucleus	50%	Lost	NA	NA	NA
PC52	Adenocarcinoma	4+5	5	33.2	negative	NA	NA	NA	Lost	NA	NA	NA
PC53	Adenocarcinoma	5+3	4	48.4	negative	NA	NA	NA	Lost	NA	NA	NA
PC54	Adenocarcinoma	4+3	3	24.1	positive	Strong	Nucleus	75%	Lost	NA	NA	NA
PC55	Adenocarcinoma	4+4	4	18.9	negative	NA	NA	NA	Lost	NA	NA	NA
PC56	Adenocarcinoma	5+5	4	80.1	negative	NA	NA	NA	Lost	NA	NA	NA
PC57	Adenocarcinoma	4+3	3	19.7	negative	NA	NA	NA	Lost	NA	NA	NA
PC58	Adenocarcinoma	5+4	5	50.3	negative	NA	NA	NA	Lost	NA	NA	NA
PC59	Adenocarcinoma	3+4	3	17.2	positive	Strong	Nucleus	68%	Lost	NA	NA	NA

PC60	Adenocarcinoma	3+3	2	10.4	negative	NA	NA	NA	Lost	NA	NA	NA
PC61	Adenocarcinoma	4+4	4	19.5	negative	NA	NA	NA	Lost	NA	NA	NA
PC62	Adenocarcinoma	4+5	5	40.1	positive	Strong	Nucleus	90%	Lost	NA	NA	NA
PC63	Adenocarcinoma	4+4	4	25.2	positve	Strong	Nucleus	95%	Lost	NA	NA	NA
PC64	Adenocarcinoma	4+4	4	18.7	negative	NA	NA	NA	Lost	NA	NA	NA
PC65	Adenocarcinoma	5+4	5	53.2	positive	Strong	Nucleus	70%	Lost	NA	NA	NA
PC66	Adenocarcinoma	3+4	2	16.8	negative	NA	NA	NA	Lost	NA	NA	NA
PC67	Adenocarcinoma	4+5	5	41.5	positive	strong	nucleus	100%	Lost	NA	NA	NA
PC68	Adenocarcinoma	3+3	2	9.1	negative	NA	NA	NA	Lost	NA	NA	NA
PC69	Adenocarcinoma	3+3	2	10.2	positive	Strong	Nucleus	90%	Lost	NA	NA	NA
PC70	Adenocarcinoma	4+5	5	38.4	negative	NA	NA	NA	Lost	NA	NA	NA
PC71	Adenocarcinoma	4+5	5	39.2	negative	NA	NA	NA	Lost	NA	NA	NA
PC72	Adenocarcinoma	4+5	5	40.5	negative	NA	NA	NA	Lost	NA	NA	NA
PC73	Adenocarcinoma	5+5	5	85.5	negative	NA	NA	NA	Lost	NA	NA	NA
PC74	Adenocarcinoma	5+4	5	50.9	negative	NA	NA	NA	Lost	NA	NA	NA
PC75	Adenocarcinoma	4+5	5	36.3	negative	NA	NA	NA	Lost	NA	NA	NA

PC76	Adenocarcinoma	4+5	5	34.1	negative	NA	NA	NA	Lost	NA	NA	NA
PC77	Adenocarcinoma	4+4	4	21.3	negative	NA	NA	NA	Lost	NA	NA	NA
PC78	Adenocarcinoma	4+3	3	22.7	positive	Strong	Nucleus	70%	Lost	NA	NA	NA
PC79	Adenocarcinoma	4+5	5	38.6	negative	NA	NA	NA	Lost	NA	NA	NA
PC80	Adenocarcinoma	4+5	5	42.1	positive	Strong	Nucleus	80%	Lost	NA	NA	NA
PC81	Adenocarcinoma	5+5	5	85.7	negative	NA	NA	NA	Lost	NA	NA	NA
PC82	Adenocarcinoma	5+5	5	80.5	negative	NA	NA	NA	Lost	NA	NA	NA
PC83	Adenocarcinoma	4+4	4	20.6	negative	NA	NA	NA	Lost	NA	NA	NA
PC84	Adenocarcinoma	4+5	5	34.3	negative	NA	NA	NA	Lost	NA	NA	NA
PC85	Adenocarcinoma	4+4	4	23.7	positive	Strong	Nucleus	90%	Lost	NA	NA	NA

Appendix table 2: Homologous recombination repair and Mismatch repair gene mutations with various clinical significance result status

Gene	Chromosome	Ref allele	Alt allele	Type	Impact	Prediction Tool (SIFT)	Nucleotide Change	Nucleotide Change Frequency	Protein Change	Clinical Significance
ATM	chr11	C	T	SNP	missense_variant	Damaging	c.6176C>T	1	p.Thr2059Ile	likely_benign
ATM	chr11	T	A	SNP	missense_variant	Tolerated	c.2289T>A	1	p.Phe763Leu	likely_pathogenic
ATM	chr11	C	T	SNP	missense_variant	Tolerated	c.1810C>T	2	p.Pro604Ser	likely_benign
ATM	chr11	C	A	SNP	missense_variant	Damaging	c.2442C>A	1	p.Asp814Glu	VUS
ATM	chr11	A	G	SNP	missense_variant	Damaging	c.131A>G	1	p.Asp44Gly	likely_pathogenic
ATM	chr11	G	A	SNP	missense_variant	Tolerated	c.1541G>A	3	p.Gly514Asp	VUS
ATM	chr11	C	T	SNP	missense_variant	Damaging	c.4138C>T	1	p.His1380Tyr	likely_benign
ATM	chr11	C	A	SNP	missense_variant	Damaging	c.4792C>A	1	p.Leu1598Ile	likely_benign
ATM	chr11	A	G	SNP	missense_variant	Damaging	c.3383A>G	3	p.Gln1128Arg	likely_benign
ATM	chr11	T	A	SNP	missense_variant	Damaging	c.4633T>A	1	p.Leu1545Ile	likely_benign
ATM	chr11	C	T	SNP	missense_variant	Tolerated	c.2614C>T	2	p.Pro872Ser	likely_benign
ATM	chr11	T	A	SNP	missense_variant	Tolerated	c.378T>A	18	p.Asp126Glu	likely_benign
ATM	chr11	T	C	SNP	missense_variant	Tolerated	c.6995T>C	1	p.Leu2332Pro	likely_benign

BRCA1	chr17	G	T	SNP	missense_variant	Damaging	c.557C>A	1	p.Ser186Tyr	VUS
BRCA1	chr17	A	C	SNP	missense_variant	Damaging	c.1137T>G	1	p.Ile379Met	VUS
BRCA1	chr17	C	T	SNP	missense_variant	Tolerated	c.4600G>A	1	p.Val1534Met	likely_benign
BRCA1	chr17	T	C	SNP	missense_variant	Tolerated	c.3113A>G	9	p.Glu1038Gly	likely_benign
BRCA1	chr17	T	C	SNP	missense_variant	Tolerated	c.1427A>G	1	p.His476Arg	likely_benign
BRCA1	chr17	T	C	SNP	missense_variant	Tolerated	c.2458A>G	1	p.Lys820Glu	likely_benign
BRCA1	chr17	T	C	SNP	missense_variant	Damaging	c.4837A>G	5	p.Ser1613Gly	likely_benign
BRCA1	chr17	G	A	SNP	missense_variant	Tolerated	c.2612C>T	17	p.Pro871Leu	likely_benign
BRCA2	chr13	A	G	SNP	missense_variant	Damaging	c.7712A>G	1	p.Glu2571Gly	likely_benign
BRCA2	chr13	C	T	SNP	missense_variant	Damaging	c.9875C>T	1	p.Pro3292Leu	likely_benign
BRCA2	chr13	A	C	SNP	missense_variant	Tolerated	c.4090A>C	1	p.Ile1364Leu	likely_benign
BRCA2	chr13	A	G	SNP	missense_variant	Tolerated	c.5414A>G	1	p.Asn1805Ser	likely_benign
BRCA2	chr13	A	T	SNP	missense_variant	Damaging	c.8830A>T	3	p.Ile2944Phe	likely_benign
BRCA2	chr13	A	C	SNP	missense_variant	Tolerated	c.1114A>C	5	p.Asn372His	likely_benign
BRCA2	chr13	T	G	SNP	missense_variant	Damaging	c.5640T>G	1	p.Asn1880Lys	likely_benign
BRCA2	chr13	C	A	SNP	missense_variant	Tolerated	c.4681C>A	1	p.His1561Asn	likely_benign
BRCA2	chr13	G	T	SNP	missense_variant	Tolerated	c.6412G>T	2	p.Val2138Phe	likely_benign
BRCA2	chr13	C	T	SNP	missense_variant	Tolerated	c.4241C>T	2	p.Thr1414Met	likely_benign

BRCA2	chr13	G	A	SNP	missense_variant	Tolerated	c.5704G>A	2	p.Asp1902Asn	likely_benign
BRCA2	chr13	G	C	SNP	missense_variant	Damaging	c.7017G>C	1	p.Lys2339Asn	likely_benign
BRCA2	chr13	A	G	SNP	missense_variant	Tolerated	c.7319A>G	1	p.His2440Arg	likely_benign
BRCA2	chr13	G	A	SNP	missense_variant	Tolerated	c.9730G>A	1	p.Val3244Ile	likely_benign
BRCA2	chr13	C	A	SNP	missense_variant	Tolerated	c.7150C>A	1	p.Gln2384Lys	likely_benign
BRCA2	chr13	A	G	SNP	missense_variant	Tolerated	c.10234A>G	9	p.Ile3412Val	likely_benign
BRCA2	chr13	T	C	SNP	missense_variant	Tolerated	c.7397T>C	29	p.Val2466Ala	likely_benign
CHEK2	chr22	A	C	SNP	missense_variant	Tolerated	c.1343T>G	2	p.Ile448Ser	VUS
MLH1	chr3	C	T	SNP	missense_variant	Damaging	c.2152C>T	6	p.His718Tyr	likely_benign
MLH1	chr3	A	G	SNP	missense_variant	Tolerated	c.1963A>G	2	p.Ile655Val	likely_benign
MLH1	chr3	A	G	SNP	missense_variant	Tolerated	c.655A>G	5	p.Ile219Val	likely_benign
MSH2	chr2	A	G	SNP	missense_variant	Damaging	c.380A>G	1	p.Asn127Ser	VUS
MSH6	chr2	A	C	SNP	missense_variant	Tolerated	c.155A>C	1	p.Glu52Ala	likely_benign
MSH6	chr2	C	A	SNP	missense_variant	Damaging	c.15C>A	1	p.Ser5Arg	likely_benign
PALB2	chr16	T	C	SNP	missense_variant	Tolerated	c.721A>G	1	p.Asn241Asp	likely_benign
PALB2	chr16	G	A	SNP	missense_variant	Tolerated	c.629C>T	2	p.Pro210Leu	likely_benign
PALB2	chr16	T	C	SNP	missense_variant	Tolerated	c.925A>G	2	p.Ile309Val	likely_benign
PALB2	chr16	T	C	SNP	missense_variant	Tolerated	c.1676A>G	18	p.Gln559Arg	likely_benign

PMS2	chr7	C	T	SNP	missense_variant	Tolerated	c.1609G>A	1	p.Glu537Lys	likely_benign
PMS2	chr7	A	G	SNP	missense_variant	Damaging	c.497T>C	1	p.Leu166Pro	likely_pathogenic
PMS2	chr7	C	A	SNP	missense_variant	Tolerated	c.1688G>T	1	p.Arg563Leu	likely_benign
PMS2	chr7	C	T	SNP	missense_variant	Tolerated	c.59G>A	3	p.Arg20Gln	likely_benign
PMS2	chr7	G	A	SNP	missense_variant	Tolerated	c.1532C>T	6	p.Thr511Met	likely_benign
PMS2	chr7	C	G	SNP	missense_variant	Tolerated	c.924G>C	8	p.Glu308Asp	likely_benign
PMS2	chr7	G	A	SNP	missense_variant	Tolerated	c.1408C>T	2	p.Pro470Ser	likely_benign
PMS2	chr7	T	C	SNP	missense_variant	Tolerated	c.1621A>G	28	p.Lys541Glu	likely_benign
TP53	chr17	G	A	SNP	missense_variant	Damaging	c.139C>T	2	p.Pro47Ser	likely_benign
TP53	chr17	G	C	SNP	missense_variant	Tolerated	c.215C>G	19	p.Pro72Arg	likely_benign

Appendix table 3:Research Work Plan

	2022						2023						2024					
Month	Jan-Feb	Mar-April	May- June	Jul-Aug	Sep -Oct	Nov- Dec	Jan-Feb	Mar-April	May- June	Jul-Aug	Sep -Oct	Nov- Dec	Jan-Feb	Mar-April	May- June	Jul-Aug	Sep -Oct	Nov- Dec
Literature review																		
Redaction of the research proposal																		
Approval Proposal.																		
Data collection																		
Data analysis																		
Manuscripts Preparation.																		
Report Preparation.																		
Submission and Defence																		

Appendix table 4: Rsearch budget estimate

S/N	Item Description	Unit price (USD)	Total Price (USD)
1	Sterile Dried Blood Sport (DBS) cards, Pack of 200 Cards	40	40
2	Qiagen DNA purification kit, 1 kit	435	435
3	PCR tubes, Eppendorf tube and consumables, pack of 500 1.5ul Tubes	250	250
4	NGS Exon Targeting Sequencing for 40 samples	500	20,000
5	Informed consent document preparation and printing	0.20	48
6	Questionnaire designing and printing	48	48
7	Stationeries, binding and dissertation preparation	50	150
8	IREC Ethical clearance –MOI University	300	300
9	NIMR Ethical Clearance	300	300
10	Charged slides,2Pack	150	300
11	Flex Minit Kit, 1 Kits required	2000	2000
12	PTEN Monoclonal Antibodies.7mls, 3 vials are required	325	975
13	ERG Monoclonal Antibodies.12mls, 2 vials are required	450	900
14	Bioinformatician and Biostatistician cost@500	500	1,000
15	Research asissistance	500	500
	GRAND TOTAL ESTIMATE		**27,681**

Appendix 5 : Questionnaire

MOI UNIVERSITY

School of Medicine

P.O Box 4606 030100 Eldoret, Kenya

Tel: +254777248196, Telephone 254 53 2061562, 254 53 2060958/9

Fax 254 53 2033041,deanmedicine@mu.ac.ke

QUESTIONNAIRE

A. DEMOGRAPHIC AND SOCIAL ECONOMIC STATUS

1) How old are you?
   a) Age in years…….. Or
   b) 45 – 59 [ ], 60- 69, [ ], 70 – 80 [ ],80 and above [ ]
2) Height in (M)……weight in (Kg)……..
3) Area of residence
   a) Region/County of origin……
   b) Ward……..
4) Marital status; Married [ ] Not Married [ ] Divorced [ ] Widowed [ ]
5) Do you have children? Yes, [ ] No [ ].If yes Mention number of children…….

6) What is your level of education?; No formal education [ ] Primary education [ ] Secondary level education [ ] Diploma [ ] University degree and above [ ]

7) Occupation? Employed [] Business/entrepreneur [ ]Retiree[ ]Agriculture[ ]Peasant[ ]

8) What is the estimate of your monthly income in

i. 0 -300,000TSh [ ],301,000- 800,000 Tsh[ ],801,000– 1,500,000Tsh.[ ],1500,001–2000Tsh.[ ],2,000, 001 na zaidi [ ]

ii. 0 -15,000Ksh. [ ],15,001- 40,000Ksh.[ ],40,001– 75,000Ksh.[ ],75,001–100,000Ksh[ ],100,001 Ksh. and above [ ]

9) What type of water does your family use for Cooking or drinking?

Natural sources (Liver, lake) [ ] Dam [ ] Well [ ] Piped wáter [ ]

B. LIFE STYLE BEHAVIOR

10) Participation in Vigorous physical activity before the diagnosis of Prostate cancer

Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

11) Tobacco Consumption or Smoking Cigarette

i. Highly smoker [ ], Moderate smoker [ ], Rarely smoker [ ], Non smoker [ ]

ii. Age at first smoking……..

12) Alcohol Consumption

i. Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

ii. Type of alcohol consumed, Beer [.],Wine [.] , Spirit [.]

iii. Age at first drinking……

13) Sexual activity/frequency of sexual intercourse

Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

14) Bicycle riding

Never[ ],Rarely [ ],1-50km/week [ ],51-100km/week [ ], 100-150/week[ ],above100km/week[.]

15) Motor Bike riding

Never[ ],Rarely[ ],1-100km/week [ ],101-200km/week [ ],201-300/week[ ],above 300km/week[.]

16) Track driving

i. Have you ever driven a track for a long distance? Yes [.] No [.]

ii. If yes, For how long 1-2 years [ ], 3-4 years [ ],5- 10 years[ ]10years and above [.]

C. DIETARY PATTERN AT LEAST IN THE PAST YEAR

17) Consumption of Vegetables and fruits

Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

18) Diary food Consumption

Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

19) Red meat Consumption

Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

20) Fish Consumption

Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

21) Food containing Saturated fats consumption eg. Food containing high amounts of animal fats

Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

22) Coffee Consumption

Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

23) Green/black tea consumption

i. Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

ii. which type of tea is mostly consumed

24) Vitamins and Mineral supplements consumption

i. Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week [ ]

ii. Which type of vitamins are consumed……or Multivitamins?[ ]

25) Tomato source Consumption

Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

26) Legume consumption

Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

27) Soya consumption

Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

28) Grains consumption

i. Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

ii. Grains consumed: Whole Grains [.] Refined Grains [.],Not specific [.]

D. FAMILY HISTORY OF PROSTATE CANCER

29)	**Family history with cancer**	**Relationship with family member**	**Yes/No**	**Age at Diagnosis 50 < (Yes/No)**
i	Family history with Prostate	Father		
		Son		
		Brother		
		Grand father-paternal		
		Grand father-maternal		
		Uncle-paternal		
		Uncle-maternal		
ii	Family history of any type of cancer	Father		
		Mother		
		Son		
		Daughter		
		Brother		
		Sister		
		Grand father-paternal		
		Grand Mother-paternal		
		Grand father-maternal		
		Grand Mother-maternal		
		Uncle-paternal		
		Uncle -maternal		
		Grand son		
		Grand daughter		
iii	Family history with Breast, Ovarian, Colorectal, Endometrial or Pancreatic cancer	Father		
		Mother		
		Son		
		Daughter		
		Brother		
		Sister		
		Grand father-maternal		
		Grand Mother-maternal		
		Grand father-paternal		
		Grand Mother-paternal		
		Uncle-paternal		
		Uncle-maternal		
		Grand son		
		Grand daughter		

E. ENVIRONMENTAL EXPOSURE TO CHEMICALS AND RADIATION

30) Have you ever worked/handling chemicals, pesticides or agricultural chemicals? Yes [ ]No [ ]

i. If yes, For how long 1-2 years [ ], 3-4 years [ ], 5- 10 years[ ] 10 years and above [.]

31) Have you ever worked in chemical laboratory, factory,mining or chemical industries Yes [ ] No [ ]

i. If yes, For how long 1-2 years [ ], 3-4 years [ ], 5- 10 years[ ] 10 years and above [.]

32) Have you ever worked with/handling the following chemicals?

i. Benzene [ ], Toluene [ ], Xylene [ ],Styrene [ ],Paints [ ] Synthetic Glues [ ]Thiner [ ]

ii. If yes, For how long 1-2 years [ ], 3-4 years [ ], 5- 10 years[ ] 10 years and above [.]

33) Have you ever worked in petrol fuel station? Yes [.] No [.]

iii. If yes, For how long 1-2 years [ ], 3-4 years [ ], 5- 10 years[ ] 10 years and above [.]

34) Have you ever worked in woodwork/dust environment? Yes [.] No [.]

i. If yes, For how long 1-2 years [ ], 3-4 years [ ], 5- 10 years[ ] 10 years and above [.]

35) Have you ever worked in a diesel engine emission environment? Yes [.] No [.]

i. If yes, For how long 1-2 years [ ], 3-4 years [ ], 5- 10 years[ ] 10 years and above [.]

36) Have you ever worked with or in lubricating oils/greasy environments?Yes [.] No [.]

i. If yes, For how long 1-2 years [ ], 3-4 years [ ], 5- 10 years[ ] 10 years and above [.]

37) Have you ever worked in a radiation environment Yes [ ] No [ ]

ii. If yes, For how long 1-2 years [ ], 3-4 years [ ], 5- 10 years[ ] 10 years and above [.]

F. MEDICAL CONDITIONS

38) Have you ever been suffering or diagnosed with any of the following diseases

i. Diabetes [ ], Erectile dysfunction [ ],syphils[ ], Gonorhea[.], Trichomoniasis[.] or any other STDs[.]…….

G. EXPOSURE TO MEDICATION

39) Have you ever used any antihypertensive drugs? Yes [.] No [.]

i. If yes, For how long 1-2 years [ ], 3-4 years [ ], 5- 10 years[ ] 10 years and above [.]

40) Have you ever used any pharmaceutical drugs for erectile dysfunction? Yes [.] No [.]

i. If yes, For how long 1-2 years [ ], 3-4 years [ ], 5- 10 years[ ] 10 years and above [.]

41) Have you ever used any herbal medicine for erectile dysfunction? Yes [.] No [.]

ii. If yes, For how long 1-2 years [ ], 3-4 years [ ], 5- 10 years[ ] 10 years and above [.]

Appendix 6 : Tool for Clinical Data collection



MOI UNIVERSITY

School of Medicine

P.O Box 4606 030100 Eldoret,Kenya

Tel: +254777248196, Telephone 254 53 2061562, 254 53 2060958/9

Fax 254 53 2033041,deanmedicine@mu.ac.ke

TOOL FOR CLINICAL DATA COLLECTION

Data Collection From Patient File

1	Hospital name:
2	Patients name:
3	Patient File or registration number
4	Hospital/clinic of Diagnosis
5	Age at Diagnosis
6	Body Mass Index Overweigh [ ]Under wight [ ] Normal [ ]
7	PSA Level in g/DL
8	Tumor grade/Gleason Score
9	Tumor pathological stage, Stage I [], Stage II [ ] Stage III [ ]Stage IV[ ]
10	Immunohistochemistry (IHC) tested for ERG Expression and loss of PTEN? Yes [ ], No [ ], if Yes Mention the results……..
11	Patient is Diabetic? [Yes [ ] No [ ]
12	Patient have any underline Condition? Yes [ ] No[ ] If Yes Please Mention
12	Patient history with Gonorhea or Clamidia? Yes [ ] No [ ]

Any Other

comments……..……

.....

...

……... ……

Signature of Primary Investigator Date



HOSPITALI YA RUFAA NA MAFUNZO /CHUO KIKUU MOI /CHUO CHA SAYANSI YA AFYA

SHULE YA TIBA

S.L.P 4606 030100 Eldoret,Kenya

Simu : +254777248196, 254 53 2061562, 254 53 2060958/9

Faksi 254 53 2033041,deanmedicine@mu.ac.ke

DODOSO

A. TAARIFA KUHUSU MSHIRIKI NA HALI YAKE YA KIJAMII NA KIUCHUMI

1) Una umri gani?
   c) Umri (miaka) …….. au
   d) 45 – 59 [ ], 60- 69, [ ], 70 – 80 [ ],80 ana Zaidi [ ]
2) Urefu (M)……Uzito (Kg)……..
3) Mahali unapoishi
   c) Mkoa/ Kaunti……
   d) Kata……..
4) Hali ya ndoa; Nmeoa [ ] Sijaoa [ ] Talaka [ ] Mgane [ ]
5) Una Watoto? Ndio, [ ] Hapana [ ].Kama ndio , wangapi?…….

6) Una kiwango gani cha elimu?. Sijasoma katika mfumo rasmi wa elimu; Elimu ya msingi [ ] Elimu ya Sekondari [ ] Diploma [ ] Chuo kikuu na zaidi[ ]

7) Unajihusisha na nini kupata kipato?U meajiliwa[ ]Biashara/Ujasiliamali[ ]Mstaafu[ ] Kilimo na ufugaji [ ] Shughuli ndogo ndogo [ ]

8) Wastani wa mapato yako kwa mwezi

0 -300,000TSh[ ],301,000- 800,000 Tsh[ ],801,000– 1,500,000Tsh.[ ],1500,001–2000Tsh.[ ],2,000, 001 na zaidi [ ]

9) Umekuwa unatumia maji kutoka wapi kwa ajili ya kunywa na kupikia katika familia?

Maji ya mtoni au ziwani [ ] kwenye mabwawa [ ] Visima [ ] Maji ya Bombani[ ]

B. MINDO WA MAISHA

10) Hali ya ushiriki wako katika mazoezi au shughuli zenye kukupa mazoezi

Sishiriki mazoezi [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

11) Matumizi ya tumbaku au uvutaji wa Sigara

i. Situmii tumbaku wala kuvuta sigara[ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

ii. Ulianza kuvuta katika umri gani?……..

12) Utumiaji wa pombe

i. Situmii [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

ii. Kama unatumia, ni aina gani kati ya hivi?, Bia [.],Waini [.] ,Pombe Kali/spiriti [.]

iii. Ulianza kunywa katika umri gani?……..

13) Ushiriki w atendo la ndoa

Sishiriki [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

14) Uendeshaji wa Baiskeli

Hapana [ ],Nadra [ ],1-50km/wiki [ ],51-100km/wki [ ], 100-150/wiki[ ],zaidi 100km/wiki[.]

15) Uendeshaji wa pikipiki

Hapana[ ],Nadra[ ],1-100km/wiki [ ],101-200km/wiki [ ],201-300/wiki[ ],above 300km/wiki[.]

16) Uendeshaji wa Malori

i. Umewahi kuendesha malori kwa umbali mrefu au masafa marefu?Ndio [.] Hapana [.]

ii. kama ndio , kwa muda gani? miaka 1-2 [ ],miaka 3-4 [ ], miaka 5- 10 [ ] miaka10 na zaidi [.]

C. HALI YA ULAJI WA CHAKULA LATIKA KIPINDI ANGALAU KWA MWAKA ULIPITA NA ZAIDI

17) Ulaji wa matunda na mboga za majani

Situmii [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

18) Unywaji wa maziwa na ulaji wa bidhaa zake

Situmii [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

19) Ulaji wa nyama nyekundu na bidhaa zake

Situmii [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

20) Ulaji wa Samaki na bidhaa zake

Situmii [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

21) Food containing Saturated fats consumption eg. Food containing high amount of animal fats

Never [ ], Rarely [ ],1-2 times/week [ ], 3-4times/week [ ], 5-7times/week[ ]

22) Unywaji wa kahawa na kutumia bidhaa zake

Situmii [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

23) Unywaji wa Chai ya Kijani au Chai nyesusi

Situmii [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

iii. Aina gani ya chai hutumia mara nyingi…….

24) Utumiaji wa vitutubisho vya vitamina au madini

i. Situmii [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

ii. Aina gani ya virutubisho vya vitamina au madini unayotumia…..or Virutubisho vingi vya vitamina kwa pamoja?[ ]

25) Utumiaji wa nyanya iliyosindika (Tomato source)

Situmii [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

26) Utumiaji wa vyakula mimea jamii ya mikunde

Situmii [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

27) Utumiaji wa vyakula vya Soya

Situmii [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

28) Utumiaji wa vyakula vya nafaka

i. Situmii [ ], Nadra/mara chache [ ],mara 1-2 kwa wiki [ ], mara 3-4 kwa wiki [ ], mara 5-7 kwa wiki [ ]

ii. Aina ya nafaka unayotumia: Isiyo kobelewa [ ], Iliyokobelewa ,Hutumia yeyote. [.]

D. HISTORIA YA SARATANI KATIKA FAMILIA

29)	**Historia ya uwepo wa Ugonjwa saratani katika Familia**	**Uhusiano na mgonjwa**	**Ndio/Hapana**	**Umri wakati wa uchungzi 50 < (Ndio/Hapana)**
i	Kuna ndugu yako wa karibu aliyewahi kuugua saratani ya Tezi dume	Baba		
		Mtoto wa kiume		
		Kaka		
		Babu		
		Babu mzaa Mama		
		Mjomba		
ii	Kuna ndugu yako wa karibu aliyewahi kuugua aina yoyote ya saratani	Baba		
		Mama		
		Mtoto wa kiume		
		Mtoto wa kike		
		Kaka		
		Dada		
		Babu mzaa baba		
		Bibi mzaa baba		
		Babu mzaa mama		
		Bibi mzaa mama		
		Mjukuu wa kiume		
		Mjukuu wa Kike		
		Mjomba		
Ii	Kuna ndugu yako wa karibu aliyewahi kuugua saratani ya matiti,ovari,Utumbo mpana,endometrial au kongosho.	Baba		
		Mama		
		Mtoto wa kiume		
		Mtoto wa kiume		
		Baba		
		Dada		
		Babu mzaa baba		
		Bibi mzaa baba		
		Babu mzaa mama		
		Bibi mzaa mama		
		Momba		
		Mjukuu wa kiume		
		Mjukuu wa Kike		

E. KAZI KATIKA MAZINGIRA YENYE KEMIKALI AU MIONZI

30) Umewahi kufanya kazi kwenye kemikali, viuatilifu au kemikali au madawa yanayotumika kwenye kilimo na ufugaji? Ndio [ ] Hapana [ ]

ii. kama ndio, kwa muda gani?.miaka 1-2 [ ], miaka 3-4 [ ], miaka 5- 10[ ] 1miaka 10 na zaidi[.]

31) umewahi kufanya kazi kwenye maabara za kemia au migodini au viwanda vinavyotumia kemikali?Ndio [ ] Hapana [ ]

i. kama ndio, kwa muda gani?.miaka 1-2 [ ], miaka 3-4 [ ],miaka 5- 10[ ]miaka 10 na zaidi[.]

32) Umewahi kutumia kemikali zifuatazo katika shughuli zako? Ndio [ ] Hapana [ ]

iii. Benzene [ ], Toluene [ ], Xylene [ ],Styrene [ ],Rangi [ ] Synthetic Glues [ ]Thiner [ ]

iv. kama ndio, kwa muda gani?.miaka 1-2 [ ], miaka 3-4 [ ], miaka 5- 10[ ]miaka 10 na zaidi[.]

33) Umewahi kufanya kazi katik vituo vya mafuta? Ndio [ ] Hapana [ ]

i. kama ndio, kwa muda gani?.miaka 1-2 [ ], miaka 3-4 [ ], miaka 5- 10[ ] 1miaka 10 na zaidi[.]

34) Umewahi kufanya kazi katika mbao au mazingira yenye vumbi la mbao au uchomaji moto mbao? Ndio [ ] Hapana [ ]

i. kama ndio, kwa muda gani?.miaka 1-2 [ ], miaka 3-4 [ ], miaka 5- 10[ ] 1miaka 10 na zaidi[.]

35) Umewahi kufanya kazi katika mzingira ya moshi unaotokana na mitambo, au mashine zinazotumia dizeli? Ndio [ ] Hapana [ ]

i. kama ndio, kwa muda gani?.miaka 1-2 [ ], miaka 3-4 [ ], miaka 5- 10[ ] 1miaka 10 na zaidi[.]

36) Umewahi kufanya kazi katika mzingira ya kutumia mafuta ya vilainishi au grisi? Ndio [ ] Hapana [ ]

i. kama ndio, kwa muda gani?.miaka 1-2 [ ], miaka 3-4 [ ], miaka 5- 10[ ] 1miaka 10 na zaidi[.]

37) Umewahi kufanya kazi katika mazingira ya mionzi hatarishi? Ndio [ ] Hapana [ ]

ii. kama ndio, kwa muda gani?.miaka 1-2 [ ], miaka 3-4 [ ], miaka 5- 10[ ] 1miaka 10 na zaidi[.]

F. MAGONJWA

38) Umewahi kuugua au kuugua moja kati ya magonjwa yafuatayo?

Kisukari [ ], kukosa nguvu za kiume [ ],Kaswende[ ], Gono [.], Trikomoniasisi[.] au ugonjwa mwingine wa zinaa[.]…….

G. UTUMIAJI WA DAWA MBALIMBALI

39) Umewahi kutumia dawa za kupunguza presha ? Ndio [ ] Hapana [ ]

i. kama ndio, kwa muda gani?.miaka 1-2 [ ], miaka 3-4 [ ], miaka 5- 10[ ] 1miaka 10 na zaidi[.]

40) Umewahi kutumia dawa za hospital za kuongeza nguvu za kiume? Ndio [ ] Hapana [ ]

i. kama ndio, kwa muda gani?.miaka 1-2 [ ], miaka 3-4 [ ], miaka 5- 10[ ] 1miaka 10 na zaidi[.]

41) Umewahi kutumia dawa za asili za kuongeza nguvu za kiume? Ndio [ ] Hapana [ ]

i. kama ndio, kwa muda gani?.miaka 1-2 [ ], miaka 3-4 [ ], miaka 5- 10[ ] 1miaka 10 na zaidi[.]

HOSPITALI YA RUFAA NA MAFUNZO /CHUO KIKUU MOI /CHUO CHA SAYANSI YA AFYA
SHULE YA TIBA
S.L.P 4606 030100 Eldoret,Kenya
Simu : +254777248196, 254 53 2061562, 254 53 2060958/9
Faksi 254 53 2033041,deanmedicine@mu.ac.ke

DODOSO LA KUCHUKULIA TAARIFA KUTOKA JALADA LA MSHIRIKI KUTOKA HOSPITALI ANAYOPATIWA HUDUMA ZA MATIBABU

1	Jina la Hospitali:
2	Jina la Mgonjwa:
3	Namba ya Jalada au Usajili wa mgonjwa
4	Hospitali aliyochunguzwa:
5	Umri wakati anagundulika kuwa na Saratani ya tezi dume
6	Hali ya uzito: Uzito uliopitiliza [ ]Uzito wa chini ya kiwango [ ] uzito wa kawaida [ ]
7	Kiwango cha PSA (g/DL)
8	Kiwango cha uvimbe wa saratani /Gleason Score:
9	Kiwango cha hatua ya kusambaa Saratani, Hatua ya I [ ],Hatua ya II [ ] Hatua ya III [ ]Hatua ya IV[ ]
10	Uchunguzi wa (IHC) ulifanyika kuangalia hali ya ERG Epression and Loss of PTEN? Ndio [ ], Hapana [ ], kama jibu ni ndio taja matokeo ya uchunguzi s……..
11	Je mshiriki ana ugonjwa wa kisukari? Ndio [ ] Hapana[ ]
12	Je mshiriki ana changamoto zingine za kiafya mbali ya Saratani? Ndio [ ] Hapana [ ] kama jibu ni ndio, Tafadhali eleza……..
13	Je Mshiriki aliwahi kuugua Gonorhea au Clamidia? Ndio [ ] Hapana [ ]

Maoni mengine

……..……

.....

...

……... …….

Sahihi ya Mtafiti/Mwakilishi Tarehe

Appendix 7: Consent Form

MOI TEACHING & REFERRAL HOSPITAL / MOI UNIVERSITY

COLLEGE OF

HEALTH SCIENCES -INSTITUTIONAL RESEARCH AND

ETHICS COMMITTEE (MTRH/MU-IREC)

INFORMED CONSENT FORM

Study Title:

Mutation Spectrum, Tumor subtypes and Risk factors of Prostate cancer seen at Ocean Road Cancer Institute, Dar es Salaam-Tanzania.

Name of Principal Investigator(s): Fidelis Charles

Co-investigator(s): Dr. Rispah Torrorey and Dr. Richard Biegon

Name of Organization: Department of Immunology, Moi University-Kenya

Address: Moi University P.O Box 4606 030100 Eldoret, Kenya Telephone: +255700094408

Name of Sponsor/Funding Agency: P4HPTII

Informed Consent Form for:

Participants to be recruited in the study

This Informed Consent Form has two parts:

• Part I: Information Sheet [to share information about the study with you]

• Part II: Certificate of Consent [for signatures if you choose to participate]

PART I: INFORMATION SHEET

Introduction:

You are being asked to take part in a research study. This information is provided to tell you about the study. Please read this form carefully. You will be given a chance to ask questions.

Taking part in this research study is voluntary. Saying no will not affect your rights to health care or any other services. Your treatment/payment or enrollment in any health plans or eligibility for benefits will not be affected if you decide not to take part. You are also free to withdraw from this study at any time. If after data collection you choose to quit, you can request that information provided by you be destroyed under supervision. This would be before data is de-identified and aggregated. You will be notified if new information becomes available about the risks or benefits of this research. You will receive a copy of this form after it is signed

Purpose of the study: To determine the mutation spectrum of *BRCA1/2, ATM, CHEK2, PALB2, MLH1, MSH2, MSH6, and PMS2* genes, tumor molecular characterization and risk factors associated with prostate cancer among cancer patients at Ocean Road Cancer Institute (OCRI) and Muhimbili National Hospital (MNH).

Study site: Dar es Salaam-Tanzania.

Study population: The Tanzanian Population will be involved. Tanzanian prostate cancer patients and non-prostate cancer patients at Ocean Road Cancer Institute will be recruited. This is because Mostly Tanzanian prostate cancer patients get specialized prostate cancer treatment from ORCI and MNH.

Study procedures: The case-control and cross-sectional study will be conducted from October 2022 to August 2024. A case-control study will be conducted to demonstrate and evaluate potential risk variables for prostate cancer, such as socioeconomic status, dietary habits, chemical exposure, medical problems, smoking, and alcohol consumption status. While the cross-sectional study will be used to determine the spectrum of genetic mutation, tumor subtypes, and their association with the grade of prostate cancer.

A questionnaire design will be used to collect both epidemiological, associated risk factors, and social economic data associated with prostate cancer. For individuals with prostate cancer, archived block tissue samples will be collected from histopathology and used for ERG expression and PTEN loss tumor subtypes characterization while dried blood sport will be collected from participants with family background or high-grade disease of prostate cancer for germline genetic mutation analysis, No biological sample will be collected from the individual with no prostate cancer, they will only be involved as the control group in establishing risk factors associated with prostate cancer.

If you agree you will do the following:For an individual with Prostate cancer to participate in this study, you will be required to authorize: Your archived biopsy/ block tissues sample used during diagnosis to be used for germline *BRCA1/2, ATM, CHEK2, PALB2, MLH1, MSH2, MSH6 and PMS2* Mutation analysis for this research, researcher to access records of your file at the hospital where you get treatment and where necessary you will contribute blood sports sample on dried blood sports card (DBS) for germline Mutation analysis and provide information as asked in the study questionnaire. Also, Sterile equipment and materials will then be used for sample

collection and transported to the laboratory for processing and analysis. For the control group, only fill study questionnaire, no sample will be taken.

While the Non-prostate cancer group will authorize a researcher to access records of your file at the hospital where you get treatment and provide information as asked in the study questionnaire.

Benefits: The novel information and validated data on risk factors, the spectrum of gene mutation as well as tumor subtypes characterization and their association with disease grade generated from the present study will provide additional lifesaving information in the proper management and prevention of prostate cancer in the Tanzanian population

Risks/Discomforts: There are no potential risks of taking part in the study

Payments and Reimbursements: There will be no payment to participate in this study

Disclosure of test results

No details connecting you to particular test results may be shared with anybody or any organization without your explicit agreement. The consent study may be the only use made of the samples, information gathered, and results of the genetic tests.

Confidentiality:

All reasonable efforts will be made to keep your protected information (private and confidential). Using or sharing ("disclosure") of such information will follow National privacy guidelines. By signing the consent document for this study, you are giving permission ("authorization") for the use and disclosure of your study information. We may need to share your protected information with the community advisory board, MTRH//MU-IREC, NACOSTI or the healthcare team. We will retain your research records for at least six years after the study is completed. At that time,

the research information is destroyed by incineration. If you decide to withdraw your permission for use of your data, contact the [PI] in writing and let them know your decision. At that time, we will stop further collection of any information about you. However, the health information collected before this withdrawal may continue to be used for reporting and research quality.

Sterile equipment and materials will then be used for sample collection and transported to the laboratory for processing and analysis.

Injury compensation:

There is no potential injury to participants in this study

PART II: CONSENT OF PATICIPANT:

I have read or have had someone read to me the description of the research study. The investigator or his/her representative has explained the study to me and has answered all the questions I have at this time. I have been told of the potential risks, discomfort, and possible benefits (if any) of the study. I freely volunteer to take part in this study.

_____	_____	_____
Name of Participant	Signature of participant/Thumbprint	Date & Time
_____	_____	_____
Name of Witness [Optional]	Signature of Witness	Date & Time
_____	_____	_____
Name of the person obtaining consent	Signature of person obtaining consent	Date & Time
_____	_____	
The printed name of the investigator	Signature of Investigator	Date & Time

Contacts for questions about the study

Questions about the study: Fidelis Charles, +25470009408, bugoye81@yahoo.co.uk

Questions about your rights as a participant: You may contact The Chairman of the National Health Research Ethics Committee, (NatHREC)-NIMR, Tanzania. Can also be contacted at +255222121400/ +255 758 587885**, or email:** nimrethics@gmail.com

OR

Institutional Ethics and Research Committee (MTRH//MU-IREC) 0787723677 or email irec@mtrh.go.ke or irecoffice@gmail.com. The MTRH//MU-IREC concerning your rights as a research volunteer. These are group of people that review studies for safety and to protect the rights of participants.

HOSPITALI YA RUFAA NA MAFUNZO /CHUO KIKUU MOI /CHUO CHA SAYANSI YA AFYA – KAMATI YA TAASISI INAYOSHUGHULIKA NA MAADILI KATIKA TAFITI

(MTRH/MU-IREC)

FOMU YA KURIDHIA KUSHIRIKI KATIKA UTAFITI

Kichwa cha Utafiti:

Wigo wa mabadiliko ya jeni, aina ndogo za uvimbe, vihatarishi vya saratani ya tezi dume zinavyo onekana miongoni mwa wagonjwa wa sarati ya tezi deme katika Hospitali ya Saratani ya Ocean Road na Hospitali ya Taifa Muhimbili, Dar es salaam-Tanzania.

Jina la Mtafiti Mkuu : Fidelis Charles

Watafiti wenza(s): Dkt. Rispah Torrorey and Dkt. Richard Biegon

Jina la Taasisi Name: Idara ya Patholojia na Immunolojia, Chuo Kikuu Moi,-Kenya

Anuani: Chuo Kikuu Moi S.L.P 4606 030100 Eldoret, Kenya Simu: +255700094408

Jina la mfadhili /Taasisis ya ufadhili: P4HPTII

Fomu ya kuridhia kwa ajili ya : Kushiriki katika utafiti

Fomu hii ina sehemu mbili:

- Sehemu I: fomu ya maelezo [kukueleza kuhusu utafiti]
- Part II: Cheti cha kuridhia kushiriki katika utafiti [kwa kuweka sahihi kama utaridhia kushiriki]

PSEHEMU YA I: FOMU YA MAELEZO

Utangulizi:

Unaomba ushiriki katika utafiti. Maelezo haya yanatolewa ili kukuambia kuhusu utafiti. Tafadhali soma fomu hii kwa makini. Utapewa nafasi ya kuuliza maswali. Kushiriki katika utafiti huu ni kwa hiari. Kusema hapana hakutaathiri haki zako za huduma ya afya au huduma zingine zozote. Matibabu/malipo au uandikishaji wako katika mipango yoyote ya afya au ustahiki wa manufaa hautaathiriwa ukiamua kutoshiriki.

Pia uko huru kujiondoa kwenye utafiti huu wakati wowote. Ikiwa baada ya kukusanya taarifa utachagua kuacha, unaweza kuomba kwamba maelezo uliyotoa yaharibiwe chini ya usimamizi. Hii itakuwa kabla ya taarifa haijatambuliwa na kujumuishwa. Utaarifiwa iwapo taarifa mpya itapatikana kuhusu hatari au manufaa ya utafiti huu. Utapokea nakala ya fomu hii baada ya kusainiwa

Madhumuni ya utafiti:

Kuchunguza wigo wa mabadiliko ya jeni *BRCA1/2, ATM, CHEK2*, *PALB2, MLH1, MSH2, MSH6* na *PMS2*, sifa za molekuli ya uvimbe mdogo na vihatarishi vinavyosababisha saratani ya tezi dume kwa wagonjwa wa saratani katika Taasisi ya Saratani ya Ocean Road (OCRI) na Hospitali ya Taifa Muhimbili (MNH).

Eneo la utafiti:

Taasisi ya Saratani ya Ocean Road Dar es Salaam-Tanzania

Wahusika katika utafiti:

Idadi ya watu wa Tanzania watahusika. Wagonjwa wa saratani ya tezi dume na wasio na Saratani ya Tezi dume kutoka Tanzania katika Taasisi ya Saratani ya Ocean Road (ORCI)

au Hospital ya Taifa ya Muhimbili. Wagonjwa wengi wa saratani ya tezi dume wa Tanzania wanapata matibabu maalumu ya saratani ya tezi dume kutoka ORCI na MNH.

Taratibu za utafiti:

Utafiti huu utafanyika kuanzia mwezi Oktoba 2022 hadi Agosti 2024. Utafiti huu utafanyika ili kubaini na kutathmini uwezekano wa viahatarishi vya saratani ya tezi dume, kama vile hali ya kijamii na kiuchumi, tabia ya ulaji wa vyakula, athahri za kemikali, matatizo ya kiafya na matibabu. , hali ya uvutaji sigara na unywaji pombe. Aidha utafiti huu pia utatumika kubainisha wigo wa mabadiliko ya jeni, aina ndogo za uvimbe na uhusiano wao na daraja la saratani ya tezi dume.

Muundo wa dodoso utatumika kukusanya taarifa ya magonjwa, vihatarishi zinazohusiana, na taarifa ya kiuchumi ya kijamii inayohusishwa na saratani ya tezi dume. Sampuli ya tishu iliyohifadhiwa kwenye itakusanywa kutoka kitengo cha histopatholojia na kutumika kwa uuchunguzi na uwekaji wa makundi madogo ya uvimbe wan saratani katika kundi hasi ama chanya ya ERG na PTEN. Vile vile sampuli ya damu itachukuliwa na kukaushwa kutoka kwa washiriki walio na historia ya saratani katika familia zao au ugonjwa wa daraja la juu wa saratani ya tezi dume ili sampulin zao zitumike katika uchunguzi wa uchanganuzi wa wigo na mabadiliko ya jeni.

Ukikubali kushirki, unaridhia yafuatayo:

Ili kushiriki katika utafiti huu utahitajika kuidhinisha: Kwa wagonjwa wa saratani ya tezi dume sampuli yako iliyohifadhiwa ya biopsy/block tissues iliyotumika wakati wa utambuzi itumike kwa katika uchungui wa wigo wa Mabadiriko ya jeni *BRCA1/2, ATM, CHEK2, PALB2, MLH1, MSH2, MSH6* na *PMS2*. Mtafiti kupata kumbukumbu za faili lako hospitalini unapopata matibabu Pamoja na kujaza dodoso la maswali na inapobidi utachangia sampuli ya damu kidogo kwenye kadi ya kuchukulia na kukaushia damu(DBS) kwa ajili ya uchungui manadiliko ya jeni. Vifaa na nyenzo safi na salama zitatumika kwa

ukusanyaji wa sampuli na kusafirishwa hadi kwenye maabara kwa ajili ya uhifadhi na uchambuzi. Kwa washiriki watakao tumika kama kundi linganishi, wanajaza dodoso la utafiti pekee, hakuna sampuli itakayochukuliwa.

Faida za utafiti huu:

Taarifa za muhimu iliyoidhinishwa kuhusu mambo ya vihatarishi, wigo wa mabadiliko ya jeni pamoja na sifa za aina ndogo za uvimbe na uhusiano wao na kiwango cha ugonjwa unaotokana na utafiti huu utatoa taarifa za ziada za kuokoa maisha katika udhibiti na uzuiaji sahihi wa saratani ya tezi dume nchini Tanzania.

Athari za ushiriki katika utafiti huu:

Hakuna athari zozote katika kushiriki katika utafiti buu

Malipo kwa washiriki:

Hakuna mailipo yoyote kwa kushiriki katika utafiti huu

Ufichuaji wa matokeo ya uchunguzi

Hakuna maelezo yanayokuunganisha kwenye matokeo mahususi ya uchunguzi katika utafiti huu yanaweza kushirikiwa na mtu yeyote au shirika lolote bila makubaliano yako ya wazi. ridhaa yako ya kushiriki katika utafiti huu utazingatiwa na matumizi ya sampuli, taarifa zinazopatikana katika utafiti huu yatatumika katika malengo yaliyokusudiwa tu.

Usiri na faragha:

Juhudi zote zinazofaa zitafanywa ili kuweka maelezo yako yaliyolindwa (ya faragha na ya siri). Kutumia au kushiriki (“kufichua”) kwa maelezo kama haya kutafuata miongozo ya faragha ya Kitaifa. Kwa kutia sahihi hati ya idhini ya utafiti huu, unatoa ruhusa ("idhini") kwa matumizi na ufichuaji wa maelezo yako ya utafiti. Huenda tukahitaji kushiriki habari zako zinazolindwa na bodi ya ushauri ya jamii, MTRH//MU-IREC, NACOSTI au timu ya afya. Tutahifadhi rekodi zako za utafiti kwa angalau miaka sita baada ya utafiti kukamilika. Wakati huo, maelezo ya utafiti yanaharibiwa na kuteketezwa. Ukiamua

kuondoa ruhusa yako ya kutumia taarifa yako ya kibinafsi, wasiliana na [PI] kwa maandishi na uwajulishe uamuzi wako. Wakati huo, tutasimamisha ukusanyaji zaidi wa taarifa yoyote kukuhusu. Hata hivyo, maelezo ya afya yaliyokusanywa kabla ya uondoaji huu yanaweza kuendelea kutumika kwa madhumuni ya kuripoti na ubora wa utafiti.

Vifaa na nyenzo safi na salama vitatumika kwa ukusanyaji wa sampuli na kusafirishwa hadi kwenye maabara kwa ajili ya uhifadhi na uchanguzi.

Fidia kwa jeraha:

Hakuna jeraha linaloweza kutokana na ushiriki wako katika utafiti huu

SEHEMU II: IDHINI YA MSHIRIKI:

Nimesoma au kuna mtu amenisomea maelezo ya utafiti huu. Mchunguzi au mwakilishi wake amenieleza kuhusu utafiti na amejibu maswali yote niliyo nayo kwa wakati huu. Nimeambiwa kuhusu athari zinazoweza kutokea, usumbufu, na manufaa yanayoweza kutokea (ikiwa yapo) ya utafiti. Ninajitolea kwa hiari kushiriki katika utafiti huu.

_____ _____ _____

Jina la Mshiriki, Sahihi ya mshiriki/alama ya dole gumba Tarehe na muda

_____ _____ _____

Jina la shuhuda [si lazima] Sahihi ya shuhuda Tarehe na muda

_____ _____ _____

Jina la aliyepokea idhini Sahihi ya aliyepokea idhini Tarehe na muda

Time

_____ _____ _____

Jina la Mtafiti. Sahihi ya Mtafiti Tarehe na muda

Mawasilkiano na maswali kuhusu utafiti huu.

Maswali kuhusu utafiti: Fidelis Charles, +25470009408, bugoye81@yahoo.co.uk

Maswali kuhusu haki zako kama mshiriki: Unaweza kuwasiliana na Mwenyekiti wa Kamati ya Kuzingatia Maadili kutoka Taasisi ya Utafiti wa Magonjwa ya Binadamu ya Tanzania (NIMR) anaweza pia kutaarifiwa kuhusu haki zako kama mshiriki katika utafiti kwa namba ya simu +255-22-2121400/ +255 758 587885**,** au barua pepe: nimrethics@gmail.co

Au

Kamati ya Maadili na Utafiti ya Kitaasisi (MTRH//MU-IREC) 0787723677 au barua pepe irec@mtrh.go.ke au irecoffice@gmail.com. MTRH//MU-IREC ni kundi la watu wanaopitia tafiti kwa ajili ya usalama na kulinda haki za washiriki.

Appendix 8:IREC Approval

MTRH/MU-INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/325/2022
Approval Number: 0004307

5th December, 2022

Fidelis Charles,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET- KENYA.

Dear Mr. Charles,

MUTATION SPECTRUM, TUMOR SUBTYPES, AND RISK FACTORS OF PROSTATE CANCER SEEN AT OCEAN ROAD CANCER INSTITUTE, DAR ES SALAAM- TANZANIA

This is to inform you that ***MTRH/MU-IREC*** has reviewed and approved the above referenced research proposal. Your application approval number is ***FAN: 0004307.*** The approval period is **5th December, 2022- 4th December, 2023.** This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, Material Transfer Agreements (MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by ***MTRH/MU-IREC***.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to ***MTRH/MU-IREC*** within 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to ***MTRH/MU-IREC*** within 72 hours.
v. Clearance for export of biological specimens must be obtained from ***MOH at the recommendation of NACOSTI*** for each batch of shipment.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to ***MTRH/ MU-IREC.***

Prior to commencing your study; you will be required to obtain a research license from the National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and other relevant clearances from study sites including a written approval from the CEO-MTRH which is mandatory for studies to be undertaken within the jurisdiction of Moi Teaching & Referral Hospital (MTRH) and its satellites sites.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
05 DEC 2022
APPROVED
P O Box 4606-30100 ELDORET

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

MTRH/MU-INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET

: IREC/325/2022

5th December, 2023

Approval Number: 0004307

Fidelis Charles,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Mr. Charles,

RE: CONTINUING APPROVAL

The Moi Teaching and Referral Hospital/Moi University College of Health Sciences- Institutional Research and Ethics Committee (MTRH/MU-IREC) has reviewed your request for continuing approval for your study titled: -

"Mutation Spectrum, Tumor Subtypes, and Risk Factors of Prostate Cancers Seen at Ocean Road Cancer Institute and Muhimbili National Hospital, Dar es Salaam-Tanzania."

Your request has been granted Approval with effect from 5th December, 2023. You are therefore permitted to continue with your study.

Note that this approval is for 1 year; it will thus expire on 4th December, 2024 if it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to MTRH/MU-IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
05 DEC 2023
APPROVED
P. O. Box 4606 - 30100 ELDORET

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: CEO - MTRH Dean - SPH Dean - SOM
Principal - CHS Dean - SOD Dean - SON

MTRH/MU- INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
COLLEGE OF HEALTH SCIENCES
P.O. BOX 4606
ELDORET
Tel: 33471/2/3

Reference: IREC/325/2022
Approval Number: 0004307

18th October, 2023

Fidelis Charles,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Mr. Charles,

RE: APPROVAL OF AMENDMENT

The Moi Teaching and Referral Hospital /Moi University College of Health Sciences -Institutional Research and Ethics Committee (MTRH/MU-IREC) has approved the requested amendment to the study titled "***Mutation Spectrum, Tumor Subtypes, and Risk Factors of Prostate Cancers Seen at Ocean Road Cancer Institute, Dar es Salaam-Tanzania.***" The amendment pertains to the modification of the study title to include "Muhimbili National Hospital" in addition to the original study site.

1. **Description of the Proposed Change**: The proposed change involves adding "Muhimbili National Hospital" to the study title. While the approved research protocol already mentioned Muhimbili National Hospital as a recruitment site, the title at the time of approval did not reflect this inclusion.

Amended Study Title: "Mutation Spectrum, Tumor Subtypes, and Risk Factors of Prostate Cancers Seen at Ocean Road Cancer Institute and Muhimbili National Hospital, Dar es Salaam-Tanzania."

MTRH/MU-IREC acknowledges and approves this change, and it will be reflected in all official documents, including the study protocol and any associated reports. Please proceed with the implementation of these approved amendments according to the revised protocol.

You are required to submit progress(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change(s) or amendment(s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

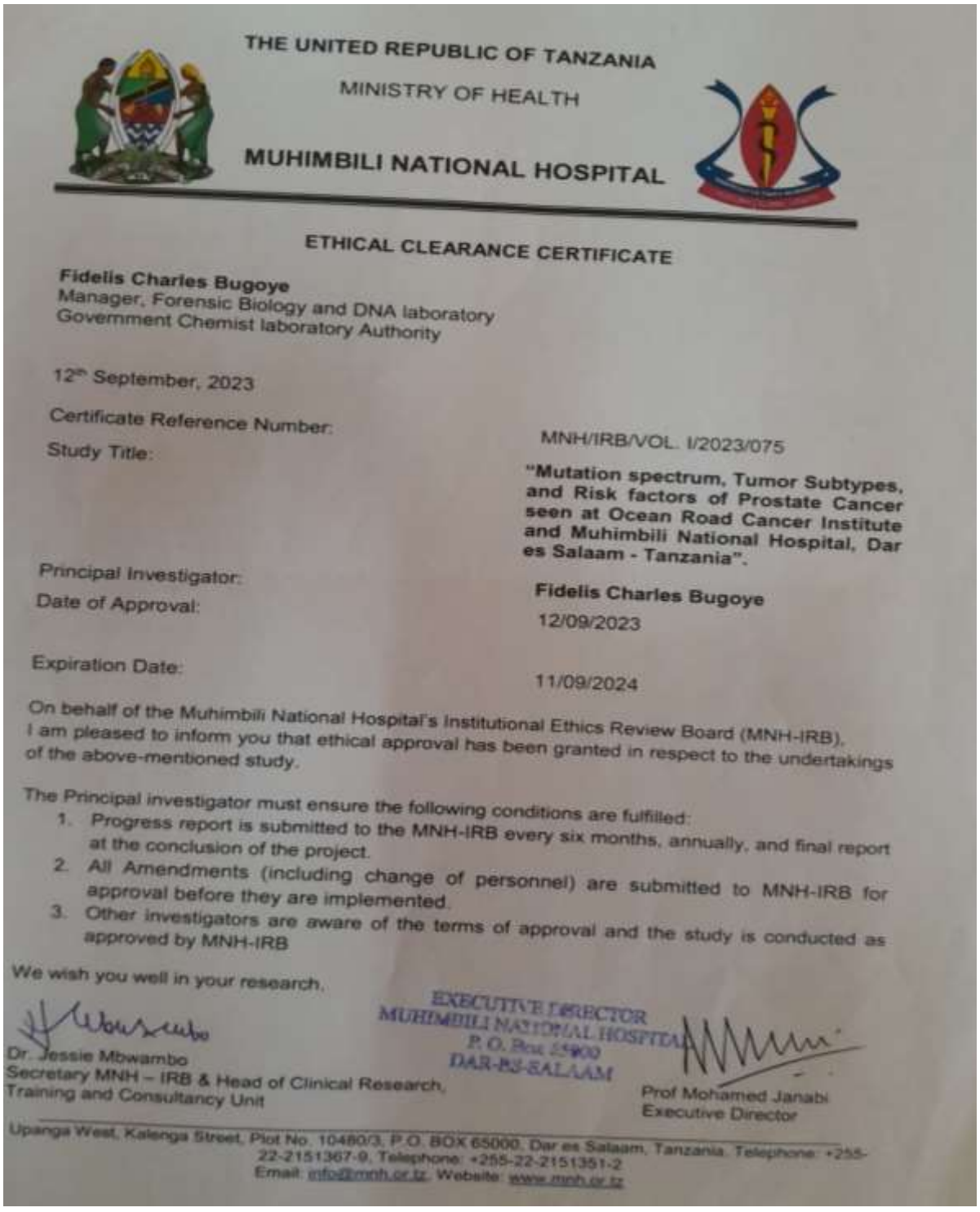
INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
18 OCT 2023
APPROVED
P. O. Box 4606 - 30100 ELDORET

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc: CEO - MTRH Dean - SPH Dean - SOM
Principal - CHS Dean - SOD Dean - SON

Appendix 9:Approval to Conduct Research



THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH

MUHIMBILI NATIONAL HOSPITAL

ETHICAL CLEARANCE CERTIFICATE

Fidelis Charles Bugoye
Manager, Forensic Biology and DNA laboratory
Government Chemist laboratory Authority

12th September, 2023

Certificate Reference Number: MNH/IRB/VOL. I/2023/075

Study Title: **"Mutation spectrum, Tumor Subtypes, and Risk factors of Prostate Cancer seen at Ocean Road Cancer Institute and Muhimbili National Hospital, Dar es Salaam - Tanzania".**

Principal Investigator: **Fidelis Charles Bugoye**

Date of Approval: 12/09/2023

Expiration Date: 11/09/2024

On behalf of the Muhimbili National Hospital's Institutional Ethics Review Board (MNH-IRB), I am pleased to inform you that ethical approval has been granted in respect to the undertakings of the above-mentioned study.

The Principal investigator must ensure the following conditions are fulfilled:

1. Progress report is submitted to the MNH-IRB every six months, annually, and final report at the conclusion of the project.
2. All Amendments (including change of personnel) are submitted to MNH-IRB for approval before they are implemented.
3. Other investigators are aware of the terms of approval and the study is conducted as approved by MNH-IRB

We wish you well in your research.

EXECUTIVE DIRECTOR
MUHIMBILI NATIONAL HOSPITAL
P. O. Box 65000
DAR-ES-SALAAM

Dr. Jessie Mbwambo
Secretary MNH – IRB & Head of Clinical Research,
Training and Consultancy Unit

Prof Mohamed Janabi
Executive Director

Upanga West, Kalenga Street, Plot No. 1048/3, P.O. BOX 65000, Dar es Salaam, Tanzania. Telephone: +255-22-2151367-9, Telephone: +255-22-2151351-2
Email: info@mnh.or.tz, Website: www.mnh.or.tz

THE UNITED REPUBLIC OF TANZANIA

National Institute for Medical Research
3 Barack Obama Drive
P.O. Box 9653
11101 Dar es Salaam
Tel: 255 22 2121400
Fax: 255 22 2121360
E-mail: ethics@nimr.or.tz

Permanent Secretary
Ministry of Health
Government City Mtumba
Health Road
P.O. Box 743
40478 Dodoma

NIMR/HQ/R.8a/Vol.IX/4299

15 May 2023

Mr. Fidelis Charles Bugoye
Government Chemist Laboratory Authority (GCLA)
P.O Box 164
Dar es Salaam

RE: ETHICAL CLEARANCE CERTIFICATE FOR CONDUCTING MEDICAL RESEARCH IN TANZANIA

This is to certify that the research entitled **"Mutation spectrum, Tumor subtypes, and risk factors of Prostate cancer seen at Ocean Road Cancer Institute and Muhimbili National Hospital, Dar es Salaam-Tanzania" (Bugoye, F. *et al.*)** has been granted ethical clearance to be conducted in Tanzania.

The Principal Investigator of the study must ensure that the following conditions are fulfilled:

1. Progress report is submitted to the Ministry of Health and the National Institute for Medical Research, Regional and District Medical Officers after every six months
2. Permission to publish the results is obtained from the National Institute for Medical Research
3. Copies of final publications are made available to the Ministry of Health and the National Institute for Medical Research
4. Any researcher, who contravenes or fails to comply with these conditions, shall be guilty of an offence and shall be liable on conviction to a fine as per NIMR Act No. 23 of 1979, PART III Section 10 (2)
5. Sites: Dar es Salaam

Approval is valid for one year: 15 May 2023 to 14 May 2024

Name: Prof. Said S. Aboud

Signature
CHAIRPERSON
MEDICAL RESEARCH
COORDINATING COMMITTEE

Name: Prof. Tumaini J. Nagu

Signature
CHIEF MEDICAL OFFICER
MINISTRY OF HEALTH

c.c: Director, Health Services-TAMISEMI, Dodoma
RMO of Dar es Salaam region
DMO/DED of Ilala district

UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH

OCEAN ROAD CANCER INSTITUTE

ORCI OCEAN ROAD CANCER INSTITUTE

On Reply Please Quote:

Ref: 10/VOL.XXI/121-B

Date: 8th February 2023

INSTITUTIONAL ACADEMICS, RESEARCH, PUBLICATIONS AND ETHICS COMMITTEE (IARPEC)

Fidelis Charles Bugoye,
PhD candidate,
MOI University,
Eldoret, Kenya.

Re: Approval to undertake a study titled 'Mutation Spectrum, Tumor Subtypes and Risk Factors of Prostate Cancer seen at Ocean Road Cancer Institute, Dar es Salaam, Tanzania'

Reference is made to the above heading and to the letter of introduction from the Department of pathology, Moi University dated 21st September 2022 requesting for permission to conduct this research at the Institute. It is noted that the study has received ethical clearance from the Moi Teaching and Referral Hospital Institutional research and Ethics Committee. It is also noted that application for research clearance has been made to the National Institute of Medical Research (NIMR).

This is to inform you that the Institutional Academics, Research, Publications and Ethics Committee has granted you permission for the implementation of the above-mentioned study at ORCI for a period of one year from the date of this letter subject to receiving clearance from NIMR.

You are required to submit a copy of NIMR clearance before beginning data collection. Upon successful completion of the study, you are obliged to submit a copy of the final document of this research project to the IARPEC.

Sincerely,

OCEAN ROAD CANCER INSTITUTE
ACADEMIC UNIT
P.O. Box 3592
DAR E. SALAAM

Dr Nazima Dharsee MD, MMed, MSc
Director- Academic, Research and Consultancy Unit

THE UNITED REPUBLIC OF TANZANIA

MINISTRY OF HEALTH

MUHIMBILI NATIONAL HOSPITAL

Ref: No.: MNH/IRB/Prov/2023/001

Date: 11/04/2023

Fidelis Charles Bugoye
Manager, Forensic Biology and DNA Laboratory
Government Chemist Laboratory Authority

REF: PROVISIONAL ETHICAL CLEARANCE

On behalf of the Muhimbili National Hospital's Institutional Review Board (MNH-IRB), I am pleased to inform you that your study **proposal titled "Mutation Spectrum, Tumor Subtypes, and Risk Factors of Prostate Cancer seen at Ocean Road Cancer Institute and Muhimbili National Hospital, Dar es Salaam - Tanzania" has** been reviewed and granted ethical clearance.

However, please note that this clearance is **provisional** and contingent upon the submission of all required documents, including the NIMR-IRB approval and DTA/MTA where applicable. If study is a clinical trial you will need TMDA approval.

A full administrative ethical clearance together with a data collection permit for study to be conducted at MNH will be awarded on successful lodging of all respective documents at Clinical Research, Training and Consultancy Unit.

We wish you well in concluding all procedures for ethical clearance, data transfer and material transfer agreement.

HEAD, TEACHING, RESEARCH & CONSULTANCY UNIT
P.O. Box 65000
DAR-ES-SALAAM

Dr. Jessie Mbwambo
Secretary MNH – IRB & Head of Clinical Research, Training and Consultancy Unit

Upanga West, Kalenga Street, Plot No. 10480/3, P.O. BOX 65000, Dar es Salaam, Tanzania. Telephone: +255-22-2151367-9, Telephone: +255-22-2151351-2
Email: info@mnh.or.tz, Website: www.mnh.or.tz