GENETIC POLYMORPHISM OF ION CHANNEL TRANSIENT RECEPTOR POTENTIAL MEMBRANE MELASTATIN AND RISK OF BENIGN PROSTATE HYPERPLASIA AMONG MEN IN KISUMU COUNTY

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DECLARATION

This thesis is my original work and has not been presented for a degree at any other University or any other award.

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DEDICATION

I humbly dedicate this thesis to the unwavering pillars of my life, expressing my deepest gratitude to my dear wife, Selah Owino. Her continuous moral and emotional support has been the guiding light throughout this academic journey. To my daughters, Myra, Asnat, and Ayli, whose presence and joy fueled my determination; to my parents, George and Pamela, for their ceaseless prayers and unwavering moral support; and to my brothers and sisters, your encouragement echoed in every chapter of this study, making the path smoother.

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LIST OF ABBREVIATIONS AND ACRONYMS

ARI	Alpha Reductase Inhibitor
AUR	Acute Urinary Retention
BPH	Benign Prostate Hyperplasia
Ca2+	Calcium Ion
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic Acid
DRE	Digital Rectal Examination
EDTA	Ethylene Diamine Tetra Acetic Acid
ERC	Ethics and Review Committee
FSH	Follicle-Stimulating Hormone
ILRI	International Livestock Research Institute
IPSS	International Prostate Symptom Score
JOOTRH	Jaramogi Oginga Odinga Teaching &Referral Hospital
LH	Luteinizing Hormone
LHRH	Luteinizing Hormone Releasing Hormone
LUTS	Lower Urinary Tract Symptoms
MAF	Minor Allele Frequency
METs	Metabolic Syndrome
Mg2+	Magnesium Ion
MDH	Malate Dehydrogenase
MOPC	Medical Outpatient Clinic
Na+	Sodium Ion
NACOSTI	National Commission for Science, Technology & Innovation
OR	Odds Ratio

Pca	Prostate Cancer
PEP	Phosphoenyl Pyruvate
PEPC	Phosphoenyl Pyruvate Carboxylase
PSA	Prostate Surface Antigen
QOL	Quality of Life
ROS	Reactive Oxygen Species
SD	Standard Deviation
SNPs	Single Nucleotide Polymorphism
TGF-β	Transforming Growth Factor-Beta
TRPM	Transient Receptor Potential Melastatin
TRPS	Transient Receptor Proteins
TRUS	Transrectal Ultrasonography
TURP	Transurethral Resection of The Prostate
UTI	Urinary Tract Infection

ABSTRACT

Benign prostate hyperplasia (BPH) is a condition that primarily affects middle-aged and older men, leading to lower urinary tract symptoms. The current understanding of genetic polymorphisms in transient potential membrane Melastatin channels provides compelling evidence of their potential role in the pathogenesis of BPH due to cation imbalances. This study aimed to determine the genetic polymorphism of transient receptor potential Melastatin channel genes as a risk factor for BPH among men in Kisumu County. The analytical cross-sectional study involved 194 BPH patients and 194 healthy controls aged 35 years and above from the urology clinic of Jaramogi Oginga Odinga Teaching and Referral Hospital. Six SNP variants were analyzed: TRPM2 (rs168355), TRPM6 (rs4745363), TRPM7 (rs8042919, rs2362295), and TRPM8 (rs10490018, rs1016062). DNA extraction was performed using the Qiagen® kit, and genotyping was done using the Illumina® I Scan Infinium and Multiplex human genotyping microarrays. Polymorphisms were analyzed in genome studio software modules, while haplotype distribution was determined using the DRAGEN haplotype variant. Serum concentrations of sodium, chloride, and potassium ions were measured using the ROCHE® AVL 9180, and concentrations of magnesium and bicarbonate ions were measured using the COBAS® integra 400 plus. Prostate surface antigen (PSA) was analyzed using the Cobas® e411 analyzer. Statistical significance was set at $p<0.05$, with 95% confidence intervals. Approval for the study was obtained from Kenyatta University's graduate school, Jaramogi Oginga Odinga Teaching and Referral Hospital's Ethics and Research Committee (IERC/JOOTRH/531/21), and NACOSTI (NACOSTI/0/22/17031). Three hundred eighty-eight adult males participated in the study: 194 BPH patients and 194 healthy individuals. The mean age for BPH patients was 65.47±12.55 years (range: 38-92 years), while the healthy control group had a mean age of 64.52±12.19 years (range: 39-91 years). Most of both groups fell within the 70-79 age range (n=53 for BPH, n=52 for controls). Statistically significant differences were found in symptom duration ($p=0.001$*) and severity of lower urinary tract symptoms (LUTS) ($p<0.001$*) between the BPH patients and controls, as well as in the association between age distribution and PSA levels ($p<0.001$*). Significant associations were also found between the genotypes TT/TG/GG of TRPM2 rs168355 and LUTS IPSS scores (mild, moderate, severe) ($p=0.021$), as well as between the genotypes CC/CT/TT of TRPM7 rs2362295 and LUTS IPSS scores ($p=0.034$). Additionally, significant relationships were observed between genotypes TT/TG/GG of TRPM2 rs168355 and PSA levels ($p=0.034$) and between CC/CT/TT of TRPM7 rs2362295 and PSA levels ($p=0.041$) among BPH patients. A significant correlation ($p\leq0.05$) was observed between rs168355 (TRPM2) and rs2362295 (TRPM7) polymorphisms. For rs168355, there was an increase in the TT genotype (92.7%) and T allele frequency (95.6%) in BPH patients compared to controls (TT, 61.6%; T, 74.9%; $p<0.001$). The GG genotype had a lower frequency among BPH patients (1.6%) compared to controls (11.9%, $p<0.001$). The TT/TG/GG genotypes and T/G alleles showed significant differences between patients and controls. This study's significance lies in providing critical insights into the genetic factors contributing to BPH risk, which may guide future diagnostic and therapeutic strategies aimed at improving patient outcomes.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background information

Benign Prostate Hyperplasia (BPH) is a common non-malignant condition affecting the prostate gland, primarily seen in ageing males. BPH is estimated to affect more than half of the global male population, with its prevalence rising significantly among men aged 80 to 89 years, with approximately three-quarters of the male population in this age group affected (Awedew *et al*., 2022). This condition is characterized by the excessive growth of stromal and epithelial cells within the prostate gland, particularly in the transitional zone (TZ) surrounding the urethra, leading to an enlarged prostate. Its pathophysiology is closely linked to impaired apoptosis, causing cellular proliferation that contributes to the prostate's enlargement (Vickman *et al*., 2020).

Several factors influence the onset and progression of BPH, key among them being biological factors such as the imbalance of androgens (e.g., testosterone and dihydrotestosterone), age-related changes in tissue, and chronic inflammation. These elements work synergistically to alter the prostate’s structural and functional integrity, resulting in BPH symptoms (Cannarella *et al*., 2021). The overgrowth of prostate tissue often gives rise to lower urinary tract symptoms (LUTS), which are classified into two main categories: irritative (e.g., urgency and frequency) and obstructive (e.g., difficulty initiating urination and weak urinary stream). These symptoms contribute significantly to the decreased quality of life among affected individuals (Lee & Kuo, 2017).

While BPH can remain asymptomatic in some men, most individuals often develop clinically significant symptoms with advancing age. Risk factors such as obesity, metabolic syndrome, and a sedentary lifestyle have been linked to worsening BPH

symptoms (Langan, 2019). Several therapeutic options are available as the condition progresses, ranging from pharmacological interventions to surgical procedures, depending on the disease's severity and the symptoms' impact on daily activities (Miernik & Gratzke, 2020).

The diagnostic approach to BPH includes several methodologies, with histopathological examination recognized as the definitive method for diagnosis (C. Gyasi-Sarpong *et al*., 2018). Other diagnostic tools used in clinical practice include the International Prostate Symptom Score (IPSS), which evaluates the severity of LUTS, along with various radiographic imaging techniques, digital rectal examinations (DRE), and laboratory tests like serum prostate-specific antigen (PSA) levels, (Lokeshwar *et al*., 2019).

Calcium ($Ca^{2+)}$ and magnesium (Mg^{2+}) ions are critical in many cellular processes, including signal transduction, enzyme activation, and cell cycle regulation. Dysregulation of these ions has been implicated in a variety of pathophysiological conditions, including cardiovascular diseases, diabetes, and BPH (Fiorentini *et al*., 2021; Wang *et al*., 2021). The loss of homeostasis in ion channel regulation, specifically the malfunction of calcium and magnesium channels, has been suggested to contribute to abnormal cellular growth in the prostate gland (Li *et al*., 2021).

The discovery of transient receptor potential (TRP) ion channels has significantly advanced our understanding of how these channels regulate Ca^{2+} and Mg^{2+} ions. Initially discovered in Drosophila, TRP channels are now known to play a crucial role in maintaining cellular ion homeostasis, with specific channels identified as regulators of prostate growth and oncogenesis (Blair *et al*., 2024; Froghi *et al*., 2021). Among the TRP channel families, the transient receptor potential melastatin (TRPM) subfamily has drawn attention for its involvement in several cancers and its regulatory

role in cell proliferation (Koivisto *et al*., 2022; Perna *et al*., 2022; Silverman *et al*., 2020). The TRPM subfamily comprises eight members: TRPM1, TRPM2, TRPM3, TRPM4, TRPM5, TRPM6, TRPM7, and TRPM8 (Jimenez *et al*., 2020).

Mutations and dysfunctions in TRPM channels have been implicated in various clinical disorders. For example, mutations in TRPM6 have been associated with hypomagnesemia, a condition marked by low magnesium levels, leading to complications such as seizures and muscle spasms (Lomelino-Pinheiro *et al*., 2020; Rather *et al*., 2023). Dysregulated TRPM7 activity has been connected to tumorigenesis, while TRPM8 has been proposed as a potential therapeutic target for prostate cancer (Ochoa *et al*., 2023).

This study aims to investigate the genetic polymorphisms of TRPM2, TRPM6, TRPM7, and TRPM8 and their association with BPH in men from Kisumu County, Kenya. Given the critical roles of Ca^{2+} and Mg^{2+} in disease pathogenesis, this study will explore whether specific variants in these channels may contribute to BPH risk, providing new insights into potential biomarkers and therapeutic targets for this prevalent condition.

1.2 Statement of the problem

Benign Prostate Hyperplasia (BPH) is a significant global health issue, particularly among ageing men. The rise in illnesses among the elderly is often attributed to degenerative changes and weakened immune systems, making them more susceptible to various conditions (Phua, 2021). BPH is one of the most prevalent conditions affecting this population, with serious complications that drastically reduce the quality of life. Symptoms of BPH, such as frequent urination, urinary intermittency, painful ejaculation, and a foul odour due to urine accumulation, are particularly distressing (Ginsberg *et al*., 2021). Left untreated, BPH can lead to urinary tract infections (UTIs)

and even renal failure. Despite the widespread prevalence of BPH, its exact aetiology remains unclear, contributing to challenges in diagnosis and treatment (Alzahrani *et al*., 2024).

A significant gap in BPH research lies in the lack of understanding of the role of genetic factors and ion channel dysfunctions in its development and progression. Current treatments often fail to address all patients' needs, with some showing resistance to available therapies. This indicates that a complex interplay of genetics, environmental influences, and other unknown factors may contribute to the disease's pathogenesis. Studies have shown that transient receptor potential (TRP) ion channels, which regulate various cellular processes, including cation and anion transport, are implicated in numerous disorders, including BPH (Duitama *et al*., 2020; Rosenbaum *et al*., 2020). However, a detailed investigation into the specific genetic polymorphisms of TRP channels and their potential association with BPH remains largely unexplored.

This study aims to fill this gap by investigating the genetic polymorphisms of TRPM2, TRPM6, TRPM7, and TRPM8 channels and their possible association with BPH in men from Kisumu County, Kenya. Understanding the role of these ion channels in BPH could uncover new biomarkers for early diagnosis and provide insights into potential therapeutic targets. By doing so, this research could contribute to improved management and treatment outcomes for men affected by BPH, particularly in the Kenyan population, where such studies are limited.

1.3 Significance of study

The significance of this study lies in its contribution to understanding the genetic factors influencing Benign Prostate Hyperplasia (BPH) within the Kenyan population. There is clear evidence linking Ca^{2+}, $Mg^{2+,}$ and other trace elements imbalances in the

aetiology and pathophysiology of BPH (Asare *et al*., 2017). Transient Receptor Potential Melastatin (TRPM) channels regulate mineral homeostasis; hence, they play a role in disease progression. In the Kenyan population, there is a lack of data regarding the role of genetic variation in TRPM channels among men with BPH. This study aimed to investigate the association of rs1618355 in TRPM2, rs4745363 in TRPM6, rs8042919 and rs2362295 in TRPM7, and rs1016062 and rs10490018 in TRPM8 polymorphisms among BPH patients. Additionally, the study sought to establish if there was a link between SNP variation or mutation and mineral concentrations to determine potential functional outcomes related to BPH.

The exclusion of TRPM1, TRPM3, TRPM4, and TRPM5, which are part of the TRPM family, was due to their limited relevance to prostate health and the specific focus of this research. TRPM1 primarily involves visual processing and melanocyte function, making it less pertinent to prostate physiology. TRPM3, although engaged in magnesium transport, overlaps with other TRPM subtypes more directly linked to prostate function, leading to its exclusion to avoid redundancy. TRPM4 regulates membrane potential in cardiac and immune cells unrelated to prostate disease. At the same time, TRPM5 is associated with taste sensation and glucose metabolism, unrelated to prostate ion channel regulation and oncogenesis.

Kisumu County was selected as the study locale due to its high prevalence of BPH cases compared to other regions in Kenya. There has been a 15% increase in BPH cases over the past five years (National Bureau of Statistics Nairobi, 2023), significantly higher than the national average of an 8% increase. The availability of well-equipped medical facilities, such as Jaramogi Oginga Odinga Teaching and Referral Hospital, also provided an ideal environment for conducting comprehensive clinical studies (MOH, 2018).

For policymakers, the research offers insights that can inform the development of targeted screening programs aimed at early detection of BPH in high-prevalence regions. Healthcare policies can prioritize genetic testing and preventative interventions by identifying specific genetic markers, potentially reducing the disease burden and healthcare costs. The study contributes to personalized treatment approaches for patients, with the potential for more effective therapies based on genetic profiles, which could lead to improved outcomes and reduced invasive treatments. Practitioners stand to benefit from the study's findings by gaining deeper insights into the genetic mechanisms of BPH, enabling them to make informed decisions on patient management. The study's unique contribution lies in filling a significant knowledge gap on TRPM genetic polymorphisms and their role in BPH among the Kenyan population, providing a foundation for future research and contributing to the global understanding of prostate disease.

1.4 Null Hypotheses

Ho_1: There is no significant difference between socio-demographic profiles, clinical characteristics, and laboratory profiles between BPH patients and control male participants in Kisumu County, Kenya.

Ho_2: There is no significant association between Transient Receptor Potential Melastatin membrane 2, 6, 7, and 8 channels and benign prostate hyperplasia among men in Kisumu County, Kenya.

Ho_2: There are no significant levels of the variant transient receptor potential melastatin 2, 6, 7, and 8 channels regulatory proteins in BPH patients and control participants in Kisumu County, Kenya.

Ho_3: There are no significant levels of electrolytes, and the transient receptor potential melastatin membrane channel expression is in BPH patients and control participants in Kisumu County, Kenya.

1.5 Objectives

1.5.1 Broad Objective

To determine the genetic polymorphism of ion channel transient receptor potential melastatin 2, 6, 7, and 8 membranes as a possible risk for benign prostate hyperplasia among men in Kisumu County, Kenya.

1.5.2 Specific Objectives

1. To establish and compare the socio-demographic profiles, clinical characteristics, and laboratory profiles between BPH patients and control participants.
2. To determine the levels of Na^+, K^+, Cl^+, $Mg^{2+,}$ and HCO3 and their association with the TRPM membrane channel expression among men in Kisumu County and the Transient Receptor Potential Melastatin membrane channel expression in BPH patients and Controls participants in Kisumu County, Kenya.
3. To determine the levels of the variant Transient Receptor Potential Melastatin membrane 2, 6, 7, & 8 channels regulatory proteins in BPH patients and Controls participants in Kisumu County, Kenya.
4. To determine the association between Transient Receptor Potential Melastatin membrane 2, 6, 7, 8 channels and benign prostate hyperplasia among men in Kisumu County, Kenya

1.6 Conceptual Framework

The conceptual framework for this study illustrates the relationship between genetic polymorphisms of TRPM channels and the risk of developing BPH among men in

Kisumu County. Independent variables include PSA, Education, occupation, and Residential, while the dependent variables are the specific TRPM gene variants (e.g., TRPM2, TRPM6, TRPM7, TRPM8) and expression of the mineral ion channels. The framework postulates that specific polymorphisms may lead to altered ion channel function, influencing prostate cell proliferation and thus increasing BPH risk. Intervening/Moderating variables such as age, lifestyle factors, and comorbidities are also considered, as they may impact the strength or direction of the relationship between TRPM polymorphisms and BPH development.

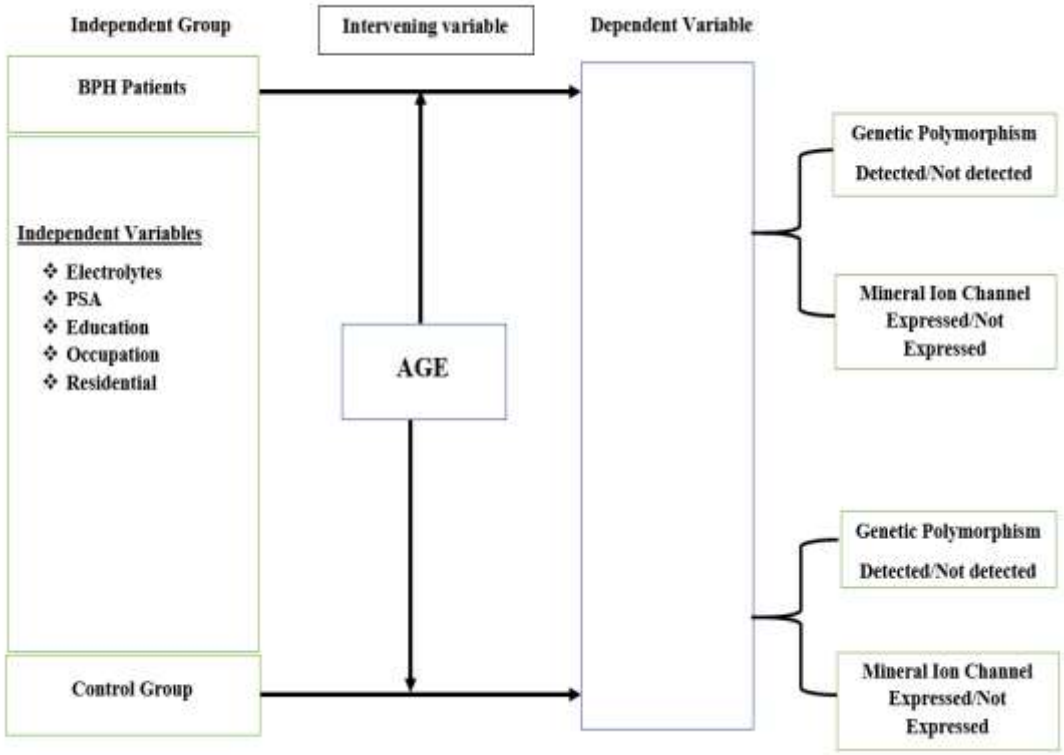

Figure 1.1 Conceptual framework

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Aetiology, epidemiology, pathogenesis, diagnosis and treatment of benign prostate hyperplasia

2.1.1 Aetiology of benign prostate hyperplasia

Benign prostate hyperplasia (BPH) is a condition characterized by the enlargement of the prostate gland due to an increase in stromal cell growth, resulting in the onset of lower tract symptoms (LUTS). The hyperplasia observed in the prostate glands is known to develop from the periurethral region, producing discomfort and lowering the quality of life (QOL) in the ageing male population (Lloyd *et al*., 2019). Studies suggest that an individual's genetic composition significantly influences benign prostate hyperplasia. In a case-control study involving men aged 64 years and older who had BPH and underwent prostatectomy, it was found that blood brothers were six times more likely to undergo surgery compared to other relatives. At the same time, kin were generally four times more likely to face an age-specific risk of such procedures. This increased risk suggests a robust familial component in BPH and prostate cancer, particularly among close male relatives. Studies have confirmed that family history, especially among first-degree relatives, significantly raises the likelihood of BPH and prostate cancer, reflecting similar patterns observed in colorectal cancer cases (Beebe-Dimmer *et al*., 2017).

2.1.2 Epidemiology of benign prostate hyperplasia

Age, genetics, androgens, lifestyle, and inflammation are among the five primary categories of risk factors linked to the development and progression of BPH (Calogero *et al*., 2019). Age is considered the most critical risk factor for BPH and LUTS development (Nickel, 2008). The risk notably rises after fifty years of age in

Caucasians and after forty years of age in black men and those with a family history of BPH. Approximately two-thirds of BPH cases are diagnosed in men aged sixty-five and above (Yeboah & Hsing, 2016). Each decade, the incidence rate accelerates by 10%, reaching an all-time high of 80% by the octogenarian age. The symptoms of benign prostate hyperplasia affect approximately 75% of men over the age of 50 and around 20-30% of those under 50. Additionally, about 20-30% of men over the age of 70 may require surgical intervention (Lokeshwar *et al*., 2019). Even though BPH is generally not expected to affect men below 40 years, an estimated 50% of the male population may have BPH complications by the age of 50 (Abt *et al*., 2021).

The most popular metric for BPH epidemiological studies is an international score for associated symptoms, calculated using a questionnaire's key (Gyasi-Sarpong *et al*., 2018). It categorizes symptoms as asymptomatic, mild, moderate, or severe. The prevalence of the score that indicated moderate to severe LUTs has been estimated to be between 12-44% among those aged 50-74. Many factors influence the presentation of a patient's symptoms with BPH, including the number of hospital visits, origin, and background (Bajunirwe *et al*., 2018; Haghsheno *et al*., 2015).

The Benign prostate hyperplasia and lower tract symptoms are eccentrically defined in terms of race and ethnicity. Several authors have reported a racially homogenous sample; therefore, they are unable to draw any conclusions about a racial variation in BPH prevalence. Nonetheless, a few small studies involving diverse populations have been conducted to see if there is a difference in race presentation. For instance, a cancer prevention trial conducted in the United States discovered that Hispanic men had a higher prevalence of BPH compared to the racial groups, while Asian men were the least affected by the condition (Jung *et al*., 2016; Lee *et al*., 2017)

A community-based study conducted in Olmsted County, Ohio, Examining the severity and impact of lower urinary tract symptoms in black and white men revealed that black men had a 41% higher prevalence compared to white men (Sarma *et al*., 2003). In a population-based study conducted in Iran involving men aged 40 and older, a 23% prevalence was discovered among the 7,374 men who had registered for the survey. Furthermore, the study noted that the prevalence of BPH rises with age, particularly among males aged 70 years and above, with the oldest having an 8.2 times higher risk of acquiring BPH than the youngest age group participating in the study (Safarinejad, 2008).

The paucity of data on the epidemiology of BPH in Kenya has led to an over-reliance on data from other countries, particularly advanced countries. However, the risk factors may vary, and the majority of Kenyan males are diagnosed with BPH at an advanced stage. According to a study conducted in Nairobi, 76% of the 108 participating males had BPH (Ngugi & Byakeka, 2016). Another study conducted in a urology clinic at one of Western Kenya's referral hospitals found that annual occurrences of prostate illnesses were 31.3%, with BPH prevalence reaching as far as 63.9%. None of the previous studies on BPH in Kenya have identified genetic variations in the ion channel transient receptors potential melastatin 2,6,7, and 8 as a potential risk factor for Benign prostate hyperplasia.

2.1.3 Pathogenesis of benign prostate hyperplasia

Hormonal changes, metabolic syndromes, and inflammation are believed to be involved in the development of BPH. Regardless of BPH's impact on the community and public health, its pathophysiology is yet to be understood. Several hypotheses have been proposed to understand the cause of BPH, but none have been successful, and the cause remains ambiguous in some aspects (Oseni *et al*., 2023). The underlying

mechanism behind BPH progression and severity is not yet fully understood. Like other chronic disorders, it is gradual, taking longer to advance from cellular modification to the formation of LUTS and the clinical manifestation of BPH (Kopp, 2018). The natural course of BPH is assumed to be controlled by two phases: pathological microscopic and pathological macroscopic. It is well thought that nearly all men who survive to be octogenarians will have developed microscopic BPH at some point (ElJalby *et al*., 2019; Foo, 2017).

Histopathologically, the accumulation of epithelial and stromal cells in the periurethral region of the prostate is characteristic of BPH. This accumulation is believed to be caused by the proliferation of the epithelial and stromal cells, which occurs due to either inadequate programmed cell death or a faulty apoptosis process, leading to cellular buildup. Other factors contributing to the hyperplastic process include dysfunction in the androgen-estrogen pathway and the involvement of growth factors and neurotransmitters (Madersbacher *et al*., 2020).

The testosterone hormone and other hormones and growth factors encourage prostate gland growth (Cannarella *et al*., 2021). Even though testosterone is the most abundant androgen in the blood, its role appears to be that of the pro-hormone, as it must be converted to dihydrotestosterone (DHT) to operate. The appropriate androgen and estrogen receptors and the 5-alpha reductase and aromatase enzymes are required to modulate BPH (Ajayi & Abraham, 2018; Dandona & Rosenberg, 2010).

2.1.4 Diagnosis and treatment of benign prostate hyperplasia

Each affected individual's BPH diagnosis is unique, considering the patient's history (Devlin *et al*., 2021). The main objective of the examination is to determine the severity of LUTS, the most common symptom of BPH that adversely affects the quality of life of affected BPH patients (Adegun *et al*., 2016). The best technique to

determine the severity of LUTS is to employ a self-administered, internationally validated questionnaire with the response score summed and interpreted against the questionnaire's score keys (Franco *et al*., 2023).

Physical examination, including a digital rectal exam, laboratory analysis of PSA levels, and imaging such as ultrasounds or trans abdominal scans are all diagnostic options for identifying BPH.

Treatment for BPH varies depending on the patient's comorbidities and the severity of the condition. It can range from close monitoring with symptom management to surgical intervention. The preferred medications for treating BPH include adrenoreceptor blockers and alpha-reductase inhibitors. These medications reduce resistance at the bladder and neck and relax smooth muscle, alleviating discomfort during urination (Bortnick *et al*., 2020).

2.2 Transient receptor potential channels

Transient receptor potential (TRP) channels are a group of ion channels found in cell membranes that are involved in various physiological processes, including sensory signaling and regulation of ion homeostasis. These channels are permeable to cations such as calcium, magnesium, and sodium, and their activation can lead to changes in membrane potential and intracellular calcium levels, ultimately influencing cellular functions (Marini *et al*., 2023).

TRP channels were first discovered in the fruit fly *Drosophila melanogaster* by Montel and Rubin in 1989, where mutations in these channels affected the response to light (Fowler & Montell, 2013; Montell, 2012). Subsequent research studies have identified TRP channels in mammals, where they play a crucial role in sensory processes such as vision, taste, and touch, as well as in regulating body temperature and pain perception (Aroke *et al*., 2020) Based on its structural similarity to other

cation channels and thorough examination of the permeation properties of the light-induced current in the mutant of the transient receptor potential gene, it was suggested that the product of this gene is a protein with six transmembrane segments that act as a cation-permeable channel (Samanta *et al*., 2018).

In mammals, there are 28 different TRP channel genes, which can be divided into six subfamilies based on gene sequence homology: TRPC (Canonical), TRPV (Vanilloid), TRPM (Melastatin), TRPA (Ankyrin), TRPML (Mucolipin), and TRPP (Polycystin). Each subfamily has distinct functional properties and is activated by different stimuli, allowing TRP channels to participate in various physiological processes (Zhang *et al*., 2023).

2.2.1 Transient receptor potential melastatin (TRPM)

The TRPM subfamily is one of the six parts and the largest subfamily of the transient receptor potential channels and shares structural similarities with other TRP subfamilies. It comprises six membrane-spanning regions, cytoplasmic Cand N terminals, and a C-terminal motif (Samanta *et al*., 2018; Sander *et al*., 2022). Among the TRPM genes, TRPM8 is predominantly expressed in vascular smooth muscles; despite their diverse electrophysiological behaviours, TRPM proteins can be classified into two major groups (Jimenez *et al*., 2020). All TRPMs can form a functional cation channel either as homo or hetero-multimers, as demonstrated by patch-clamp measurements in the mammalian cell lines transferred with TRPM plasmid DNAS (González-Muñiz *et al*., 2019).

The TRPM subfamily comprises eight members, each involved in various biological functions. Since the cloning of TRPM1 in 1998, significant progress has been made in understanding these channels' function, structure, and pharmacological properties. Structures of TRPM2, TRPM4, and TRPM8, as well as a partial structure of TRPM7,

have been determined by cryo-electron microscopy, providing insights into their assembly, ion permeation, gating mechanism, and structural pharmacology (Huang *et al*., 2020). These channels are widely expressed and contribute to cellular calcium signalling by allowing calcium entry into the cytosol in response to various stimuli. This calcium entry can affect biological processes such as the sensing of oxidative stress, regulation of endothelial permeability, magnesium homeostasis, myogenic response, and regulation of vascular tone. It can also affect the effects of other channels by modulating their membrane potentials (Froghi *et al*., 2021).

Members of the TRPM family have emerged as promising drug targets for various disorders, including neurodegenerative disorders, cardiovascular diseases, type-II - diabetes, inflammation, and inflammatory pain (Malko *et al*., 2019; Zierler *et al*., 2017).

2.2.1.1 Transient receptor potential melastatin2 (TRPM2)

Transient receptor potential melastatin type 2 (TRPM2) is a nonselective cation channel that allows the passage of calcium (Ca^{2+}), sodium, and potassium ions. Various stimuli, including oxidant stress, ADP ribose, and intracellular calcium levels, activate it. ADP-ribose is produced from NAD+, CD38, ADP-ribose breakdown, and protein de-acetylation (Cheung & Miller, 2017; Cruz-Torres *et al*., 2020). TRPM2 is a bifunctional protein containing a Nudix box in its C-terminus. This motif is common among enzymes that degrade nucleotide diphosphates and also serves as a binding site for ADP-ribose (Kühn, 2020).

TRPM2 is highly expressed in the central nervous system (CNS), particularly in regions such as the hippocampus, substantia nigra, striatum, cortex, and dorsal root ganglion sensory neurons in the spinal cord (Ji *et al*., 2022). Studies have shown that TRPM2 plays a role in cell death in response to oxidative stress in various cell types,

including those implicated in conditions like benign prostate hyperplasia (BPH) (Malko & Jiang, 2020). This suggests a potential involvement of TRPM2 in neurological disorders (Turlova *et al*., 2018). In the CNS, TRPM2 influences neurite growth and spine formation, linking reactive oxygen species (ROS) to calcium-signaling responses that can contribute to neurological disorders (Wang *et al*., 2020).

A hypothesis regarding TRPM2 and depression suggests that TRPM2 activation by ROS leads to calcium influx, resulting in neuronal injury (Zong *et al*., 2022). This unique activation mechanism of TRPM2 could be targeted for potential therapeutic intervention in depression. Additionally, selectively suppressing TRPM2 expression or blocking its transmigration into the nucleus may selectively inhibit prostate cancer growth without harming non-cancerous cells (Ali *et al*., 2021). Therefore, TRPM2 could be a potential target for controlling prostate cancer growth and cell proliferation (Miller, 2019).

2.2.1.2 Transient receptor potential melastatin6 (TRPM6)

TRPM6 is a channel that selectively transports divalent cations, showing a higher affinity for magnesium (Mg^{2+}) over calcium (Ca^{2+}). The TRPM6 gene comprises 39 exons and encodes a large protein of 2022 amino acids (Ferioli *et al*., 2017). It shares approximately 50% sequence homology with its close family member, TRPM7. TRPM6 consists of six transmembrane segments, with a putative pore region located between the fifth and sixth segments, as well as long intracellular amino (N) and carboxyl (C) termini domains (Zouharova *et al*., 2019). It is classified as a chanzyme, combining channel activity with α-serine/threonine protein 41 kinases within its C-terminal domain. While TRPM6 is primarily expressed in the intestine and kidney, recent studies have also shown its expression in the prostate gland (Ciaglia *et al*., 2023).

The channel plays a crucial role in regulating Mg^{2+} homeostasis, and its activity is triggered by a decrease in Mg^{2+} levels, leading to active (re)absorption in kidney and intestine epithelial cells. TRPM6 and TRPM7 can assemble to form a heteromeric complex, which has been observed to be essential for TRPM6 channel localization in the plasma membrane and its proper function(Morrison, 2023; Samanta *et al*., 2018). Studies have shown that micromolar concentrations of 2-aminoethoxydiphenylborate can increase TRPM6 activity, while the same concentrations inhibit TRPM7 channel activity (Andriulė *et al*., 2022).

2.2.1.3 Transient receptor potential melastatin7 (TRPM7)

Transient Receptor Potential Melastatin 7 (TRPM7) is a crucial ion channel that selectively transports divalent cations, including magnesium ($Mg^{2+),}$ calcium (Ca^{2+}), zinc (Zn^{2+}), cobalt (Co^{2+}), manganese (Mn^{2+}), nickel (Ni^{2+}), and other essential or toxic metals, (Franken *et al*., 2022). It was discovered in 2001 by multiple independent research groups and comprises six transmembrane segments, with a pore-forming loop between the fifth and sixth domains. These segments assemble into tetramers, forming functional TRPM7 channels (Jimenez *et al*., 2020).

The human TRPM7 gene is located at locus 15q21 on chromosome 15 and comprises 39 exons, encoding a protein of 1865 amino acids with a mass of 212.7 kDa (Yee, 2017). TRPM7 is distinct from its closest relative, TRPM6, in that it combines cation channel activity with a functional C-terminal alpha-type serine/threonine protein kinase domain. However, the functional interaction between these two domains remains incompletely understood (Chubanov & Gudermann, 2020).

TRPM7 is widely distributed throughout the body, with exceptionally high expression levels observed in the liver, bone, heart, and adipose tissue (Zhong *et al*., 2023). It is a magnesium-nucleotide-regulated current (MgNum) channel, with its activity

controlled by serum magnesium levels. TRPM7 channels have been detected in both normal and cancerous prostate tissue(Yee, 2017).

The expression of TRPM7 begins at an early embryonic stage and is vital for embryonic viability; deletion of the TRPM7 gene can result in embryo lethality. TRPM7 is critical in various physiological processes, including cell proliferation, survival, and differentiation. Dysregulation of TRPM7 has been implicated in several diseases, including cardiovascular disorders, neurodegenerative diseases, and cancer (Liang *et al*., 2022).

2.2.1.4 Transient receptor potential melastatin8 (TRPM8)

Transient Receptor Potential Melastatin 8 (TRPM8) is a well-studied member of the TRP channel family, known for its role in cold sensation. However, it also involves pain sensation, thermoregulation, bladder function, and cancer (Samanta *et al*., 2018). It was initially retrieved from a prostate cDNA library screen in 2001(Plaza-Cayón *et al*., 2022).TRPM8 is overexpressed in androgen receptor-positive prostate cancers, suggesting a potential role in prostate cancer progression (Grolez & Gkika, 2016).

TRPM8 is not only implicated in prostate cancer but also in a variety of other cancers, including adenocarcinoma, melanomas, lung cancer, and breast cancer (Lai *et al*., 2020). It is known to encode for a cold- and menthol-sensitive ion channel in sensory neurons, specifically in the trigeminal ganglion and dorsal root ganglion neurons (Iftinca & Altier, 2020). In the prostate, TRPM8 mRNA is expressed mainly in epithelial cells, with higher levels observed in benign prostatic hyperplasia (BPH) and prostate cancer compared to normal prostate tissue (Ciaglia *et al*., 2023).

Studies have demonstrated that TRPM8 expression is regulated by androgens, with a decrease in TRPM8 mRNA expression observed after castration in a mouse model of prostate cancer. Additionally, TRPM8 mRNA levels decrease in prostate cancer cells

(LNCaP) upon androgen withdrawal or treatment with androgen receptor (AR) antagonists (Di Donato *et al*., 2021). The functional expression of TRPM8 requires a functional AR, as transfection of AR into AR-negative cells induces TRPM8 expression, which can be reversed by siRNA-AR treatment (Grolez *et al*., 2019; Lai *et al*., 2020).

Quantitative RT-PCR studies have shown significantly increased expression of TRPM8 mRNA in malignant prostate samples compared to nonmalignant tissue, suggesting that TRPM8 levels could be used as a diagnostic marker for prostate cancer and BPH (Alaimo *et al*., 2024). Furthermore, there is a strong correlation between TRPM8 mRNA expression levels and disease relapse after radical prostatectomy, with lower levels of TRPM8 associated with a shorter time to prostate-specific antigen (PSA) relapse-free survival (Lunger *et al*., 2021).

TRPM8 may also stabilize the epithelial phenotype in prostate cells and act as an oncogene in various cancers, including prostate adenocarcinoma, colon carcinoma, melanoma, and breast and lung adenocarcinoma. Its diverse functions and dysregulation in cancer make TRPM8 a potential therapeutic target for the treatment of prostate cancer and other malignancies (Grolez *et al*., 2022).

2.3 Association between transient receptor potential melastatin membrane channels and benign prostate hyperplasia

Various transient receptor potential (TRP) channels are found in the genitourinary tract, particularly in the prostate, where they are believed to play roles in normal prostate function and diseases, especially prostate cancer development (Andersson, 2019). TRPM8, initially discovered in prostate cells, is highly expressed in sensory neurons (McKemy, 2007). Its activation is triggered by cool temperatures and cooling agents, making it essential for temperature sensation. TRPM8 and other TRP channels

like TRPV1 are also implicated in bladder sensation, making them potential targets for treating lower urinary tract dysfunction (Plaza-Cayón *et al*., 2022).

TRPM2 has been recently identified in laser-micro dissected tumoral epithelial cells from human prostate tissue. The analysis revealed significantly higher levels of TRPM2 transcripts in malignant epithelial cells compared to benign cells from identical specimens (Ongonga *et al*., 2024). TRPM2 RNA was also detected in prostate cancer cell lines, such as LNCaP and PC-3. In PC-3 cells, TRPM2 was found not only in the plasma membrane, cytosol, and the nucleus, a pattern not seen in benign cell lines (Hantute-Ghesquier *et al*., 2018). Additionally, TRPM2 is expressed in lysosomes and may function as a lysosomal calcium release channel. Knocking down TRPM2 with siRNA inhibited cell growth in PC-3 cells, indicating its importance in prostate cancer cell proliferation (Ali *et al*., 2023).

Studies have shown that TRPM2 channels are expressed in the plasma membrane in benign prostatic hyperplasia (BPH), associated with oxidative stress-induced cell injury (Hwang *et al*., 2021). These channels mediate sodium and calcium influx, leading to membrane depolarization and calcium imbalance, which can promote cell injury. TRPM8 channels in hyperplastic prostate cells are found in the plasma membrane and endoplasmic reticulum (Prevarskaya *et al*., 2018). There is a close relationship between TRPM8 and TRPM2 expression in hyperplastic prostate cells, suggesting a role in regulating intracellular calcium ions, which in turn affects cell proliferation and programmed cell death (Chinigò *et al*., 2022; Hantute-Ghesquier *et al*., 2018). In BPH, TRPM2 proteins are clustered in patterns in the plasma membrane and cytoplasm.

2.4 Electrolytes levels and TRPM channel expression in BPH patients

Dietary minerals are essential in regulating biochemical and molecular processes in the body. In addition to acting as a stabilizer of protein structures, several of them act as co-factors in enzyme reactions. Some of the elements carry their roles by acting as ligands or by altering the permeable structure and form of the cell membrane in order to prevent specific chemicals from entering the cells(Liu *et al*., 2023). When the components are deficient, average, or overexpressed in the body system, alternative pathways can be promoted, leading to the development and progression of an illness. The concentrations of these substances in the body can be assessed in a clinical setting through various analytical methods (Cedzyński *et al*., 2019).

Calcium, phosphorus, magnesium, and sodium are essential components in the body system that must be consumed in large amounts (Babaali *et al*., 2020). The concentrations of $Ca^{2+,}$ $Mg^{2+,}$ and Na^{+}, among other metals, increase in metastatic prostate cancer compared to benign prostatic tissue (Asare *et al*., 2017). The hyperplastic and normal prostates have calcium, magnesium, and sodium in diverse regions (Sarwar *et al*., 2017).

Studies have identified associations between genetic variants in TRPM channels and disease risk in BPH. Specifically, TRPM2 and TRPM7 polymorphisms are more likely to enhance severe lower urinary tract symptoms (LUTS) in BPH patients (Cheung & Miller, 2017). These discoveries indicate that dysregulated TRPM channel expression may contribute to cellular dysfunction and proliferation in the prostate gland, potentially exacerbating BPH symptoms.

The interplay between electrolytes and TRPM channels is crucial in maintaining cellular function. When neither system is appropriately regulated, it can disrupt

cellular balance and contribute to developing diseases like BPH (Rather *et al*., 2023; Yue & Xu, 2021).

2.4.1 Calcium-magnesium hemostasis and imbalance

The perfect balance of intestinal absorption, renal reabsorption, and bone metabolism regulates total calcium and magnesium levels in the body (Beggs & Alexander, 2017). The transcellular pathways operate as a regulator of calcium and magnesium ion absorption in the gut and kidney's distal convoluted tubules, and the apical entry channels (TRPV5/6 for calcium and TRPM 6/7 for magnesium) are considered to represent rate-limiting steps in the process, (Kiela & Ghishan, 2018). Calcium shortage and decreased bone mineral density are seen in TRPV5 and TRRPV6 knock-out mice. In individuals with renal hypomagnesemia, mutations in the TRPM6 gene, as well as other genes (FXYD2, HBF1B, KCNA1, EGF), have been found to affect TRPM6-Mediated transcellular Mg^{2+} reabsorption. These data imply that the above apical entry channels are essential in calcium or magnesium homeostasis in the whole body (Tseng *et al*., 2022; Viering *et al*., 2017).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The study was conducted at Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH) in Kisumu, Kenya. JOOTRH is one of Western Kenya's most significant teaching and referral hospitals, making it an ideal site for the current Benign Prostate Hyperplasia (BPH) study. It is a significant regional health hub, attracting patients from across western Kenya and parts of the Southern Rift Valley. It also has a significant number of elderly male patients, a key demographic for this research. This extensive catchment area makes JOOTRH a strategic location for studying conditions such as BPH, which predominantly affects aging men (JOOTRH, 2024).

Western Kenya, where JOOTRH is situated, comprises nine counties: Migori, Homa Bay, Kisumu, Vihiga, Busia, Kakamega, Bungoma, Siaya, and Nyamira. The region covers a total area of 17,750 km² and has a population of 9,620,852 people, with 43.74% (4,208,521) being men (KNBS, 2019). The diverse patient base at JOOTRH allowed for a sufficient sample size of men with varying degrees of BPH symptoms, helping to ensure the study's findings are robust and applicable across the region.

JOOTRH’s infrastructure and specialized medical departments made it an ideal location for the current study. The hospital is equipped with modern diagnostic and laboratory facilities. This enabled the research team to perform detailed investigations into genetic polymorphisms related to transient receptor potential melastatin (TRPM) channels, which are hypothesized to be involved in the pathogenesis of BPH. Access to state-of-the-art equipment and a trained research staff at JOOTRH facilitated collecting, analyzing, and storing biological samples, which were essential for achieving the study’s objectives.

JOOTRH's teaching and research programs also foster collaboration with academic and research institutions, providing a conducive environment for translational research that integrates clinical and molecular data. This made it possible to investigate the complex genetic interactions hypothesized in this study, contributing to the potential for future research and treatment innovations in BPH.

Geographically, JOOTRH is located at approximately 0.0917° S latitude and 34.7680° E longitude in Kisumu County, western Kenya. Kisumu lies along the shores of Lake Victoria and is accessible to patients from neighbouring counties, such as Siaya, Homabay, Kericho, and Vihiga, and neighbouring countries, including Uganda and Tanzania. The geographical location enhances the hospital's role as a regional referral centre, making it an optimal site for studying a condition like BPH, which has significant implications for men's health across the region.

3.2 Study design

This study applied the analytical cross-sectional design, recruiting all the biopsy-confirmed BPH patients and the comparative group that acted as a control. The study participants were drawn from the JOOTRH -Kisumu, Kenya, and it sought to establish whether the ion channel transient receptor membrane 2, 6, 7, and 8 genetic polymorphism is linked to BPH among men in Kisumu County.

3.3 Study population

The study recruited BPH patients and a comparative group of male participants. In order to control for confounding variables of age, the study sought to recruit participants in both arms of the study population, who were considered a risk group (35 years and above). Clinical baseline data was acquired from the patient's medical records. Patients with histopathological diagnosed BPH aged forty years and above were included in the study. The study involved confirmed BPH patients attending the

urology clinic and unmatched healthy males who served as controls and consented to be included**.** Patients and participants with other comorbidities were excluded from the study**.** The parameters obtained from the patient's biodata were demographic characteristics and laboratory diagnosis results.

3.4 Inclusion and exclusion criteria

3.4.1 Inclusion criteria.

Only individuals who agreed to take part in the study and gave consent were included in the research, which included male patients diagnosed with BPH aged 35 and above and healthy controls aged 35 and above.

3.4.2 Exclusion criteria

Patients with chronic illnesses like diabetes and hypertension, metabolic syndrome, malnutrition-related diseases, or conditions that could affect electrolyte measurements, such as dehydration, and those taking medications like diuretics or dietary supplements were not included in the study. Female participants and men under the age of 35 were excluded. Patients who were receiving supplement therapy, in addition to those who refused to give consent, were included in the study.

3.5 Sample size calculation

The sample size was computed using the formula provided by Wang & Chow (2007), with the case and control groups assigned equal samples.

$$n1 = n2 = \frac{\left(Z_{\alpha/2} + Z_{\beta}\right)^2 x\left(P_1(1-P_1) + P_2(1-P_2)\right)}{(P_1 - P_2)^2}$$

In the equation: -

n_1 =the desired sample size

n_2 =the desired sample size for the control group

$Z_{\alpha/2}$ =Normal distribution critical value at $\alpha/2$ (α=0.05, the critical value was 1.96)

Z_β =Normal distribution critical value at β (power of 80%, β=0.2 and critical value is 0.84)

P_1= the prevalence of BPH in Kenya (63.9%), the prevalence rate used for sample size calculation (63.9%) is consistent with the hospital's observed prevalence (63%)

P_2=Since the proportion for the controls is uncertain, 50% is used.

$$n1 = n2 = \frac{(1.96 + 0.84)^2 x(0.639(1 - 0.639) + 0.5(1 - 0.5))}{(0.639 - 0.5)^2}$$

=194 patients with BPH and 194 healthy individuals were recruited into the study.

As a result, 388 participants were included in the study. The study site's prevalence aligns with national data, thus validating that the study results should apply to a broader population with similar BPH prevalence.

3.6 Sample collection

3.6.1 Sampling technique

The recruitment process for this study was conducted at the JOOTRH urology clinic, focusing on patients who had been histologically diagnosed with Benign Prostate Hyperplasia (BPH). Consecutive sampling was employed to select participants who met the inclusion criteria, continuing until the desired sample size was reached. In addition to the BPH patients, the control group was composed of healthy individuals recruited from blood donors, patient escorts, and members of the surrounding community. All the participants were informed about the study's purpose and procedures, and their participation was voluntary. They provided written consent by completing a consent form, ensuring they fully understood and agreed to the study's requirements.

3.6.2 Collection of social demographic data.

Social demographic data for both cases and controls were collected through structured questionnaires administered during recruitment. These questionnaires gathered information on age, occupation, education level, marital status, income, and other relevant socio-demographic characteristics.

3.6.3 Blood sample collection

Participants were provided with standard pre-collection instructions. Trained research assistants, all qualified medical laboratory technologists collected blood samples. The samples were drawn using sterile siliconized polypropylene plain vacutainers with a clot activator for serum collection and vacutainers with EDTA anticoagulant for DNA extraction. This meticulous approach to sample collection and handling was crucial for maintaining the integrity of the specimens and ensuring the accuracy of the study's results.

3.7 Laboratory procedures

3.7.1 Electrolytes estimation

3.7.1.1 Sodium potassium and chloride ions estimation

The serum concentrations of sodium, chloride, and potassium were measured using the ROCHE® AVL 9180 equipment under the principle of ion-selective electrode measurement. These electrodes included sensors for each ion to be measured and a reference electrode. Each electrode had an ion-selective membrane that interacted specifically with the ions in the analyzed sample. The membrane, which is an ion exchange material, responds to the ion's electrical charge, leading to a change in membrane potential or the measuring voltage across the membrane.

The galvanic measuring chain within the electrode detected the difference in potential values on either side of the membrane. When there was a difference in ion concentration between the inner electrolyte and the sample, an electrochemical potential developed across the membrane of the active electrode. This potential was conducted by a highly conductive inner electrode to an amplifier.

The ion concentration in the sample was then determined using a calibration curve established by measuring points from a standard solution with precisely known ion concentrations.

3.7.1.2 Magnesium and bicarbonate ions estimation

The concentration of magnesium and bicarbonates was measured using the COBAS® integra 400 plus equipment which is an automatic analyzer with a high through output. The analyzer is equipped with measuring modules for absorbance photometry and ion-selective electrode measurements. The serum samples were placed on the primary tubes which allowed continuous access to the samples. The ion concentrations in the sample were then determined using a calibration curve established by measuring points from a standard solution with precisely known ion concentrations.

The magnesium samples were assessed using a colorimetric endpoint method. In this method, magnesium reacted with xylidyl blue diazonium salt in an alkaline solution, forming a purple-colored complex. The concentration of magnesium was then determined by measuring the decrease in absorbance of xylidyl blue using photometric techniques.

The serum bicarbonate levels were determined using an enzymatic method. In this method, bicarbonate reacted with phosphoenyl pyruvate (PEP) in the presence of phosphoenolpyruvate carboxylase (PEPC) to produce oxaloacetate and phosphate.

This reaction was coupled with another reaction involving the transfer of a hydrogen ion from an NADH analogue to oxaloacetate, catalyzed by malate dehydrogenase (MDH). The consumption of the NADH analogue resulted in a decrease in absorbance at 409 nm, which was directly proportional to the concentration of bicarbonate in the serum.

3.7.2 Prostate surface antigen electrochemiluminescence immunoassay "ECLIA"

The prostate surface antigen (PSA) was analyzed using the Cobas® e411 analyzer utilizing the sandwich principle, where 20 ul of serum; a biotinylated PSA, and a specific antibody labelled with a ruthenium complex were used to create a sandwich complex. Streptavidin-coated microparticles were then added, and the complex was bound to a solid phase through the interaction of biotin and streptavidin. The reaction mixture was transferred into a measuring cell, where the microparticles were captured magnetically onto the surface of the electrode. Any unbound substances were removed with a solution called procell/procell M. Applying a voltage to the electrode induced chemiluminescent emission, which was measured by a photomultiplier. The results were determined using a calibration curve, which was generated specifically for the instrument through a 2-point calibration and a master curve provided via the reagent barcode. The analyzer automatically calculated the concentration of PSA in each sample in ng/ml.

3.7.3 Genotyping

The genomic DNA was extracted from peripheral blood leukocytes using the salting out method, and the genotype was determined by the Illumina® I scan Infinium using the Multiplex human genotyping microarrays, polymorphism was analyzed in genome studio software modules. The criteria for choice of SNP's used were:

i. SNPs with moderate to high minor allele frequency (MAF)

ii. SNPs with the ability to efficiently capture the genetic variation in a region,

iii. The tag SNPs used for Illumina

iv. SNPs with known genotyping performance and SNPs that are compatible with the microarray assay design used by the Illumina.

In this study six SNP's TRPM2: rs168355, TRPM6: rs 4745363, TRPM7: rs 8042919, rs232295, TRPM8: rs 10490018, and rs 1016062 were analyzed for TRPM gene polymorphism. The whole process of genotyping was carried out in three days

3.7.3.1 DNA extraction

The DNA extraction was carried out using the QIAamp blood kit (Cat. NO 51106; Qiagen Inc., Valencia, CA, http://www.qiagen.com). Whole blood (200ul), Qiagen protease (20ul), and Buffer AL (200ul) were combined in a 1.5ml Eppendorf tube and vortexed to mix. The mixture was incubated at 56°C for 10 minutes, then briefly centrifuged to remove any drops inside the tube. 98% ethanol (200ul) was added, mixed by vortexing, and applied to a QIAamp spin column. The column was centrifuged at 15,000 RPM for 1 minute to bind the DNA to the filters.

The filtrate-containing tube was discarded, leaving the DNA bound to the filters in the spin columns. Buffer AW1 (500ul) was added to the spin column, which was then centrifuged at 15,000g for 1 minute. The spin column was transferred to a new collection tube, and buffer AW2 (500ul) was added, followed by centrifugation at 15,000g for 3 minutes. The filtrate-containing tube was discarded, and the spin column was transferred to a new collection tube and centrifuged for 1 minute to remove residual buffer AW2.

The spin column was placed in a clean 1.5ml low-binding microcentrifuge tube, and buffer AE (200ul) was added to elute the DNA. The column was incubated at room

temperature for 5 minutes to facilitate elution. The extracted DNA was then stored at 4°C.

3.7.3.2 Day one (preparation and amplification)

In the preparation stage, 200ng of DNA was placed into a deep-well, 96-sample plate and labelled with a barcode sticker. After centrifugation, the plate was left in a fume hood overnight to allow the liquid to evaporate and then covered with a lid to prevent dust contamination.

For the amplification process, tubes labelled "Pre" MA1, MA2, and MSM were thawed from the -20°C freezer. The oven was set to 37°C, and using a reagent basin and a 10ul, 8-channel pipette, 4ul of DNA resuspension buffer was added to each well to rehydrate the samples. Next, 20ul of MA1 was added to each well, and the plate was covered, vortexed for 1 minute at 1600 rpm on a microplate shaker, and incubated for 30 minutes at room temperature. Then, 4ul of 0.1N NaOH was added to each well, covered, vortexed again for 1 minute at 1600 rpm, and incubated at room temperature for 10 minutes. Finally, 34ul of MA2 and 38ul of MSM reagents were added to each well, the plate was sealed, pulse centrifuged, vortexed for 1 minute at 1600 rpm, and placed in the oven for 24 hours.

3.7.3.3 Day two (fragmentation, precipitation, resuspension, and hybridization)

The second day began with the fragmentation step, where FMS tubes were thawed and FMS was dispensed into the DNA plate wells, followed by incubation at 37°C. After thawing, the sample plates were removed from the oven, pulse centrifuged, and 25ul of FMS was dispensed into each well of the DNA plate. The plate was covered, pulse centrifuged, vortexed at 1600 rpm for 1 minute, and incubated in a heat block for 1 hour.

For precipitation, the PM1 tube was removed from the "post 3" 4°C box and warmed to room temperature. The plate was removed from the heat block, pulse centrifuged, and 50µl of PM1 was dispensed into each well of the plate. The plate was pulse centrifuged, vortexed at 1600 rpm for 1 minute, and incubated in the heat block for 5 minutes. Then, 155 ul of 100% isopropanol was dispensed into each well, covered tightly, manually inverted multiple times to mix, placed at 4°C for 30 minutes, and centrifuged at 4°C for 20 minutes at 3000g to form blue pellets. The liquid was removed by quick inversion of the plate and tapping forcefully on the benchtop covered in paper towels. The plate was set on a test tube rack inverted to dry and then incubated for 1 hour at room temperature.

For resuspension, RA1 was removed from the "post-2" -20°C box and thawed in a room-temperature water bath. Once thawed, 23ul of RA1 was dispensed into each well of the sample plate, sealed, pulse centrifuged, and placed in the oven for 1 hour. The plate was then removed from the oven and vortexed at 1800rpm for 1 minute.

Hybridization involved setting the heat block at 95°C and the oven at 48°C. Once the temperature stabilized, the plate was incubated on the heat block for 20 minutes while the bead chips were set at room temperature from 4°C storage. 330 ml of 100% ethanol was added to a Bottle of XC4, shaken, and incubated at room temperature overnight. The sample plate was removed from the heat block and incubated for 30 minutes. As the plate cooled, the hyb chamber was prepared, and the bead chips were scanned using the barcodes and recorded according to their loading order on the track sheet. The chips were then placed on the hyb chamber insert, and 15µl of sample from the sample plate was dispensed onto the inlet to the array. The chips were visually inspected for bubbles and regions not coated in liquid, and the hyb chamber insert was placed over the humidifying reservoirs, aligning the chip barcode with the barcode

etched into the hyb chamber base. The hyb chamber cover was then replaced, covering and closing all four clasps by closing the clasps on diagonally opposite sides first.

3.7.3.4 Day three (staining preparation, staining, extension, washing, sealing, and scanning)

In preparation for staining, the process began with the removal of hyb chambers from the oven and their incubation at room temperature for 25 minutes. Meanwhile, tubes XC1, XC2, TEM STM, and ATM were taken from the "post-1" -20°C box and placed on the bench to thaw. RA1 bottle was thawed from the "post-2" -20°C box at room temperature in a water bath, and a tube of 95% formamide was also thawed. For each chip processed, one glass slide, one plastic flow-through brace, two metal clasps, and one plastic spacer were used. The glass slides were cleaned by spraying them with ethanol and wiping them dry. The hot water bath attached to the flow-through chamber was set at 44°C, and the chamber was gently shaken to dislodge any bubbles. After the hyb chambers had cooled for 25 minutes, a wash dish was filled with PB1 (200ml).

To start the staining process, one hyb chamber was opened, and the chip was immediately placed in a chip tray and submerged in PB1 without touching the beads and ensuring they did not dry out. The tray was gently agitated to remove any bubbles, and the chips were left submerged for 1 minute. Then, a second wash dish was filled with PB1, and the process was repeated. The tray of beads was moved into the second wash dish, gently agitated, left to soak for 1 minute, and agitated again. This process was repeated for other chips that could fit within the assembly station. A clear plastic spacer was placed on top of each chip submerged in PB1, and the plastic assembly bar in the assembly station was used to place a glass slide on top of the

plastic spacer, ensuring a gap between the bead chip and the slide at the barcode end. The process was repeated for all the chips submerged in PB1, and any bubbles were checked between the chip and slides and wiped away. Two metal clasps were then placed around the glass slide, one towards the barcode end and one towards the rear, ensuring they gripped the plastic flow-through brace under the chip. The completed flow-through assemblies were removed and placed horizontally on the bench, taking care not to tip them vertically. Once all bead chips were in the flow-through assemblies, a pair of scissors was used to cut both ends of the spacer off as near to the glass slide as possible.

For the staining and extension steps, the flow-through chamber rack temperature was confirmed at 44°C in multiple positions using a temperature probe. A bead chip flow-through assembly was placed on the chamber rack by sliding the assembly down and hooking the brace at the top. 150ul RA1 was pipetted into the flow-through assembly, and incubated for 30 seconds, and this process was repeated five times. 450ul XC1 was pipetted into the flow-through assemblies, and incubated for 10 minutes. 200ul TEM was added to the flow-through assemblies, and incubated for 15 minutes. 450ul of 95% formamide/ETDA mix was added to the flow-through assemblies, incubated for 1 minute, and this process was repeated. The flow-through assemblies were then incubated for 5 minutes. The hot water bath was set to 37°C, and 450ul XC3 was added to the flow-through assemblies, incubated for 1 minute, and this process was repeated. When the water bath reached the desired temperature, 250 ul STM was added to the flow-through assemblies, and incubated for 10 minutes. 450 ul XC3 was added again to the flow-through assemblies, incubated for 1 minute, and this process was repeated. The flow-through assemblies were incubated for 5 minutes. 250ul STM was added to the flow-through assemblies, and incubated for 10 minutes. 450 ul XC3

was added to the flow-through assemblies, incubated for 1 minute, and repeated. The flow-through assemblies were then incubated for 5 minutes. The process of adding the reagents was repeated, the flow-through assemblies were removed from the chamber rack, and the water bath was turned off.

For washing and sealing, the stained bead chip was filled with 315 ml of PB1, and the flow-through assembly was disassembled by inserting a thin metal bar between the clasps and the brace and then pivoting. The glass slide was set aside, and the bead chip was removed and immediately placed in a vertical bead chip tray and submerged in PB1. This process was repeated for every flow-through assembly, minimizing their exposure to air. The bead chip tray was gently agitated to remove bubbles, and the bead chips were submerged in PB1 for 5 minutes. A second vertical wash dish was filled with XC4 prepared on day 2, and the bead chips were agitated again. In one smooth motion, the bead chip tray was pulled from the wash dish and set on a test tube rack. Using self-locking tweezers, bead chips were slid out of the tray and placed on a test tube rack. This process was repeated for every bead chip. The test tube rack with the chips was carefully transferred to a vacuum desiccator, the vacuum was turned on to ensure a proper seal, and it was incubated for 55 minutes.

During scanning, the vacuum was turned off, and the pressure returned to atmospheric pressure. The bead chips were ensured to be dry, and the edges and bottom of the chip were wiped using a paper towel to remove any liquid or debris. The scanning software was activated, and the bead chips were moved to the scanning tray. The chip decode files were downloaded by activating the decode file client software and inputting the bead chip's barcodes along with corresponding box IDs. The decode files were recognized by the software and scanned accordingly.

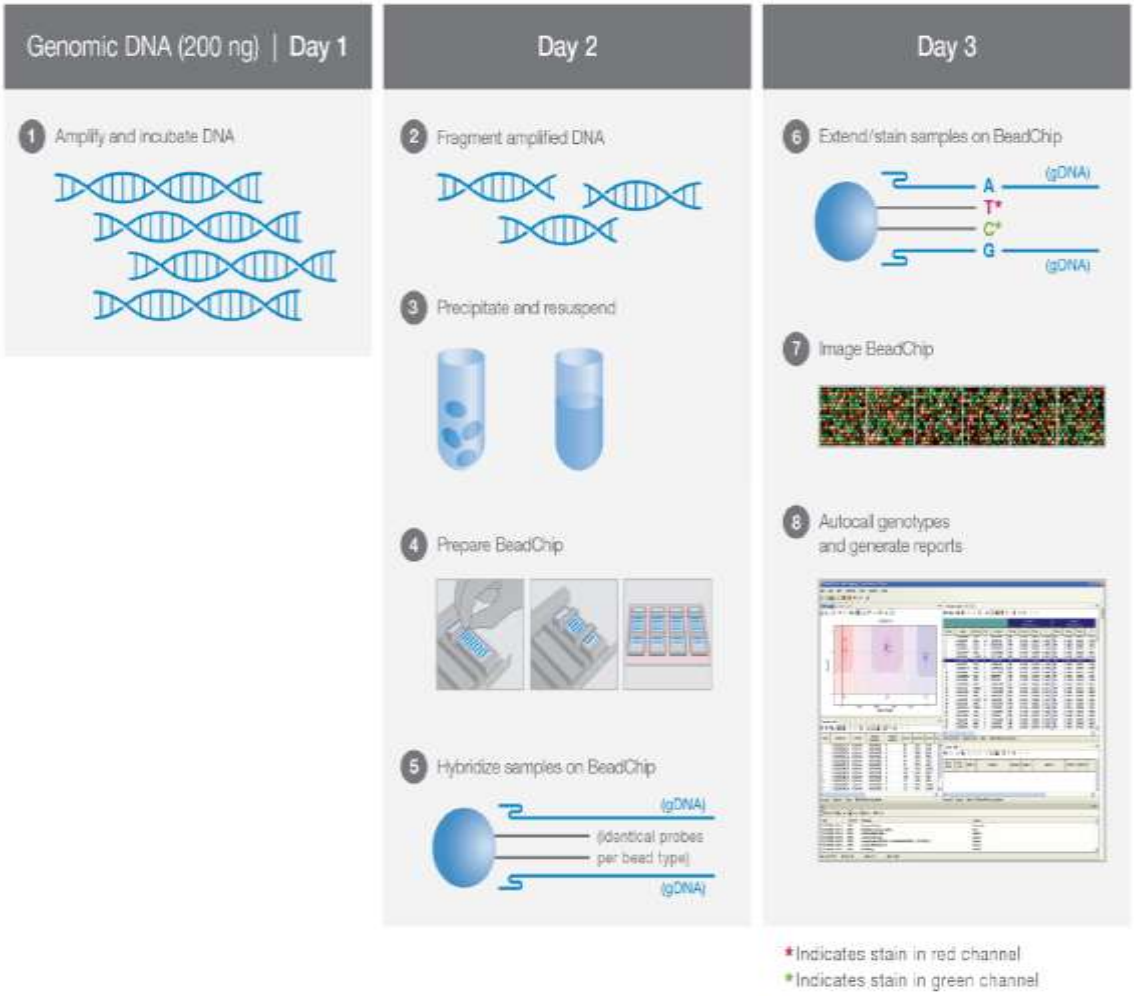

Figure 2.1: Schematic flow of the genotyping procedure

3.8 Data analysis

The data analysis for this study utilized the Statistical Package for Social Sciences (SPSS) version 27.0, chosen to align with the study's objectives of exploring genetic polymorphisms in ion channel transient receptor potential melastatin (TRPM) 2, 6, 7, and 8 as potential risk factors for Benign Prostate Hyperplasia (BPH) among men in Kisumu County, Kenya. Descriptive statistics were first used to present data through box plots and tables, which illustrated the median and interquartile range (IQR) for continuous variables and summarized categorical data as proportions. This visualization aided in understanding the data distribution and identifying any outliers.

To compare continuous variables, such as age, education, and occupation among others between BPH patients and control participants, unpaired t-tests were employed. This method was suitable for evaluating differences between the two independent groups, fulfilling the objective of contrasting socio-demographic and clinical characteristics. The Chi-Square test was used to analyze genotype frequencies, electrolyte levels, and Prostate-Specific Antigen (PSA) levels across genotype carriers. This test was ideal for categorical data and for determining associations between TRPM channel expression and various biochemical markers.

Ordinal regression analysis was applied to investigate the relationship between genotypes and the Lower Urinary Tract Symptoms International Prostate Symptom Score (LUTS IPSS) among BPH patients. This method was chosen for its ability to handle ordinal outcomes and examine associations with predictor variables, addressing the objective of exploring the link between genotypes and symptom severity. Genetic analysis included calculating Hardy-Weinberg equilibrium using the Genome Studio Genotyping Module of Illumina® Infinium assay to assess genotype frequency distributions and determining haplotype distribution with the DRAGEN Haplotype Variant Caller algorithm, which provided insights into the genetic structure relevant to BPH.

The statistical results were reported with a 95% confidence interval, and significance was determined at a p-value of ≤ 0.05. These choices in statistical methods were strategically selected to address the study's objectives comprehensively, from comparing clinical profiles to analyzing genetic and biochemical markers associated with BPH, thereby offering a thorough examination of the TRPM channels' role in the disease.

3.9 Ethical consideration

The study received approval from the graduate school board of Kenyatta University (Appendix IV). Ethical approval was obtained from JOOTRH-ERC under reference no. (IERC/JOOTRH/531/21), and a research license was secured from the National Commission for Science and Technology (NACOSTI) under reference number NACOSTI/P/22/17031 (Appendix III). All participants were required to fill and sign an informed consent form developed in English, Kiswahili, and Dholuo (Appendix IV). Potential participants were provided with a verbal explanation of the nature of the research and written information in their preferred language. They were given sufficient time to read through the consent form and ask for clarification before agreeing to participate in the study.

To ensure the protection of participants, confidentiality was strictly maintained throughout the research. No personal identifiers were collected, and identification-coded numbers were assigned to each participant instead. The risk of harm to participants was minimal, with the only potential risk being during blood drawing and the possible disclosure of personal information. However, robust measures were put in place to safeguard participant confidentiality.

The blood samples collected for this study are being securely stored in a -80°C refrigerator to preserve their integrity for potential future use. Should there be a need to conduct further studies using these samples, fresh ethical approvals will be sought from the relevant authorities to ensure that all protocols and ethical standards are adhered to. This ensures that participants' rights continue to be respected, and their personal information remains protected.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio-demographic, clinical characteristics, and laboratory profiles in BPH patients and control participants.

The socio-demographic and clinical characteristics of the study participants are summarized in Table 4.1. A total of 388 adult males were included in the study, comprising 194 BPH patients and 194 healthy control individuals without any known conditions. The mean age ± SD was 65.47 ± 12.55 years (range 38-92 years) for BPH cases and 64.52 ± 12.19 years (range 39-91 years) for the healthy control group. The majority of both BPH patients and the control group were in the age group of 70-79 years (n=53 for both groups). The age group with the least number of participants was 90-99 years for the cases (n=2) and 30-39 years for the control group (n=1). Regarding educational status, 59.27% (n=115) of the BPH patients had no formal education, while 53.09% (n=103) of the control group had no formal education. Additionally, 67.01% (n=130) of the patients resided in a rural area, while 55.15% (n=107) of the control participants lived in a rural area. The majority of the patients 63.92%, (n=124) had experienced BPH symptoms for 6-12 months, while none of the healthy control participants had these symptoms. Furthermore, the severity of lower urinary tract symptoms (LUTS) was classified as severe in the majority of the patients 63.40%, (n=123), moderate 22.68%, (n=44), and mild 13.92%, (n=27) respectively, with none of the control participants presenting with LUTS.

Table 4.1: Socio-demographic and clinical profiles of the study participants

Characteristics	Categories	BPH Patients (n=194)	Control Group (n=194)	P-Value
Ages (yrs.)	35-39	03 (1.54%)	01 (0.52%)	0.827
	40-59	25 (12.89%)	21 (10.82%)	
	50-59	41 (21.13%)	45 (23.19%)	
	60-69	46 (23.71%)	51 (26.29%)	
	70-79	53 (27.32%)	52 (26.80%)	
	80-89	24 (12.37%)	22 (11.34%)	
	90-99	02 (1.03%)	02 (1.03%)	
Education	Formal	79 (40.72%)	91 (46.90%)	0.342
	Informal	115 (59.27%)	103 (53.09%)	
Occupation	Salaried	38 (14.43%)	44 (22.68%)	0.067
	Farmer	51 (26.29%)	59 (30.41%)	
	Business	56 (28.87%)	63 (32.47%)	
	Unemployed	49 (25.26%)	28 (14.43%)	
Residence	Urban	64 (32.99%)	87 (44.85%)	0.007*
	Rural	130 (67.01%)	107(55.15%)	
Duration of BPH Symptoms	0-6 months	21 (10.82%)	00 (0.00%)	0.001*
	6-12months	124 (63.91%)	00 (0.00%)	
	13-18months	46 (23.71%)	00 (0.00%)	
	>18months	03 (1.55%)	00 (0.00%)	
LUTS Severity	Mild IPSS 0-7	27 (13.91%)	00 (0.00%)	0.000*
	MODERATE IPSS (8-19)	44 (22.68%)	00 (0.00%)	
	Severe IPSS (20-35)	123 (63.40%)	00 (0.00%)	

*Statistically significant at $P \leq 0.05$

In patients, the mean PSA levels were 135.76 ± 578.03, whereas the control participants had a mean of 2.01 ± 1.09. For sodium levels, the mean was 137.17 ± 9.84 among patients, and control participants had a mean of 143.64 ± 5.95. The mean for potassium ions was 4.15 ± 0.89 in patients and 4.4 ± 0.66 in the control group. As for hydrogen carbonate, patients had a mean of 24.86 ± 3.10, while the control participants had a mean of 1.99 ± 0.14, the mean for patients' magnesium was 0.61 ±0.17 while the cases had a mean of 0.19± 0.01 (Table 4.2).

Table 4.2: Laboratory profile of BPH patients and the control participants

	Group	**N**	**Mean**	**STD**	**SEM**
Ages	Cases	194	65.41	12.55	0.90
	Control	194	64.57	12.06	0.87
PSA	Cases	194	135.76	578.03	41.50
	Control	194	2.01	1.09	0.079
Na+	Cases	194	137.17	9.84	0.71
	Control	194	143.64	5.95	0.43
K+	Cases	194	4.15	0.89	0.06
	Control	194	4.47	0.66	0.04
Cl-	Cases	194	101.06	4.63	0.33
	Control	194	101.77	6.48	0.47
HCO_3	Cases	194	24.86	3.10	0.22
	Control	194	24.49	1.99	0.14
Mg2+	Cases	194	0.61	0.17	0.01
	Control	194	0.86	0.19	0.01

PSA biomarker measurements showed that 1.54% (n=3) of the patients had normal secretion, 9.79% (n=19) had mild secretion, 21.13% (n=41) had moderate secretion, and 67.52% (n=131) had severe secretion. In contrast, none of the control group participants had moderate or severe PSA secretion. However, 1.54% (n=3) of the

control participants had mild secretion and 98.45% (n=191) had normal secretion. The study found a statistically significant difference in PSA levels between the BPH patients and the control group (p <0.001), with a chi-square value of 365.82 and a degree of freedom of 3. (Table 4.4).

Table 4.3: PSA levels among the patients and control groups

PSA levels	Normal	Mild	Moderate	Severe	Total	P.value
BPH Patients	03 (1.54%)	19 (9.79%)	41 (21.13%	13 (67.53%)	194 (100%)	0.000*
Control group	191 (98.45%)	03 (1.54%)	00 (0.00%)	00 (0.00%	194 (100%)	
TOTAL	**194 (50.00%**	**22 (5.67%)**	**41 (10.57%)**	**131 (33.76%)**	**388 (100%)**	

Key: Normal (0- 4 mmol/L), Moderate (10- 20 mmol/L), Severe (> 20 mmol/L)

4.1.1 Association between age distribution and PSA Level.

The study evaluated the association between age and prostate-specific antigen (PSA) levels among benign prostatic hyperplasia (BPH) patients (Table 4.4) with PSA levels categorized as Normal (0-4 mmol/L), Moderate (10-20 mmol/L), and Severe (>20 mmol/L). The case-control analysis was performed across seven age groups (30-99 years), examining the distribution of PSA levels in both case and control populations.

In the 30-39 age group, there was no significant association between PSA levels and BPH, as only 2 cases exhibited elevated PSA levels, leading to a non-significant p-value of 0.148. The association began to intensify in the 40-49 age group, where 25 cases had elevated PSA levels: 6 individuals were categorized under the Moderate range (10-20 mmol/L), while 18 fell into the Severe range (>20 mmol/L). In contrast, only 2 controls exhibited severe PSA levels, resulting in a highly significant p-value of <0.001, indicating a strong correlation between increasing age and elevated PSA levels.

The trend persisted in the 50-59 age group, with 41 cases displaying elevated PSA levels, including 4 Moderate and 35 Severe cases, compared to only 1 control with Severe PSA levels. This yielded a p-value of <0.001, further solidifying the association. Similarly, in the 60-69 age group, 46 cases had elevated PSA levels, with 10 falling into the Moderate category and 32 in the Severe range. The absence of controls with PSA levels above 4 mmol/L further strengthened the association, with a p-value of <0.001.

In the 70-79 age group, 53 cases presented elevated PSA levels, with 12 Moderate and 39 Severe cases. Again, none of the controls exhibited PSA levels beyond 4 mmol/L, contributing to a significant p-value of <0.001. The 80-89 age group followed a similar pattern, with 24 cases showing elevated PSA levels, 6 of which were Moderate and 17 Severe, while controls remained within the normal range. This resulted in a p-value of <0.001, underscoring the strong association. Finally, in the 90-99 age group, despite a smaller sample size, a significant association between PSA levels and BPH was observed, with a p-value of 0.035.

Table 4.4: Association between age distribution and PSA Level.

			PSA					P-Value
Age			**0-4**	**4.0-9.0**	**10.0-20.0**	**>20.0**	**Total**	
30-39	Group	sample	0	0	1	1	2	0.148
		control	1	0	0	0	1	
	Total		1	0	0	1	3	
40-49	Group	sample	0	1	6	18	25	<0.001
		control	19	0	0	2	21	
	Total		19	1	6	20	46	
50-59	Group	sample	0	2	4	35	41	<0.001
		control	44	0	0	1	45	
	Total		44	2	4	36	86	
60-69	Group	sample	0	4	10	32	46	<0.001
		control	51	0	0	0	51	
	Total		51	4	10	32	97	
70-79	Group	sample	0	2	12	39	53	<0.001
		control	52	0	0	0	52	
	Total		52	2	12	39	105	
80-89	Group	sample	0	1	6	17	24	<0.001
		control	22	0	0	0	22	
	Total		22	1	6	17	46	
90-99	Group	sample	0	0	1	2	3	0.035
		control	2	0	0	0	2	
	Total		2	0	0	2	5	
Total	Group	sample	0	10	40	144	194	<0.001
		control	191	0	0	3	194	
	Total		191	10	40	147	388	

Key: Normal (0- 4 mmol/L), Moderate (10- 20 mmol/L), Severe (> 20 mmol/L)
*Statistically significant at P<0.05

4.1.2 Association between sodium levels and PSA level.

The data presented in Table 4.5 highlights the association between sodium levels and Prostate-Specific Antigen (PSA) levels across different groups. In the case group, a significant association was observed (P-value = 0.000). Specifically, among individuals with hyponatremia, n=5 had normal PSA levels, n=3 had mild PSA elevations, n=23 had moderate PSA elevations, and n=69 had severe PSA elevations. For those with normal sodium levels, the distribution was n=4 with normal PSA, n=10

with mild elevations, n=13 with moderate elevations, and n=55 with severe elevations. Among individuals with hypernatremia, n=0 had normal PSA, n=1 had mild elevations, n=1 had moderate elevations, and n=10 had severe elevations. Conversely, the control group showed no significant association (P-value = 0.163). Most of the control individuals with hyponatremia n=11 had normal PSA levels, while the majority of those with normal sodium levels n=189 also had normal PSA. None of the controls with hypernatremia exhibited PSA abnormalities. The overall trend shows that abnormal sodium levels, particularly hyponatremia and hypernatremia, are more associated with higher PSA levels in the case group compared to the control group.

Table 4.5: Association between sodium levels and PSA level.

			PSA LEVELS				P-Value
Groups			**Normal**	**mild**	**Moderate**	**Severe**	
Cases	Sodium	Hyponatremia	5	3	23	69	0.163*
		Normal	4	10	13	55	
		Hypernatremia	0	1	1	10	
Controls	Sodium	Hyponatremia	11	0	0	69	0.000*
		Normal	189	0	0	55	
		Hypernatremia	0	0	0	10	
Total			**200**	**17**	**37**	**134**	

*Statistically significant at P<0.05

4.1.3 Association between potassium levels and PSA level

The data in (Table 4.6) illustrates the association between potassium levels and PSA levels across different groups. In the case group, no significant association was found between potassium levels and PSA levels (P-value = 0.678). Among individuals with hypokalemia, n=3 had normal PSA levels, n=2 had mild elevations, n=12 had moderate elevations, and n=36 had severe elevations. In individuals with normal potassium levels, n=6 had normal PSA, n=10 had mild elevations, n=23 had moderate

elevations, and n=87 had severe elevations. For those with hyperkalemia, n=0 had normal PSA, n= n=2 had mild elevations, n=2 had moderate elevations, and n=11 had severe elevations. Similarly, in the control group, there was no significant association (P-value = 0.954). Most of the control individuals with normal potassium levels n=188 had normal PSA levels, while individuals with hypokalemia and hyperkalemia primarily had severe PSA elevations. Overall, the results suggest that there is no clear association between potassium levels and PSA levels in both the case and control groups.

Table 4.6: Association between potassium levels and PSA level.

			PSA LEVELS				P-Value
Groups			**Normal**	**mild**	**Moderate**	**Severe**	
Cases	Potassium	Hypokalemia	3	2	12	36	0.678
		Normal	6	10	23	87	
		Hyperkalemia	0	2	2	11	
Controls	Potassium	Hypokalemia	1	0	0	36	0.954
		Normal	188	3	0	87	
		Hyperkalemia	2	0	0	11	
Total			200	17	37	134	

4.2 Electrolyte levels among BPH patients and controls.

In the case group, 53.61% (n=104) of patients exhibited hyponatremia, while only 6.19% (n=12) had hypernatremia (Table 4.7). Hypokalemia affected 27.32% (n=53) of the patients, and 7.73% (n=15) had hyperkalemia. Hypochloraemia was found in 19.07% (n=37), and 9.28% (n=18) had hyperchloremia. Hypomagnesemia was observed in 66.49% (n=129) of the patients, and 3.09% (n=6) had hypermagnesemia. Acidosis was noted in 14.43% (n=28) of the patients, while 14.94% (n=29) exhibited alkalosis. In the control group, general electrolyte imbalance affected 13.40% (n=26). Among them, 3.09% (n=6) had hyponatremia, while no individuals presented with

hypernatremia. Potassium ion imbalances were found in 0.6% (n=0.52) with hypokalemia and 1.03% (n=2) with hyperkalemia. Hypomagnesemia was observed in 3.09% (n=6), while hypermagnesemia was found in 2.06% (n=4). Acidosis affected 2.57% (n=5), and 1.03% (n=2) experienced alkalosis.

Table 4.7: Electrolyte levels among BPH patients and controls

Electrolytes	Variable	BPH patients		Controls		Total	
Sodium (Na^+)	Hyponatremia	104	53.61%	06	3.09%	110	28.3%
	Normonatremia	78	40.20%	188	96.90%	266	68.60%
	Hypernatremia	12	6.19%	00	0.00%	12	3.10%
Potassium (K^+)	Hypokalaemia	53	27.32%	01	0.52%	54	13.92%
	Normokalaemia	126	64.95%	191	98.45%	317	81.70%
	Hyperkalaemia	15	7.73%	02	1.03%	17	4.38%
Chloride (Cl-)	Hypochloraemia	37	19.07%	04	2.06%	41	10.57%
	Normochloremia	139	71.65%	178	91.8%	317	81.70%
	Hyperchloremia	18	9.28%	12	6.19%	30	7.73%
Magnesium (Mg+)	Hypo-magnesium	129	66.49%	06	3.09%	135	34.79%
	Normo-magnesium	59	30.41%	184	94.84%	243	62.62%
	Hyper-magnesium	06	3.09%	04	2.06%	10	2.57%
Bicarbonate (HCO_3)	Acidosis	28	14.43%	05	2.57%	33	8.51%
	Normal	137	70.62%	187	96.39%	324	83.50%
	Alkalosis	29	14.95%	2	1.03%	31	7.99%

4.2.1 Electrolyte imbalances and their association to TRPM channel expression

The study examined various genotypes of the TRPM ion channels in relation to electrolyte imbalances (natremia, kalemia, chloremia, bicarbonate, and magnesemia) among BPH patients (Table 4.8), (Table 4.9). Significant differences were observed in the distribution of genotypes for specific TRPM variants. The TRPM2-rS168355 genotype showed significant associations with natremia, kalemia, chloremia, and magnesemia, with p-values of 0.002, 0.001, 0.002, and 0.001, respectively, indicating that both hypo- and hypernatremia, hypokalemia, hypochloraemia, and

hypomagnesemia were affected by these genotypes. Similarly, TRPM6-rS4745363 showed a statistically significant association with kalemia ($p = 0.033$). For TRPM7-rS8042919, a significant relationship was observed with magnesemia ($p = 0.019$). TRPM7-rs2362295 showed significant associations with kalemia, chloremia, bicarbonate, and magnesemia, with p-values of 0.004, 0.018, 0.031, and 0.004, respectively. Lastly, TRPM8-rs10490018 was significantly associated with kalemia ($p = 0.034$), while TRPM8-rs1016062 showed a significant association with magnesemia ($p = 0.008$). These findings suggest that specific TRPM genotypes are strongly associated with electrolyte imbalances in BPH patients, particularly affecting sodium, potassium, chloride, bicarbonate, and magnesium levels.

Table 4.8: Electrolyte level Imbalance among the BPH Patients Genotype

BPH PATIENTS											
		Natremia		Kalemia		Chloremia		Bicarbonate		Magnesemia	
		Hypo, (n, %)	Hyper, (n, %)	Hypo, (n, %)	Hyper, (n, %)	Hypo, (n, %)	Hyper, (n, %)	Hypo, (n, %)	Hyper, (n, %)	Hypo, (n, %)	Hyper, (n, %)
TRPM2 rS168355	**TT**	55, (28.50%)	5, (2.59%)	33, (17.10%)	8, (4.15%)	33, (17.10%)	10, (5.18%)	21, (10.88%)	19, (9.84%)	81, (41.97%)	2, (1.03%)
	TG	21, (10.88)	3, (1.55%)	8, (4.15%)	7, (3.67%)	16, (8.29%)	6, (3.11%)	10, (5.18%)	4, (2.07%)	35, (18.13%)	2, (1.03%)
	GG	10, (5.18%)	0, (0.00%)	10, (5.18%)	0, (0.00%)	6 (3.10%)	2 (1.04%)	3 (1.55%)	4 (2.07%	12, (6.22%)	2, (1.03%)
P-value		**0.002***		**0.001***		**0.002***		**0.047***		**0.001***	
TRPM6 rS4745363	**TT**	81 (41.97%	7 (3.61%)	45, (23.32%	14 (7.22%)	49 (25.25%)	17 (8.76%)	34 (17.52%)	27 (13.92%)	180, (93.26%)	6, (3.09%)
	TA	6, (3.10%)	1 (0.52%)	5 (2.59%)	1 (0.52%)	5 (2.59%)	1 (0.52%)	0 (0.00%)	0 (0.00%)	11, (5.69%)	0 (0.00%)
	AA	3, (1.55%)	0 (0.00%)	3 (1.55%)	0 (0.00%)	2 (1.03%)	0 (0.00%)	0 (0.00%)	1 (0.52%)	3, (1.55%)	0 (0.00%)
P-value		**0.302**		**0.033***		**0.417**		**0.149**		**0.792**	
TRPM7 rs8042919	**GG**	67 (34.72%)	5 (2.59%)	34 (17.71%)	12 (6.25%)	42 (21.87%	17 (8.81%)	20 (10.42%)	19 (9.89%)	84, (43.75%)	6 (3.25%)
	GA	19, (9.84%)	2 (1.03%)	15, (7.82%)	3 (1.56%)	13 (6.77%)	2 (1.04%)	13 (6.78%)	6 (3.10%)	42 (21.88%)	0, (0.00%
	AA	3 (1.55%)	1 (0.52%)	3 (1.55%)	0 (0.00%)	1 (0.52%)	0 (0.00%)	1 (0.52%)	1 (0.52%)	2 (1.04%)	0 (0.00%)
P-value		**0.081**		**0.226**		**0.469**		**0.354**		**0.019***	

*Statistically significant at p-value $\leq$0.05

Table 4.9: Electrolyte level Imbalance among the BPH Patients Genotype

TRPM7 rs2362295	**CC**	51 (26.42%)	4 (2.07%)	28 (14.51%)	9 (4.66%)	33 (17.09%)	10 (5.21%)	15 (7.78%)	12 (6.22%)	67 (34.71%)	5 (2.59%)
	CT	30 (15.54%)	4 (2.07%)	20 (10.36%)	5 (2.59%)	15 (7.77%)	6 (3.10%)	18 (9.33%)	12 (6.22%)	53 (27.46%)	0 (0.00%)
	TT	9 (4.66%)	0 (0.00%)	5 (2.59%)	1 (0.52%)	8 (4.14%)	1 (0.52%)	1 (0.52%)	3 (1.55%)	8, (4.15%)	1 (0.52%)
P-value		**0.554**		**0.004***		**0.018***		**0.031**		**0.004***	
TRPM8 rs10490018	**CC**	44 (22.91%)	6 (3.12%	27 (14.06%)	3 (1.56%)	26 (13.47%)	8 (4.17%)	20 (10.36%)	16 (8.33%)	71, (36.97%)	4 (2.08%)
	CT	38 (19.79%)	2 (1.04%)	19 (9.89%)	11 (5.73%)	24 (12.5%)	9 (4.66%)	10 (5.21%)	12 (6.25%)	45, (23.44%)	4 (2.08%)
	TT	7 (3.65%)	0 (0.00%)	6 (3.13%)	1 (0.52%)	5 (2.60%)	0 (0.00%)	4 (2.08%)	0 (0.00%)	11, (5.73%)	1 (0.52%)
P-value		**0.664**		**0.034***		**0.404**		**0.360**		**0.219**	
TRPM8 rs1016062	**GG**	49 (25.26%)	45 (23.19%)	22 (14.51%)	9 (4.64%)	31 (15.98%)	11 (5.67%)	15 (7.73%)	17 (8.76%)	59, (30.73%)	5 (2.59%)
	GA	37 (19.07%)	37 (19.07%)	27 (13.92%)	6 (3.09%)	24 (12.50%)	6 (3.09%)	17 (8.76%)	9 (4.64%)	66, (34.37%)	1 (0.52%)
	AA	4, (2.08%)	2 (1.04%)	4 (2.06%)	1 (0.52%)	1 (0.52%)	1 (0.52%)	2 (1.04%)	2 (1.04%)	4, (2.08%)	0 (0.00%)
P-value		**0.137**		**0.751**		**0.302**		**0.697**		**0.008***	

*Statistically significant at p-value ≤0.05

Table 4.10: Electrolyte levels imbalance among the control participants' genotypes

		Control									
		Natremia		**Kalemia**		**Chloremia**		**HCO3**		**Magnesemia**	
		Hypo (n, %)	**hyper (n, %)**	**Hypo (n, %)**	**hyper (n, %)**	**hypo (n, %)**	**hyper (n, %)**	**Acidosis (n, %)**	**Alkalosis (n, %)**	**Hypo (n, %)**	**hyper (n, %)**
TRPM2 RS168355	TT	58 (30.05%)	5 (2.59%)	0 (0.00%)	0 (0.00%)	8 (4.12%)	8 (4.12%)	4 (2.07%)	1 (0.52%)	3 (1.55%)	2 (1.03%)
	TG	21 (10.82%)	3 (1.55%)	0 (0.00%)	1 (0.52%)	1 (0.52%)	3 (1.55%)	1 (0.52%)	1 (0.52%)	3 (1.55%)	1 (0.52%)
	GG	10 (5.15%)	0 (0.00%)	1 (0.52%)	1 (0.52%)	0 (0.52%)	2 (1.03%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.52%)
P-value		**0.681**		**0.002***		**0.511**		**0.793**		**0.307**	
TRPM6 RS4745363	TT	81(41.97 %)	7 (3.62%)	1(0.52%)	1 (0.52%)	7 (3.62%)	13 (6.74%)	4 (2.07%)	2 (1.03%)	6 (3.10%)	4 (2.07%)
	TA	6 (3.10%)	1 (0.525)	0 (0.00%)	1 (0.52%)	1 (0.52%)	0 (0.00%)	1, (0.52	0 (0.00%)	0 (0.05%)	0 (0.00%)
	AA	3 (1.56%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.52%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
P-value		**0.302**		**0.112**		**0.126**		**0.407**		**0.637**	
TRPM7 RS8042919	GG	67 (34.89%)	5 (2.60%)	1 (0.52%)	2 (1.04%)	6 (3.11%)	8 (4.17%)	3 (1.57%)	1 (0.52%)	5 (2.60%)	4 (2.08%)
	GA	19 (9.90%)	2 (1.04%)	0 (0.00%)	1 (0.52%)	3 (1.56%)	5 (2.59%)	2 (1.04%)	1 (0.52%)	1 (0.52%)	2 (1.04%)
	AA	3 (1.57%)	1 (0.52%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)
P-value		**0.081**		**0.884**		**0.736**		**0.859**		**0.786**	

*Statistically significant at p-value $\leq$0.05

Table 4.11: Electrolyte levels imbalance among the control participants' genotypes

TRPM7 RS2362295	CC	4 (2.07%)	0 (0.00%)	0 (0.00%)	2 (1.04%)	4 (2.07%)	7 (3.67%)	2 (1.04%)	0 (0.00%)	4 (2.08%)	2 (1.04%)
	CT	1 (0.52%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	4 (2.07%)	6 (3.11%)	3 (1.56%)	1 (0.52%)	2 (1.04%)	2 (1.04%)
	TT	1 (0.52%)	0 (0.00%)	1 (0.52%)	0 (0.00%)	1 (0.52%)	0 (0.00%)	0 (0.00%)	1 (0.52%)	0 (0.00%)	0 (0.00%)
P-value		**0.454**		**0.003***		**0.769**		**0.001***		**0.003***	
TRPM8 RS10490018	CC	4 (2.08%)	6 (3.13%)	1 (0.52%)	1 (0.52%)	4 (2.07%)	7 (3.67%)	3 (1.56%)	1 (0.52%)	3 (1.56%)	3 (1.56%)
	CT	2 (1.04%)	2 (1.04%)	0 (0.00%)	0 (0.00%)	4 (2.07%)	6 (3.11%)	2 (1.04%)	0 (0.00%)	2 (1.04%)	1 (0.52%)
	TT	0 (0.00%)	0 (0.00%)	0 (0.00%)	1 (0.52%)	1 (0.52%)	0 (0.00%)	0 (0.00%)	1 (0.52%)	1 (0.52%)	0 (0.00%)
P-value		**0.644**		**0.152**		**0.823**		**0.183**		**0.801**	
TRPM8 RS1016062	GG	4 (2.08%)	0 (0.00%)	1 (0.52%)	0 (0.00%)	1 (0.52%)	0 (0.00%)	1 (0.52%)	1 (0.52%)	2 (1.04%)	2 (1.04%)
	GA	1 (0.52%)	0 (0.00%)	0 (0.00%)	1 (0.52%)	0 (0.00%)	1 (0.52%)	4 (2.06%)	1 (0.52%)	2 (1.04%)	2 (1.04%)
	AA	1 (0.52%)	0 (0.00%)	0 (0.00%)	1 (0.52%)	0 (0.00%)	1 (0.52%)	0 (0.00%)	0 (0.00%)	2 (1.04%)	0 (0.00%)
P-value		**0.137**		**0.051**		**0.643**		**0.584**		**0.089**	

*Statistically significant at p-value $\leq$0.05

4.3 Association of the TRPM genotypes and the LUTS, IPSS among the BPH patients

The study investigated the association between genetic variants and the severity of Lower Urinary Tract Symptoms (LUTS). The results, shown in Table 4.10, revealed a significant association, particularly with TRPM2 and TRPM7. For TRPM2 (rs168355), patients with the GG genotype had a higher risk of severe LUTS (OR=1.88, 95% CI: 1.17-3.91, p=0.021). Similarly, for TRPM7 (rs2362295), the CT and TT genotypes were linked to an increased risk of severe LUTS (OR=1.71, 95% CI: 1.18-2.88, p=0.034). However, TRPM6 (rs4745363) (OR=1.57, 95% CI: 1.09-2.26, p=0.296), TRPM7 (rs8042919) (OR=1.71, 95% CI: 1.18-2.88), TRPM8 (rs10490018) (OR=108, 95% CI: 0.74-1.56), and TRPM8 (rs1016062) (OR=1.28, 95% CI: 0.98-1.68) gene variants did not show a statistically significant association with LUTS severity.

Table 4.12: Association between the genotypes and LUTS IPSS score among the BPH patients

Gene	Genotypes	LUTS IPSS score (n=194)			OR (95%CI)	P-value
		mild	moderate	severe		
TRPM2 rs168355	TT/TG/GG	11 (5.67%)	18 (9.28%)	56 (28.87%)	1.88 (1.17-3.91)	0.021*
TRPM6 rs4745363	TT/TA/AA	1 (0.52%)	3 (1.55%)	11 (5.67%)	1.57 (1.09-2.26)	0.296
TRPM7 rs8042919	GG/GA/AA	2 (1.03%)	2 (1.03%)	6 (3.09%)	1.31 (0.76-1.92)	0.351
TRPM7 rs2362295	CC/CT/TT	8 (4.12%)	19 (9.79%)	39 (20.10%)	1.71 (1.18-2.88)	0.034*
TRPM8 rs10490018	CC/CT/TT	2 (1.03%)	1 (0.52%)	8 (4.12%)	1.08 (0.74-1.56)	0.963
TRPM8 rs1016062	GG/GA/AA	3 (1.55%)	1 (0.52%)	3 (1.55%)	1.28 (0.98-1.68)	0.519
		27 (13.92%)	44 (22.68%)	123 (63.40%)		

*Statistically significant at P≤0.05

4.3.1 Association between the 6 SNP's and the PSA level among the BPH patients

The study investigated the association between six SNP's and PSA levels among BPH patients, revealing a significant association as presented in Table 4.11. For TRPM2

(rs168355), the GG genotype was significantly associated with reduced odds of severe PSA levels compared to the TT genotype (OR=0.34, 95% CI: 0.12-0.96, P=0.034). Similarly, TRPM7 (rs2362295) showed an association, with the CC genotype indicating increased odds of severe PSA levels (OR=1.48, 95% CI: 1.08-3.56, P=0.041). However, the other genes studied did not exhibit any statistical significance in their association with PSA levels

Gene	Genotype	PSA level (n=194)				OR (95% CI)	P-value
		Normal (<4ng/ml)	Mild (4.1-10.0ng/ml)	Moderate (10.1-20.0ng/ml)	Severe >20ng/ml		
TRPM2 rs168355	TT	00 (0.00%)	01 (0.52%)	04 (2.06%)	33 (17.01%)	2.49 (2.27-5.09)	0.034*
	TG	00 (0.00%)	00 (0.00%)	03 (1.55%)	12 (6.19%)	1.42 (1.24-3.65)	0.768
	GG	00 (0.00%)	00 (0.00%)	04 (2.06%)	16 (8.24%)	0.34 (0.12-0.96)	0.456
TRPM6 rs4745363	TT	00 (0.00%)	00 (0.00%)	01 (0.52%)	05 (2.58%)	1.88 (1.39-2.56)	0.296
	TA	00 (0.00%)	00 (0.00%)	00 (0.00%)	03 (1.55%)	1.49 (0.63-3.21)	0.345
	AA	00 (0.00%)	00 (0.00%)	01 (0.52%)	04 (2.06%)	1.34 (0.86-1.99)	0.561
TRPM7 rs8042919	GG	01 (0.52%)	03 (1.55%)	04 (2.06%)	04 (2.06%)	1.34 (0.79-1.95)	0.534
	GA	00 (0.00%)	04 (2.06%)	02 (1.03%)	02 (1.03%)	1.24 (0.64-3.43)	0.674
	AA	01 (0.52%)	06 (3.09%)	01 (0.52%)	01 (0.52%)	1.35 (0.27-6.32)	0.891
TRPM7 rs2362295	CC	00 (0.00%)	00 (0.00%)	08 (4.12%)	22 (11.34%)	1.48 (1.08-3.56)	0.041*
	CT	00 (0.00%)	00 (0.00%)	04 (2.06%)	12 (6.19%)	0.85 (0.32-1.89)	0.287
	TT	00 (0.00%)	00 (0.00%)	03 (1.55%)	08 (4.12%)	0.53 (0.15-3.25)	1.023
TRPM8 rs10490018	CC	01 (0.52%)	02 (1.03%)	02 (1.03%)	02 (1.03%)	1.16 (0.64-1.43)	0.789
	CT	00 (0.00%)	03 (1.55%)	01 (0.52%)	01 (0.52%)	1.47 (0.42-4.38)	0.764
	TT	00 (0.00%)	00 (0.00%)	01 (0.52%)	02 (1.03%)	1.20 (0.04-2.71)	0.589
TRPM8 rs1016062	GG	00 (0.00%)	00 (0.00%)	02 (1.03%)	01 (0.52%)	1.35 (0.88-1.60)	0.591
	GA	00 (0.00%)	00 (0.00%)	00 (0.00%)	03 (1.55%)	1.07 (0.61-1.88)	1.004
	AA	00 (0.00%)	00 (0.00%)	00 (0.00%)	00 (0.00%)	0.83 (0.51-1.29)	0.456
Total		03 (1.54%)	19 (9.79%)	41 (21.13%)	131 (67.53%)		

Table 4.13:Association between genotypes and the PSA levels among the BPH patients

4.4 Levels and distributions of TRPM genotypes variants and allele frequencies among the study participants

The summary for the distribution of genotypes and allele frequencies for TRPM channel gene polymorphisms in BPH patients and control group participants is indicated in Table 4.12.

The distribution of genotypes and allele frequencies for TRPM channel gene polymorphisms in BPH patients and control participants revealed several statistically significant associations. In the rs168355 (TRPM2) polymorphism, BPH patients demonstrated a marked increase in the frequency of the TT genotype at 92.70% (n=178) compared to the control group at 61.65% (n=119), with the T allele frequency reaching 95.57% (n=367) in patients compared to 74.87% (n=289) in controls. Conversely, the GG genotype was significantly less frequent in BPH patients at 1.56% (n=3) compared to controls at 11.91% (n=23), with a highly significant p-value of <0.001. The observed differences in TT/TG/GG genotypes and T/G alleles between the two groups suggest a strong association with BPH susceptibility.

Similarly, for the rs4745363 (TRPM6) polymorphism, a significant association was found with BPH, as indicated by a p-value of 0.035. The distribution of the TT/TA/AA genotypes among BPH patients was 176/11/4, while in controls, it was 180/11/3. The differences in T/A allele frequencies between patients and controls further supported this association, though the significance was less pronounced than in TRPM2.

In the case of the rs2362295 (TRPM7) polymorphism, there was a significant association with BPH, with a p-value of 0.002. The genotype distribution among BPH patients (CC = 139, CT = 42, TT = 11) differed significantly from that of the control group (CC = 107, CT = 74, TT = 12). The C/T alleles also showed notable differences

between patients and controls, suggesting a potential involvement of TRPM7 in BPH susceptibility.

The rs10490018 (TRPM8) polymorphism also demonstrated a significant association with BPH, with a p-value of 0.006. BPH patients exhibited a higher frequency of the CC genotype 61.11%, (n=110) compared to controls 53.64% (n=103), while the CT genotype was more frequent in controls 39.06% (n=75) than in patients 28.88%, (n=52). The distribution of C/T alleles between the two groups did not show a statistically significant difference, indicating a weaker relationship between TRPM8 and BPH susceptibility.

The rs1016062 (TRPM8) polymorphism also showed a significant association with BPH, with a p-value of 0.038. The distribution of GG/GA/AA genotypes among BPH patients was 86/85/21, compared to controls with 96/86/12. The G/A alleles also demonstrated no significant differences between the two groups, further supporting a weaker involvement of TRPM8 in BPH susceptibility, although the association was weaker than for rs10490018.

These findings suggest that polymorphisms in TRPM2, TRPM6, TRPM7, and TRPM8 are likely involved in the genetic predisposition to BPH, with the strongest associations observed in rs168355 (TRPM2), rs4745363 (TRPM6) and rs2362295 (TRPM7).

Table 4.14: Genotype and allele frequencies of TRPM2,TRPM6,TRPM7 and TRPM8 gene polymorphism among BPH patients and control participants.

Gene SNP	Genotype/ Alleles	Patients	N	Controls	N	P-Value
TRPM2	TT/TG/GG	178/11/3	192	119/51/23	193	0.002**
rs168355	T/G	367/17		289/97		<0.001**
TRPM6	TT/TA/AA	176/11/4	191	180/11/3	194	0.014**
rs4745363	T/A	363/19		371/17		0.035**
TRPM7	GG/GA/AA	130/58/5	193	139/49/4	192	0.445
rs8042919	G/A	318/68		327/57		0.786
TRPM7	CC/CT/TT	139/42/11	192	107/72/14	193	0.002**
rs2362295	C/T	320/64		286/100		0.014
TRPM8	CC/CT/TT	110/52/18	180	103/75/14	192	0.006
rs10490018	C/T	272/88		281/103		0.081
TRPM8	GG/GA/AA	86/85/21	192	96/86/12	194	0.038
rs1016062	G/A	257/127		278/110		0.092

*The actual number does not usually sum up to the total number of participants numbers since there are missing values on Biodynamic array system SNP's
**Significance at 95% Confidence Interval (CI)

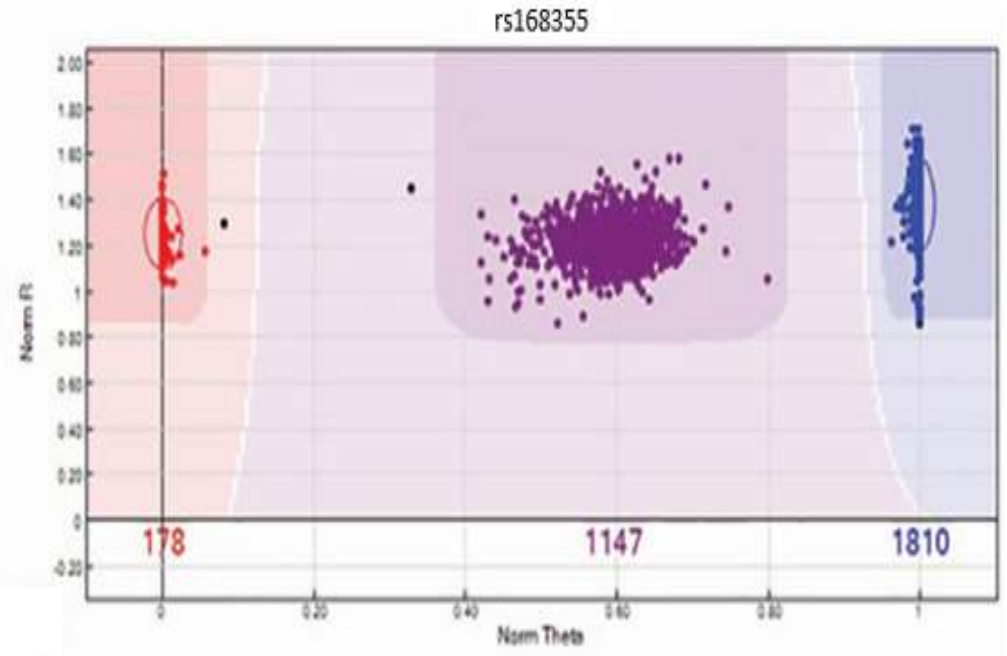

Figure 4.1: SNP distinct clusters for rs168355: TA, TT and AA.
A valid SNP with three separate clusters indicating TA, TT, and AA genotypes, represented in red, purple, and blue.

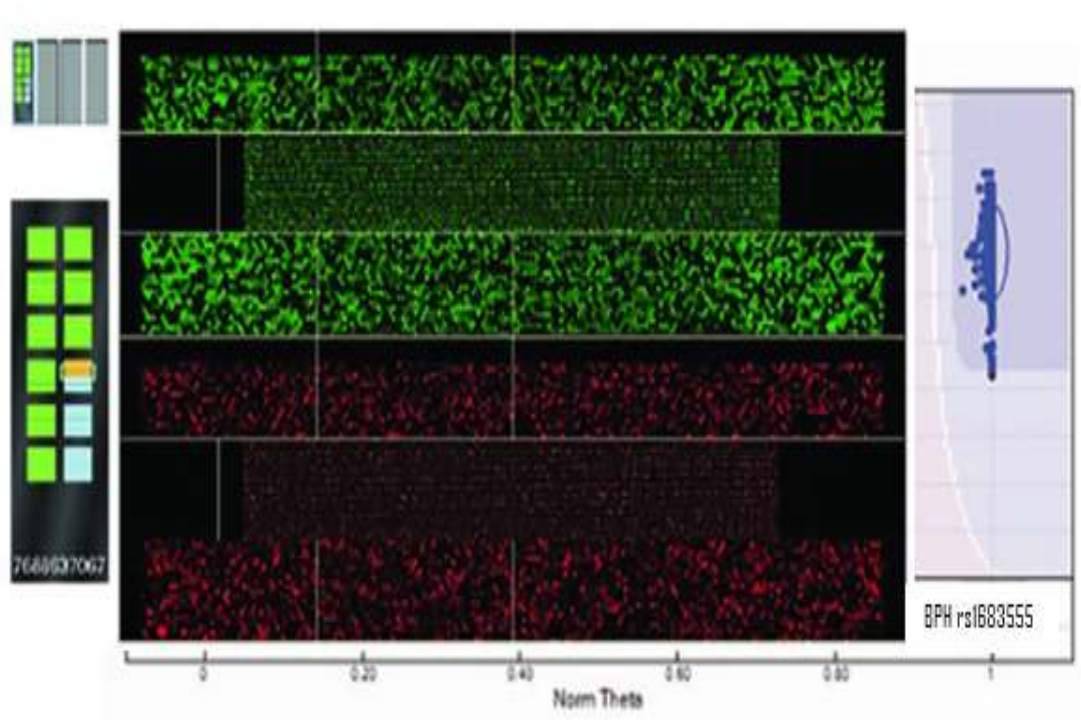

Figure 4.2: Bead chip scan by green and red lase
The Bead Chip is scanned using both red and green lasers, with the scanning software showing both colours at once. Sections that pass intensity quality control (QC) will be highlighted in green on the Bead Chip display to the left, while sections that fail intensity QC will be highlighted in red on the display.

4.5 Association between TRPM genotypes and electrolyte profiles among study participants

4.5.1 Association between TRPM2 rs168355 with electrolytes

4.5.1.1 rs168355 Genotypes and sodium levels

Sodium levels in BPH are shown in Figure 4.3. The serum sodium level was comparable across the different genotypes for SNPS rs168355 among BPH patients (P=0.096), TG carriers had lower levels compared to TT carriers (Median, 136.0; IQR, 48 mmol/l vs Median 135.0; IQR, 44 mmol/l; p=0.822). Similarly, GG carriers had lower levels compared to TT carriers (Median, 136.0, IQR, 28 mmol/l; vs Median 135.0; IQR, 44 mmol/l p=0.662). Marked Hyponatremia (n=55, 28.50%) was

observed in TT carriers while hypernatremia was observed in a small number, (n=05, 2.59%), as shown in table 4.11.

The serum level of sodium in the control group is shown in Figure 4.3, the circulating sodium levels were proportionate across different genotypes for SNPs rs168355 among the control group (p=0.085), TT carriers had the same number of participants as TG carriers having high levels of sodium (median, 143.0; IQR, 25 mmol/l vs median, 143.0; IQR, 22 mmol/l) GG carriers had the lowest levels of sodium in serum (median 143.0 IQR 22mmol/l). Marked hyponatremia was seen in TT carriers (n=58, 30.05%, p=0.681) while there was no difference in the number observed with hypernatremia to the BPH patients' group as shown in Table 4.7.

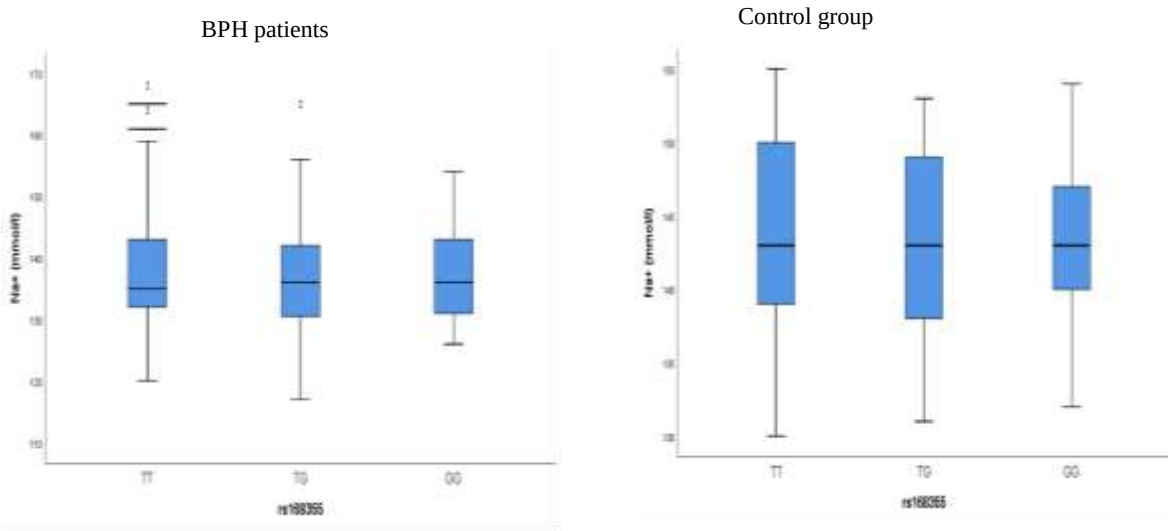

Figure 4.3: Serum sodium levels across genotype carriers SNP rs168355 among BPH patients and control group.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.1.2 rs 168355 Genotypes and potassium levels

potassium levels in BPH patients are shown in Figure 4.4 the potassium levels were comparable across the different genotypes for SNP rs168355 among BPH patients (p=0.354), TT carriers had the highest levels of potassium ions concentration in the serum compared to the TG carriers, (median, 4.0; IQR, 3.5 mmol/l vs median 4.1,

IQR 3.6 mmol/l p=0.286), GG carriers had the lowest concentration of potassium ions compared to TG carriers (median 3.5; IQR 2.4 mmol/l vs median 4.1 IQR 3.6 mmol/l; p=0.476). Marked hypokalemia was observed in TT carriers compared to GG carriers (n=33, 17.09% vs n=10, 5.18%), TG carriers presented with the least number of participants with hypokalemia (n=8, 4.15%). Hyperkalemia was observed in TT carriers more than in TG carriers (n=8,4.15%, n=7, 3.63%), Hyperkalemia was not observed among the GG carriers patients as shown in table 4.11.

The serum potassium level in the control group is shown in Figure 4.4 The levels were comparable across the different genotypes for SNPs rs168353 among the control participants (p=0.020). The TT carriers showed the highest levels of potassium concentration compared to both the TG and GG carriers (median 4.5; IQR; 2.0 mmol/l, vs median 4.3; IQR 2.1mmol/l, vs median 4.6; IQR 2.4 mmol/l p=0.039), no hypokalemia nor hyperkalemia was observed in TT carriers among the control participants, notably, hypokalemia was not observed among TG carriers but there was insignificant observation of hyperkalemia (n=1, 0.52%). There was a singular occurrence in both hypokalemia and hyperkalemia among GG carriers (n=1, 0.5%), as shown in Table 4.12.

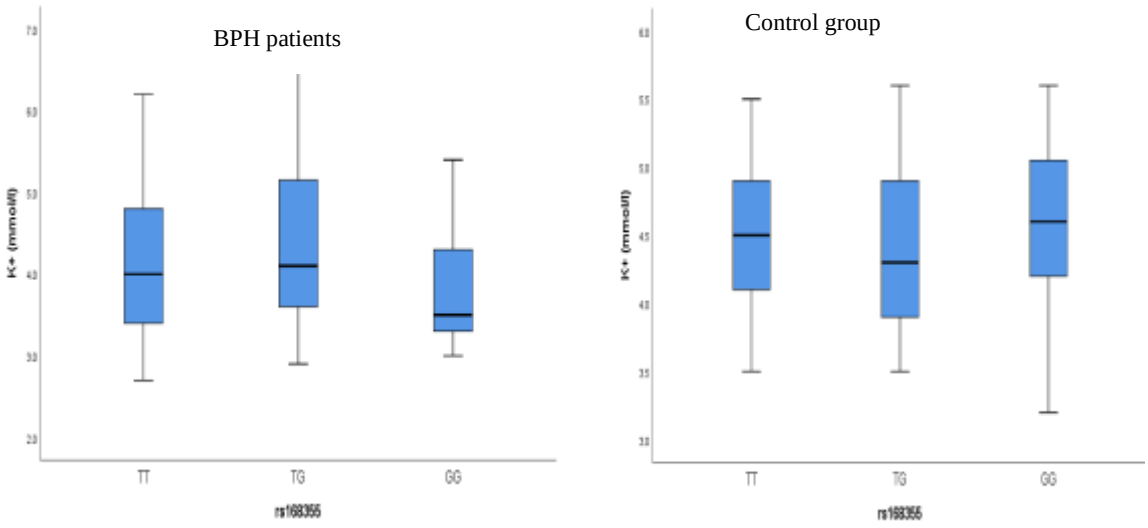

Figure 4.4 Serum potassium levels across genotype carriers at SNP rs168355 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.1.3 rs 168355 Genotypes and chloride levels

Chloride levels in BPH patients are shown in Figure 4.5 the serum chloride levels were comparable across the different genotypes for SNP rs168355 among BPH patients (p=0.498), TT carriers had the highest levels of chloride ions concentration in the serum compared to the TG carriers, (median, 100.0; IQR, 26.0 mmol/l vs median 100.0, IQR 20.3 mmol/l p=0.498), GG carriers had the lowest concentration of chloride ions compared to TG carriers (median 100.4; IQR 13.4 mmol/l vs median 100.0, IQR 20.3 mmol/l; p=0.646). Marked hypochloremia was observed in TT carriers compared to TG carriers (n=33, 17.10% vs n=16, 8.29%), GT carriers presented with the least number of participants with hypochloremia (n=6, 3.11%). Hyperchloremia was observed in TT carriers more than in TG carriers (n=10,5.18%, n=6, 3.11%), and few GG carriers presented with hyperchloremia (n=2, 1.04%) as shown in Table 4.11.

The chloride serum level among the Control group is shown in Figure 4.5, the serum chloride concentration was comparable among the different genotypes for SNP rs168355 among the control group (p=0.511), The TT carriers showed the highest levels of chloride concentration compared to TG (median 101.4; IQR; 13.5 mmol/l, vs median 102.4; IQR 10.4 mmol/l) GG carriers showed low levels of chloride concentration compared to TG carriers (median 102.4 medians; IQR, 9.5mmol/l vs median 102.4; IQR 10.4 mmol/l p=0.461), TT carriers had an equal observation for hypochloremia and hyperchloremia (n=8, 4.12%) the TT carriers had an insignificant observation in both hypochloremia and hyperchloremia (n=1, 0.52%, n=3, 1.55%), no observation was made for hypochloremia in GG carriers however, negligible observation was made for hyperchloremia in GG carriers (n=2, 1.03%) as shown in table 4.12.

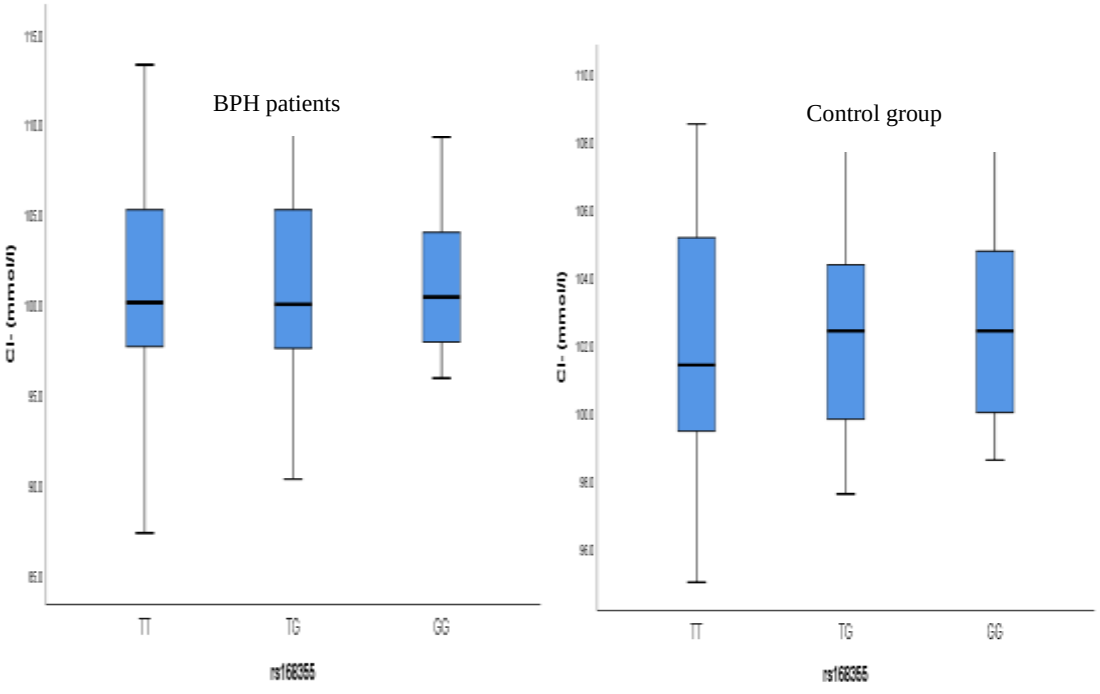

Figure 4.5:Serum chloride levels across genotype carriers at SNP rs168355 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.1.4 rs 168355 Genotypes and bicarbonate levels

Bicarbonate levels in BPH patients are shown in Figure 4.6 the serum bicarbonate levels were comparable across the different genotypes for SNP rs168355 among BPH patients (p=0.646), TT carriers had the highest levels of Bicarbonate ions concentration in the serum compared to the TG carriers, (median, 24.9; IQR, 12.0 mmol/l vs median 25.1, IQR 11.3 mmol/l p=0.692), GG carriers had the lowest concentration of bicarbonate ions compared to TG carriers (median 25.7; IQR, 9.8 mmol/l vs median 25.1, IQR 11.3 mmol/l; p=0.692). Marked acidosis was observed in TT carriers compared to TG carriers (n=21, 10.88% vs n=10, 5.18%), GT carriers presented with the least number of participants with acidosis (n=3, 1.55%). Alkalosis was observed in TT carriers more than in TG carriers (n=19,9.85%, n=4, 2.07%), GG carriers had the same observation in acidosis with TG carriers (n=4, 2.07%) as shown in table 4.11.

The bicarbonate serum level among the Control group is shown in Figure 4.6, the serum bicarbonate concentration was comparable among the different genotypes for SNP rs168355 among the control group (p=0.793), The TT carriers showed the highest levels of bicarbonate concentration compared to TG (median 24.3; IQR; 9.3 mmol/l, vs median 25.1; IQR 7.5 mmol/l) GG carriers showed no change in bicarbonate concentration (median 24.4; IQR, 6.3mmol/l), TT carriers had a minimal observation for acidosis (n=4, 2.07%) while TG carriers had a single observation for acidosis (n=1, 0.52%) the TT and TG carriers had a singular observation in alkalosis (n=1, 0.52%), there was no observation made for both acidosis and alklosis among the GG carriers as shown in table 4.12.

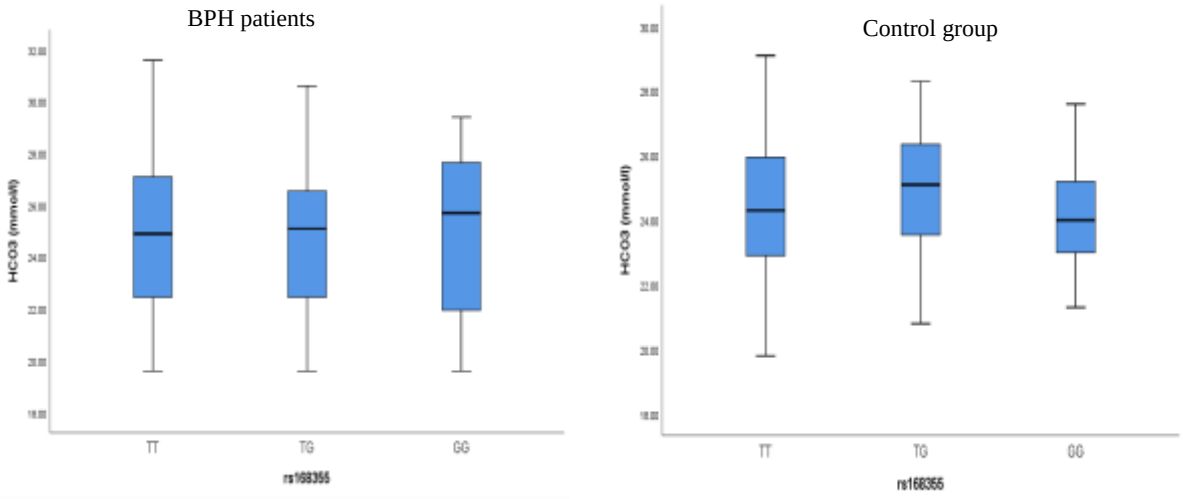

Figure 4.6:Serum bicarbonate levels across genotype carriers at SNP rs168355 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.1.5 rs 168355 Genotypes and magnesium levels

Serum Magnesium levels in BPH patients are shown in Figure 4.7 the serum magnesium levels were comparable across the different genotypes for SNP rs168355 among BPH patients (p=0.554), TT carriers had the highest levels of magnesium ions concentration in the serum compared to the TG carriers, (median,0.6; IQR, 0.81 mmol/l vs median 0.6, IQR 0.79 mmol/l p=0.298), GG carriers had the lowest concentration of magnesium ions compared to TG carriers (median 2.0; IQR 1.0 mmol/l vs median 0.6, IQR 0.79 mmol/l p=0.326). Marked hypomagnesemia was observed in TT carriers compared to TG carriers (n=81, 41.97% vs n=35, 18.13%), GG carriers presented with the least number of participants with hypomagnesemia (n=12, 6.22%). Hypermagnesemia observed across all the gene carriers was relatively low in the same proportion (n=2,1.04%) as shown in Table 4.11.

The magnesium serum level among the Control group is shown in Figure 4.7, the serum magnesium concentration was comparable among the different genotypes for

SNP rs168355 of the control group (p=0.587), The TT carriers showed the highest levels of magnesium concentration compared to TG (median 0.82; IQR; 1.57 mmol/l, vs median 0.87; IQR 1.65 mmol/l) GG carriers showed low levels of magnesium concentration compared to TG carriers (median 0.88; IQR, 1.15mmol/l vs median 0.87; IQR 1.65 mmol/l p=0.319), Both TT carriers and TG carriers had an equal low observation for hypomagnesemia (n=3, 1.55%), There was no observation made among the GG carriers for hypomagnesemia. The TG and GG carriers had an insignificant observation in hypermagnesemia (n=1, 0.52%,) but lower than the observation in TT carriers (n=2, 1.03%) as shown in table 4.12.

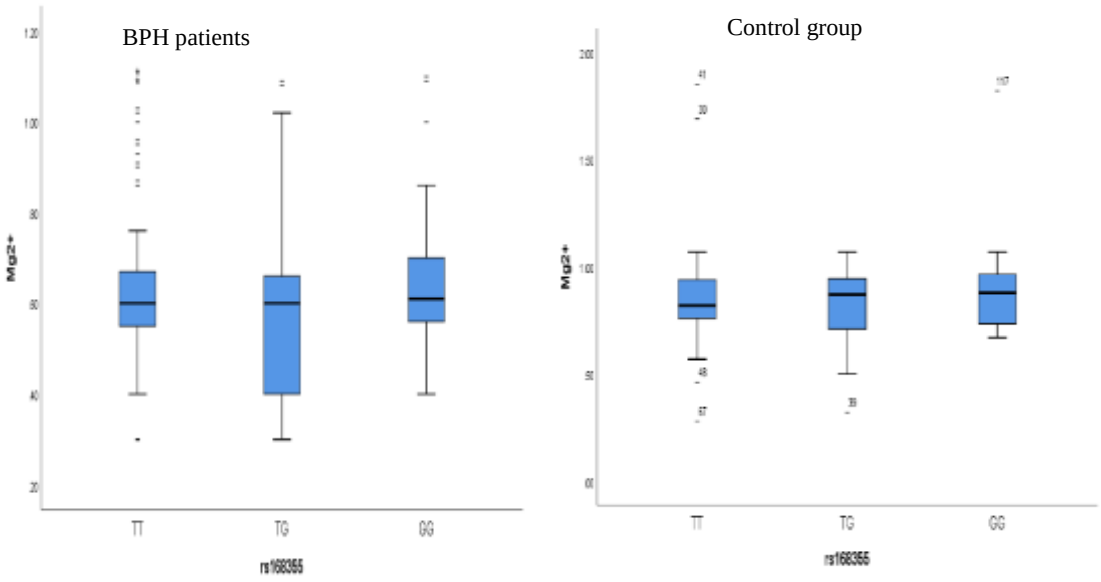

Figure 4.7: Serum magnesium levels across genotype carriers at SNP rs168355 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.2 Association of TRPM6 rs4745363 with electrolytes

4.5.2.1 rs4745363 Genotypes, sodium levels and imbalance

Sodium levels in BPH are shown in Figure 4.8. The serum sodium level was comparable across the different genotypes for SNPS rs 4745363 among BPH patients

(P=0.498), TA carriers had lower levels compared to TT carriers (Median, 133.0; IQR, 35 mmol/l vs Median 136.0; IQR, 51 mmol/l; p=0.987). Similarly, AA carriers had lower levels than TA carriers (Median, 129.0, IQR, 04 mmol/l; vs Median 133.0; IQR, 35 mmol/l p=0.662). Marked Hyponatremia (n=81, 41.97%) was observed in TT carriers than it was observed among the TA and AA carriers, AA carriers did not depict hypernatremia, while TA had a singular observation which was less than in TT carriers was observed in a small number, (n=07, 3.67%) as shown in table 4.11.

The serum level of sodium in the control group is shown in Figure 4.8, the circulating sodium level was proportionate across different genotypes for SNPs rs4745363 among the control group (p=0.681), TT carriers had the same number of participants as TG carriers having high levels of sodium (median, 135.0; IQR, 48 mmol/l vs median, 136.0; IQR, 48 mmol/l) GG carriers had the lowest levels of sodium in serum (median 136.0 IQR 28mmol/l). Marked hyponatremia was seen in TT carriers (n=58, 30.05%, p=0.681) while there was no difference in the number observed with hypernatremia to the BPH patients' group as shown in Table 4.12.

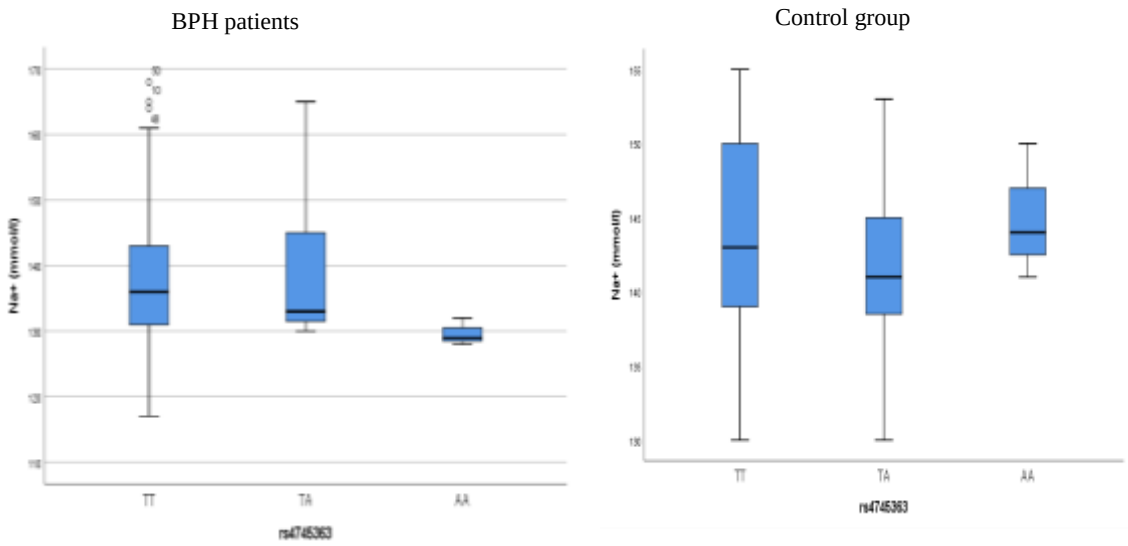

Figure 4.8: Serum Sodium levels across genotype carriers at SNP rs4745363 among BPH patients
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.2.2 rs4745363 Genotypes and Potassium Levels

potassium levels in BPH patients are shown in Figure 4.9 the potassium levels were comparable across the different genotypes for SNP rs4745363 among BPH patients (p=0.443), TT carriers had the highest levels of potassium ions concentration in the serum compared to the TA carriers, (median, 4.0; IQR, 3.6 mmol/l vs median 3.8, IQR 3.1 mmol/l p=0.336), AA carriers had the lowest concentration of potassium ions compared to TA carriers (median 3.2; IQR 0.2 mmol/l vs median 3.8, IQR 3.1 mmol/l; p=0.876). Marked hypokalemia was observed in TT carriers (n=45, 23.32%) compared to TA carriers (n=5, 2.59%), AA carriers presented with the least number of participants with hypokalemia (n=8, 4.15%). Hyperkalemia was observed in TT carriers more than in all the carriers (n=14, 7.22%), TA carriers had a single observation (n=1, 0.52%) while there was no Hyperkalemia among the AA carriers as shown in Table 4.11.

The serum level in the control group is shown in Figure 4.9. The levels were comparable across the different genotypes for SNPs rs4745363 among the control participants (p=0.468). The TT carriers showed the highest levels of potassium concentration compared to both the TA and AA carriers (median 4.5; IQR; 2.4 mmol/l, vs median 4.5; IQR 2.0 mmol/l, vs median 3.9; IQR 1.5 mmol/l p=0.044), TT carriers had a single observation for both hypokalemia and hyperkalemia (n=1, 0.52%) hypokalemia was not observed among TA carriers but there was insignificant observation of hyperkalemia (n=1, 0.52%). There was no observation made for both hypokalemia and hyperkalemia among AA carriers as shown in Table 4.12

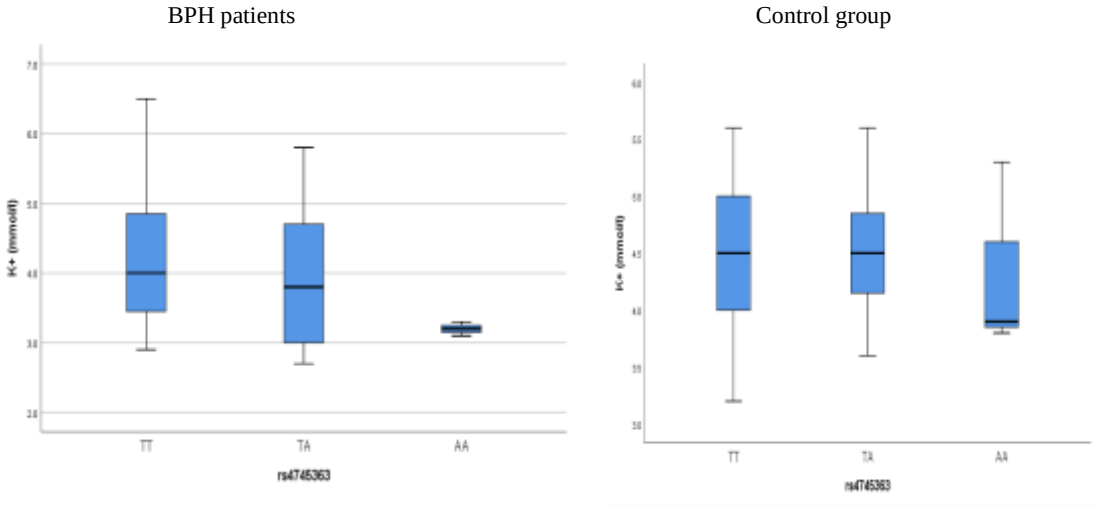

Figure 4.9: Serum Potassium levels across genotype carriers at SNP rs4745363 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.2.3 rs4745363 Genotypes and Chloride Levels

Chloride levels in BPH patients are shown in Figure 4.10 the serum chloride levels were comparable across the different genotypes for SNP rs4745363 among BPH patients (p=0.978), TT carriers had the highest levels of chloride ions concentration in the serum compared to the TA carriers, (median, 100.1; IQR, 26.0 mmol/l vs median 101.0, IQR 13.9 mmol/l p=0.263), AA carriers had the lowest concentration of

chloride ions compared to TA carriers (median 97.9; IQR 4.4 mmol/l vs median, 100.1; IQR, 26.0 mmol/l; p=0.661). Marked hypochloremia was observed in TT carriers compared to TA carriers (n=49, 25.25% vs n=5, 2.59%), AA carriers presented the least number of participants with hypochloremia (n=2, 1.03%). Hyperchloremia was observed in TT carriers more than in TA carriers which had an insignificant observation (n=17, 8.76%, n=1, 0.52%), AA carriers presented with no hyperchloremia as shown in Table 4.11.

The chloride serum level among the Control group is shown in Figure 4.10, the serum chloride concentration was comparable among the different genotypes for SNP rs4745363 among the control group (p=0.934), The TT carriers showed the highest levels of chloride concentration compared to TA (median 101.7; IQR; 13.5 mmol/l, vs median 100.8; IQR 9.4 mmol/l) AA carriers showed low levels of chloride concentration compared to TA carriers (median 98.6 median; IQR, 6.2mmol/l vs median 100.8; IQR 9.4 mmol/l p=0.126), Hypochloremia was observed among the TT carriers (n=7, 3.63%) however there was had an equal observation for hypochloremia among the TA and AA carriers (n=1,0.52%), marked hyperchloremia was observed among the TT carriers (n=13, 6.74%) while both the TA and AA carriers had no observation for hyperchloremia as shown in table 4.12.

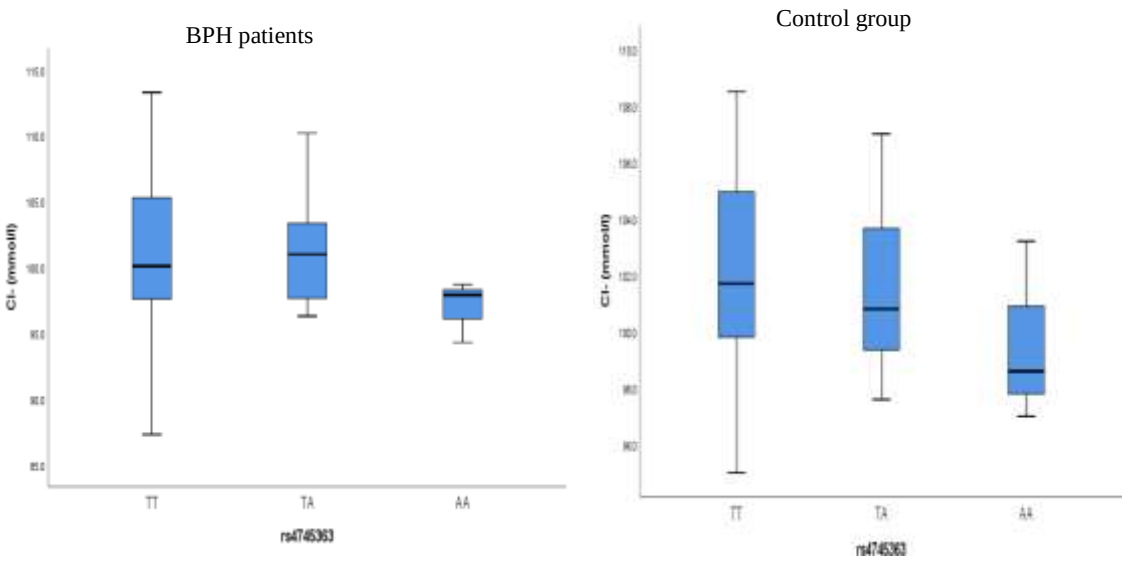

Figure 4.10: Serum chloride levels across genotype carriers at SNP rs4745363 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles.

4.5.2.4 rs4745363 Genotypes and bicarbonate levels

Bicarbonate levels in BPH patients are shown in Figure 4.11 the serum bicarbonate levels were comparable across the different genotypes for SNP rs4745363 among BPH patients (p=0.040), TT carriers had the highest levels of Bicarbonate ions concentration in the serum compared to the TA carriers, (median, 25.1; IQR, 12.0 mmol/l vs median 24.8, IQR 6.60 mmol/l p=0.181), AA carriers had the lowest concentration of bicarbonate ions compared to TA carriers (median 27.3; IQR 4.50 mmol/l vs median 24.8, IQR 6.60 mmol/l; p=0.036). Marked acidosis was observed among TT carriers (n=34,17.62%), there was no observation of acidosis among both the TA and AA carriers. Marked Alkalosis was observed among TT carriers(n=27,13.99%) while AA carriers showed an insignificant observation (n=1, 0.52%), there was no observation made for alkalosis among the TA carriers as shown in Table 4.11.

The bicarbonate serum level among the Control group is shown in Figure 4.11, the serum bicarbonate concentration was comparable among the different genotypes for SNP rs4745363 among the control group (p=0.407), The TT carriers showed the highest levels of bicarbonate concentration compared to TA (median 24.4; IQR; 9.3 mmol/l, vs median 25.7; IQR 7.5 mmol/l), AA carriers showed a minimal change in bicarbonate concentration (median 23.5; IQR, 4.40mmol/l), TT carriers had a minimal observation for acidosis (n=4, 2.07%) while TA carriers had a single observation (n=1, 0.52%), there was a single observation for acidosis among the TA carriers who did not have any observation for alkalosis, AA carriers had no observation for both acidosis and alkalosis as shown in table 4.12.

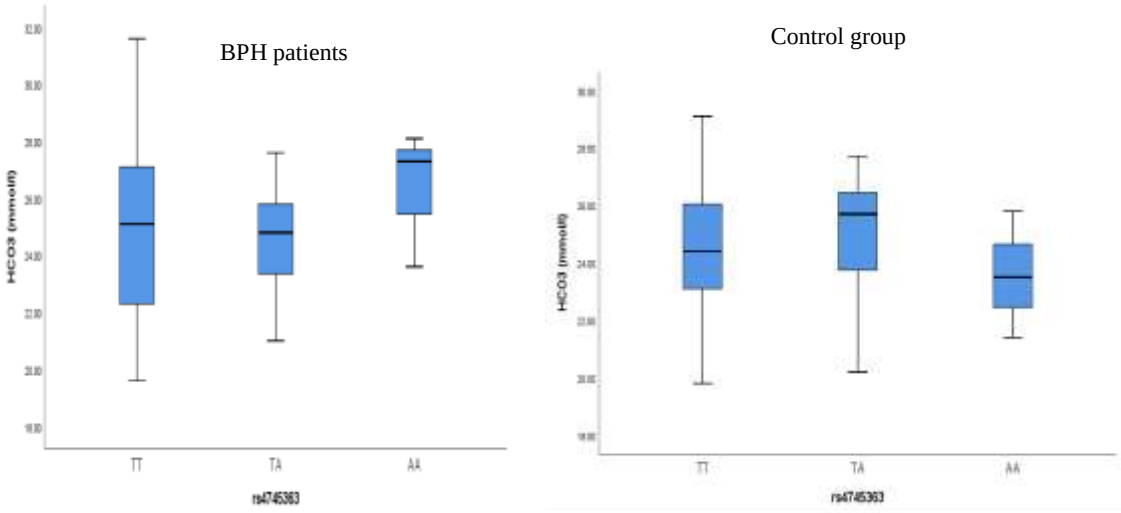

Figure 4.11: Serum bicarbonate levels across genotype carriers at SNP rs4745363 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.2.5 rs 4745363 Genotypes and magnesium levels

Serum Magnesium levels in BPH patients are shown in Figure 4.12 the serum magnesium levels were comparable across the different genotypes for SNP rs4745363 among BPH patients (p=0.336), TT carriers had the highest levels of magnesium ions concentration in the serum compared to the TA carriers, (median,0.6; IQR, 0.81

mmol/l vs median 0.57, IQR 0.38 mmol/l p=0.181), AA carriers had the lowest concentration of magnesium ions compared to TA carriers (median 0.61; IQR 0.5 mm/l vs median 0.57, IQR 0.38 mmol/l p=0.661). Marked hypomagnesemia was observed in TT carriers compared to TA carriers (n=180, 93.26% vs n=11, 5.69%), AA carriers presented with the least number of participants with hypomagnesemia (n=3,1.55%). Hypermagnesemia was only observed among the TT carriers across (n=6, 3.11%) as shown in Table 4.11.

The magnesium serum level among the Control group is shown in Figure 4.12, the serum magnesium concentration was comparable among the different genotypes for SNP rs4745363 of the control group (p=0.637), The TT carriers showed the highest levels of magnesium concentration compared to TA (median 2.00; IQR; 2.0 mmol/l, vs median 2.0; IQR 0.00 mmol/l), AA carriers showed low levels of magnesium concentration compared to TA carriers (median 2.0; IQR, 0.00 mmol/l vs median 2.0; IQR 0.00 mmol/l p=0.738), TT carriers low observation for both hypomagnesemia and hypermagnesemia (n=6, 3.11%, n=4, 2.07%) There was no observation made among both TA and AA carriers for hypomagnesemia and hypermagnesemia as shown in table 4.12.

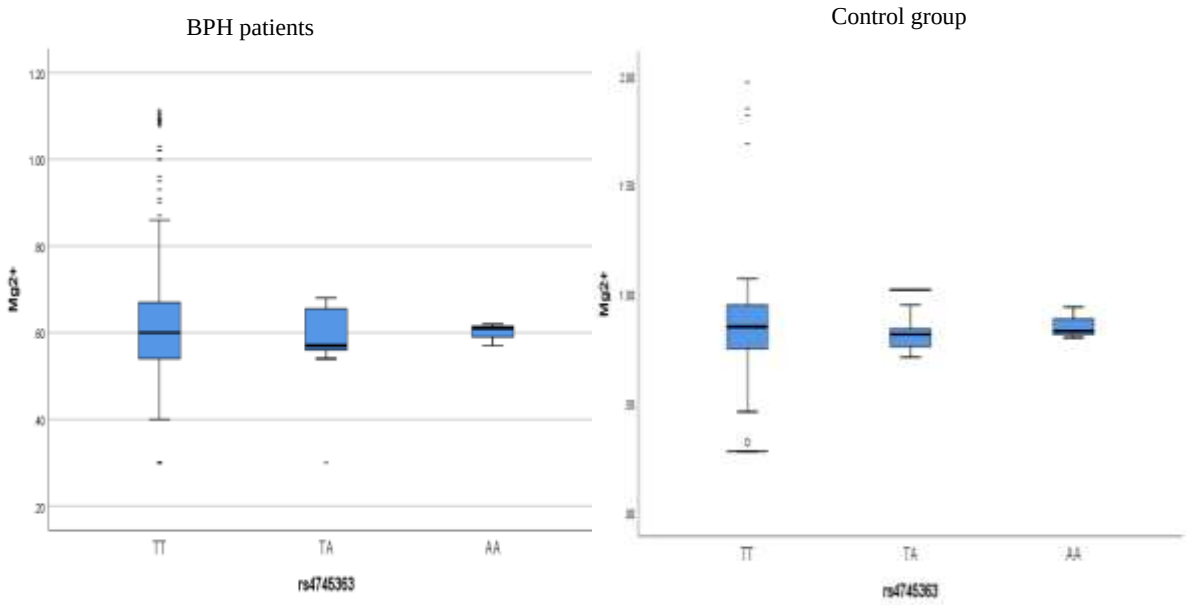

Figure 4.12: Serum magnesium levels across genotype carriers at SNP rs4745363 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.3 Association between TRPM7 rs8042919 and electrolytes

4.5.3.1 rs8042919 Genotypes and sodium levels

Sodium levels in BPH are shown in Figure 4.13. The serum sodium level was comparable across the different genotypes for SNPS rs8042919 among BPH patients (P=0.022), GG carriers had lower levels compared to GA carriers (Median, 135.0; IQR, 48 mmol/l vs Median 138.0; IQR, 41 mmol/l; p=0.206). Similarly, AA carriers had lower levels than GA carriers (Median, 129.0, IQR, 42 mmol/l; vs Median 138.0; IQR, 41 mmol/l p=0.034). Marked Hyponatremia (n=67, 34.72%) was observed in GG carriers. In contrast, hypernatremia was observed in a small number, (n=05, 2.59%) as compared to GA (n=19, 9.84%), AA carriers demonstrated a low level of hyponatremia (n=3, 1.55%) and a single observation of hypernatremia (n=1, 0.52%) as shown in table 4.11.

The serum level of sodium in the control group is shown in Figure 4.13, the circulating sodium levels were proportionate across different genotypes for SNPs rs8042919 among the control group (p=0.206), GG carriers had higher levels than the GA carriers (median, 143.0; IQR, 24 mmol/l vs median, 144.0; IQR, 12 mmol/l) AA carriers had the lowest levels of sodium in serum (median 140.0 IQR 12mmol/l). Hyponatremia was seen in TT carriers (n=5, 2.59%, p=0.569) while a single observation was seen among the TA carriers (n=1, 0.52%), AA carriers did not depict any sort of Sodium imbalance.

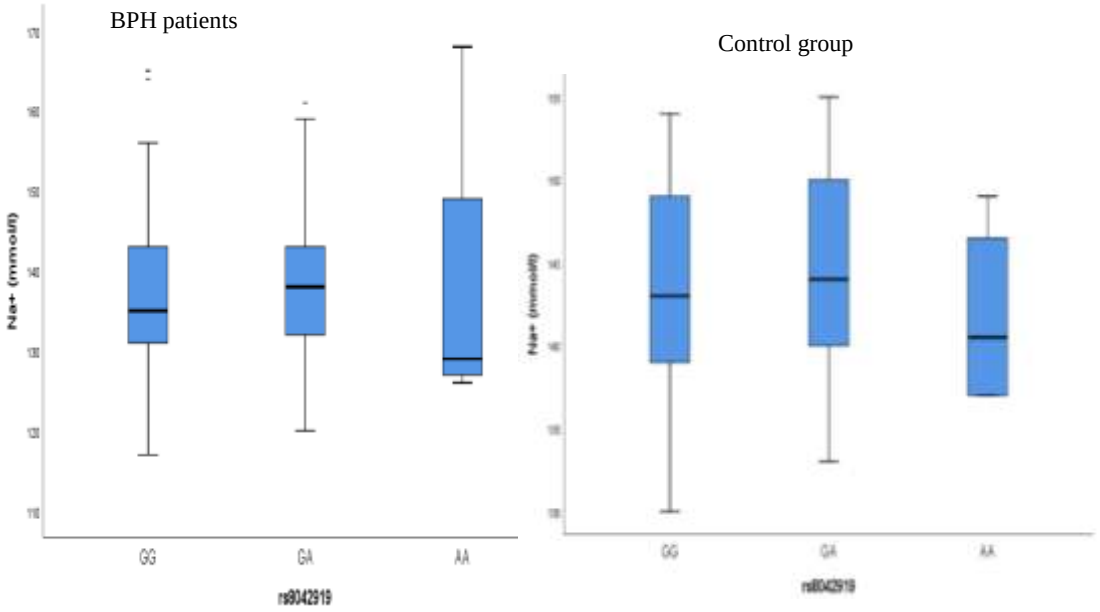

Figure 4.13: Serum Sodium levels across genotype carriers at SNP rs8042919 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.3.2 rs8042919 Genotypes and potassium levels

Potassium levels in BPH patients are shown in Figure 4.14 the potassium levels were comparable across the different genotypes for SNP rs8042919 among BPH patients (p=0.867), GG carriers had the highest levels of potassium ions concentration in the serum compared to the GA carriers, (median, 4.0; IQR, 3.8 mmol/l vs median 4.0 IQR

3.2 mmol/l p=0.346), AA carriers had the lowest concentration of potasium ions compared to GA carriers (median 3.2; IQR 1.1 mmol/l vs median 4.0 IQR 3.2 mmol/l; p=0.206). Marked hypokalemia was observed in GG carriers (n=34, 17.71%) compared to GA carriers (n=15, 7.82%), AA carriers presented with the least number of participants with hypokalemia (n=3, 1.56%). Hyperkalemia was higher in GG carriers compared to GA carriers (n=12, 7.25% vs n=3, 1.56%) however, there was no observation made for Hyperkalemia among the AA carriers as shown in Table 4.11.

The serum level in the control group is shown in Figure 4.14. The levels were comparable across the different genotypes for SNPs rs8042919 among the control participants (p=0.363). The GG carriers showed the highest levels of potassium concentration compared to both the GA and AA carriers (median 4.5; IQR; 2.4 mmol/l, vs median 4.3; IQR 2.0 mmol/l, vs median 4.6; IQR 1.4 mmol/l p=0.084), GG carriers had a single observation for hypokalemia (n=1, 0.52%) and a shallow level of hyperkalemia (n=2, 1.04%), there was no observation of any imbalance made among the GA and AA carriers as shown in table 4.12.

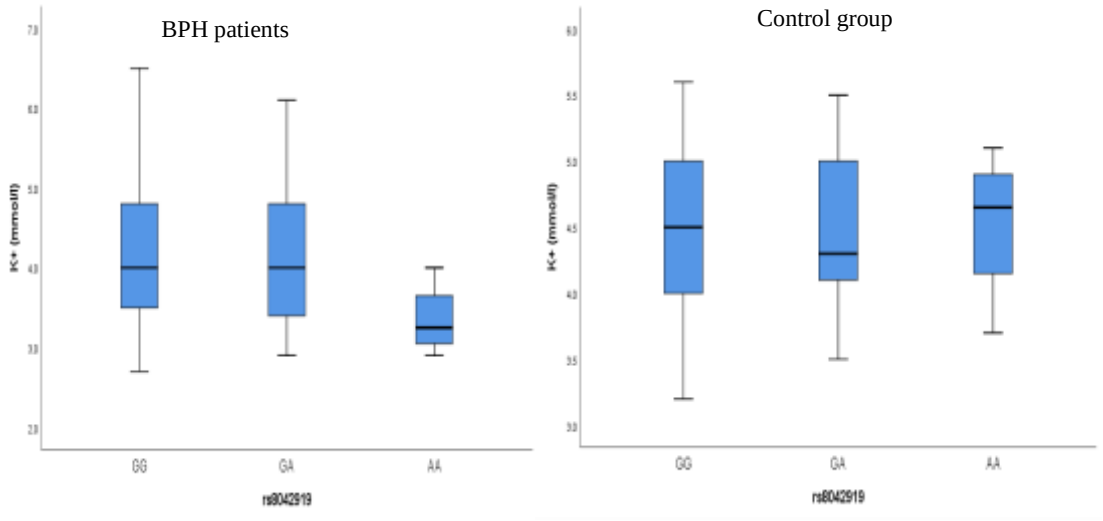

Figure 4.14: Serum Potassium levels across genotype carriers at SNP rs8042919 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.3.3 rs8042919 Genotypes and chloride levels

Chloride levels in BPH patients are shown in Figure 4.14 the serum chloride levels were comparable across the different genotypes for SNP rs8042919 among BPH patients (p=0.408), GG carriers had the highest levels of chloride ions concentration in the serum compared to the GA carriers, (median, 100.1; IQR, 23.5 mmol/l vs median 101.0, IQR 18.0 mmol/l p=0.230), AA carriers had the lowest concentration of chloride ions compared to GA carriers (median 99.0; IQR 3.5 mmol/l vs median, 100.1; IQR,18.0 mmol/l; p=0.408). Marked hypochloremia was observed in GG carriers compared to GA carriers (n=42, 21.87% vs n=13, 6.77%), and AA carriers presented with a single participant with hypochloremia (n=1, 0.52%). Hyperchloremia was observed in GG carriers more than in GA carriers which had an insignificant

observation (n=17, 8.81%, n=2, 1.04%), AA carriers presented with no hyperchloremia as shown in Table 4.11.

The chloride serum level among the Control group is shown in Figure 4.14, the serum chloride concentration was comparable among the different genotypes for SNP rs8042919 among the control group (p=0.261), The GG carriers showed the highest levels of chloride concentration compared to GA (median 101.4; IQR; 13.1 mmol/l, vs median 102.4; IQR 11.7 mmol/l), AA carriers showed lowest levels of chloride concentration compared to GA carriers (median 101.6 medians; IQR, 3.5 mmol/l vs median 102.4; IQR 11.7 mmol/l p=0.641), High levels of Hypochloremia was observed among the GG carriers (n=6, 3.11%) compared to GA carriers(n=3, 1.56%), however, there was no observation for both hypochloremia and hyperchloremia among the AA carriers, marked hyperchloremia was observed among the GG carriers (n=8, 4.17%) higher than one observed among the GA carriers (n=5, 2.59%) as shown in table 4.12.

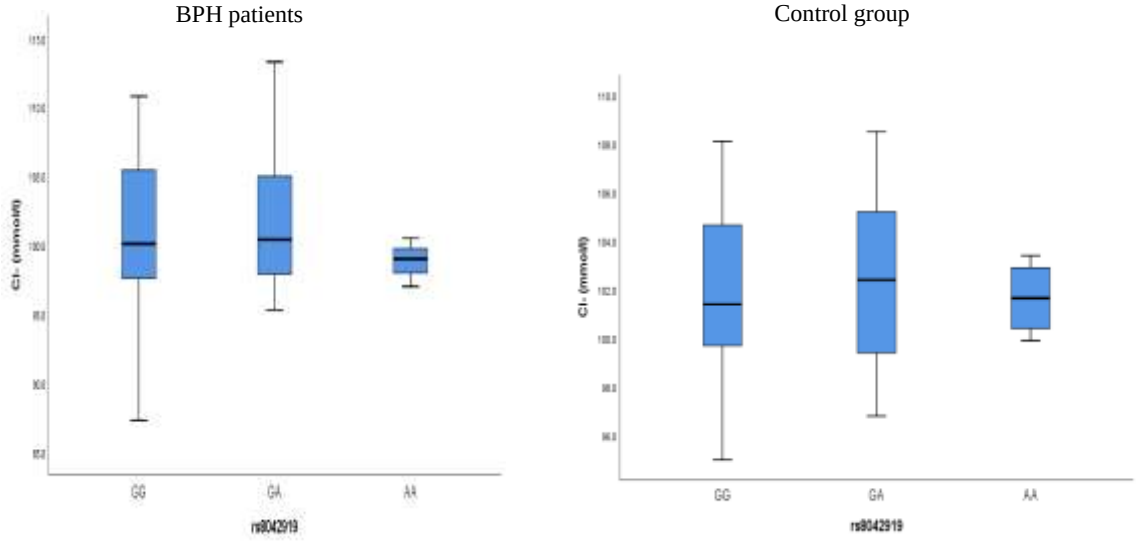

Figure 4.15: Serum Chloride levels across genotype carriers at SNP rs8042919 among BPH patients.

The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.3.4 rs8042919 Genotype and bicarbonate levels

Bicarbonate levels in BPH patients are shown in Figure 4.15 the serum bicarbonate levels were comparable across the different genotypes for SNP rs8042919 among BPH patients (p=0.982), GG carriers had the highest levels of Bicarbonate ions concentartion in the serum compared to the GA carriers, (median, 25.5; IQR, 11.6 mmol/l vs median 23.9, 11.80 mmol/l p=0.367), AA carriers had the lowest concentration of bicarbonate ions compared to GA carriers (median 25.6; IQR 8.0 mmol/l vs median 23.9, 11.80 mmol/l; p=0.243). Marked acidosis was observed among GG carriers (n=20,10.42%), higher than the observation made among the GA carriers (n=13, 6.77%), there was a single observation for acidosis among both the AA carriers (n=1, 0.52%). Higher observation of Alkalosis was observed among GG carriers (n=19,9.89%) compared to GA carriers (N=6, 3.13%), there was a singular observation (n=1, 0.52%), among the AA carriers for Alkalosis as shown in table 4.11.

The bicarbonate serum level among the Control group is shown in Figure 4.15, the serum bicarbonate concentration was comparable among the different genotypes for SNP rs8042919 among the control group (p=0.354), The GG carriers showed the highest levels of bicarbonate concentration compared to GA (median 24.2; IQR; 8.90 mmol/l, vs median 24.9 IQR 8.50 mmol/l), AA carriers showed a minimal change in bicarbonate concentration (median 25.3; IQR, 3.40mmol/l), GG carriers had a minimal observation for acidosis (n=3, 1.56%) more than observed in GA carriers (n=2, 1.04%), there was a single observation among both the GG and GA carriers for alkalosis (n=1, 0.52%), AA carriers had no observation for both acidosis and alkalosis singular as shown in table 4.12.

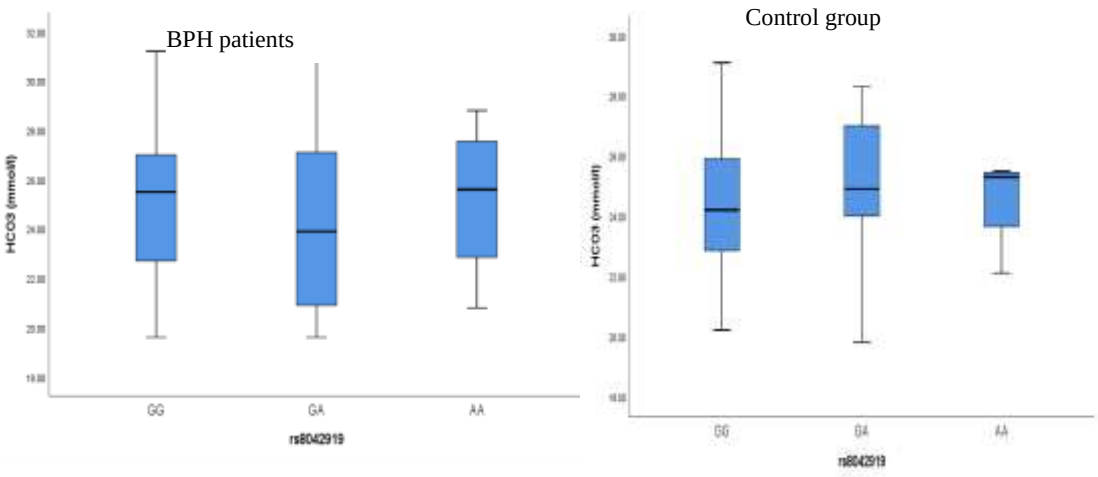

Figure 4.16: Serum Bicarbonate levels across genotype carriers at SNP rs8042919 among control participants.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.3.5 rs8042919 Genotypes and magnesium levels

Serum Magnesium levels in BPH patients are shown in Figure 4.16 the serum magnesium levels were comparable across the different genotypes for SNP rs8042919 among BPH patients (p=0.822), GG carriers had the highest levels of magnesium ions concentration in the serum compared to the GA carriers, (median,0.6; IQR, 0.81 mmol/l vs median 0.58, IQR 0.73 mmol/l p=0.181), AA carriers had the lowest concentration of magnesium ions compared to GA carriers (median 0.65; IQR 0.5 mm/l vs median 0.58, IQR 0.73 mmol/l p=0.341). Marked hypomagnesemia was observed in GG carriers compared to GA carriers (n=84, 43.75% vs n=6, 3.13%), AA carriers presented with the least number of participants with hypomagnesemia (n=2, 1.04%). Hypermagnesemia was only observed among the GG carriers across (n=6, 3.25%) as shown in Table 4.11.

The magnesium serum level among the Control group is shown in Figure 4.16, the serum magnesium concentration was comparable among the different genotypes for

SNP rs8042919 of the control group (p=0.879), The GG carriers showed the highest levels of magnesium concentration compared to GA (median 0.83; IQR; 1.54 mmol/l, vs median 0.86; IQR 1.47 mmol/l), AA carriers showed low levels of magnesium concentration compared to Gcarriers (median 0.82; IQR, 0.017 mmol/l vs median 0.86; IQR 1.47 mmol/l p=0.437), GG carriers low observation for both hypomagnesemia and hypermagnesemia (n=5, 2.60%, n=4, 2.08%) compared to the GA carriers (n=1, 0.52%, n=2, 1.04%) respectively. There was no observation made among AA carriers for both hypomagnesemia and hypermagnesemia as shown in table 4.12.

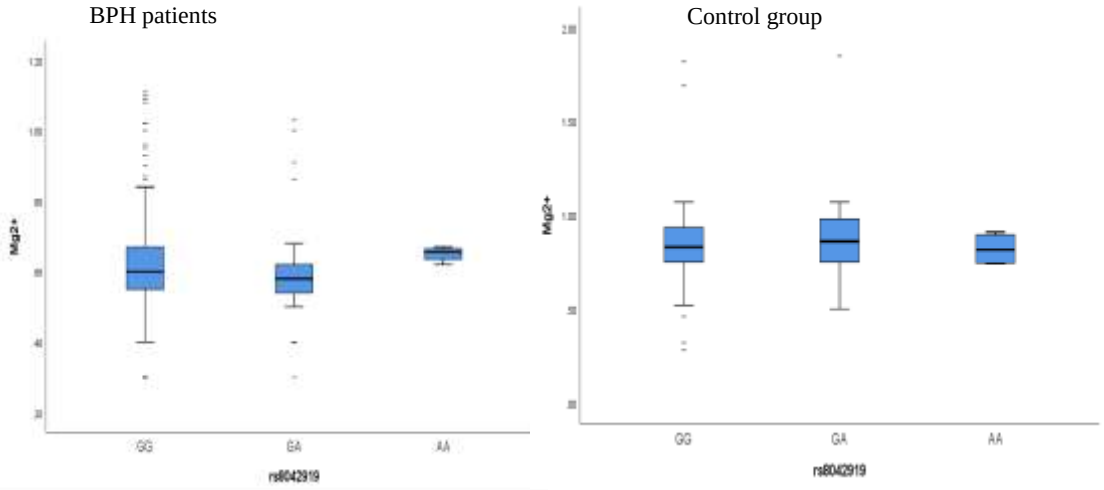

Figure 4.17:serum Magnesium levels across genotype carriers at SNP rs8042919 among control participants.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.4 Association between TRPM7, rs2362295 genotypes and electrolytes

4.5.4.1 rs2362295 Genotypes and sodium levels

Sodium levels in BPH are shown in Figure 4.17. The serum sodium level was comparable across the different genotypes for SNPS rs2362295 among BPH patients (P=0.025), CC carriers had higher levels of Sodium concentration compared to CT carriers (Median, 135.0; IQR, 51 mmol/l vs Median 136.0; IQR, 45 mmol/l; p=0.360).

Similarly, TT carriers had lower levels compared to CT carriers (Median, 129.5, IQR, 32 mmol/l; vs Median 136.0; IQR, 45 mmol/l p=0.028). Marked Hyponatremia (n=51, 26.42%) was observed among CC carriers higher than in CT carriers (n=30, 15.54%), TT carriers had a lower observation of hyponatremia compared to CT carriers (n=9, 4.67% vs n=30, 15.54%), TT carriers had observation lower than CT in hyponatremia (n=9, 4.66%). Observation of hypernatremia was the same in both CC and CT carriers (n=4, 2.07%). as shown in Table 4.11.

The serum level of sodium in the control group is shown in Figure 4.17, the circulating sodium levels were proportionate across different genotypes for SNPs rs2362295 among the control group (p=0.132), CC carriers had higher levels than the CT carriers (median, 142.0; IQR, 24 mmol/l vs median, 143.0; IQR, 22 mmol/l) TT carriers had the lowest levels of sodium in serum (median 144.0 IQR 19mmol/l). Hyponatremia was observed among CC carriers (n=4, 2.07%, p=0.454), while a single observation was observed among both the CT and TT carriers (n=1, 0.52%), Hypernatremia was not observed among all the carriers as shown in table 4.12.

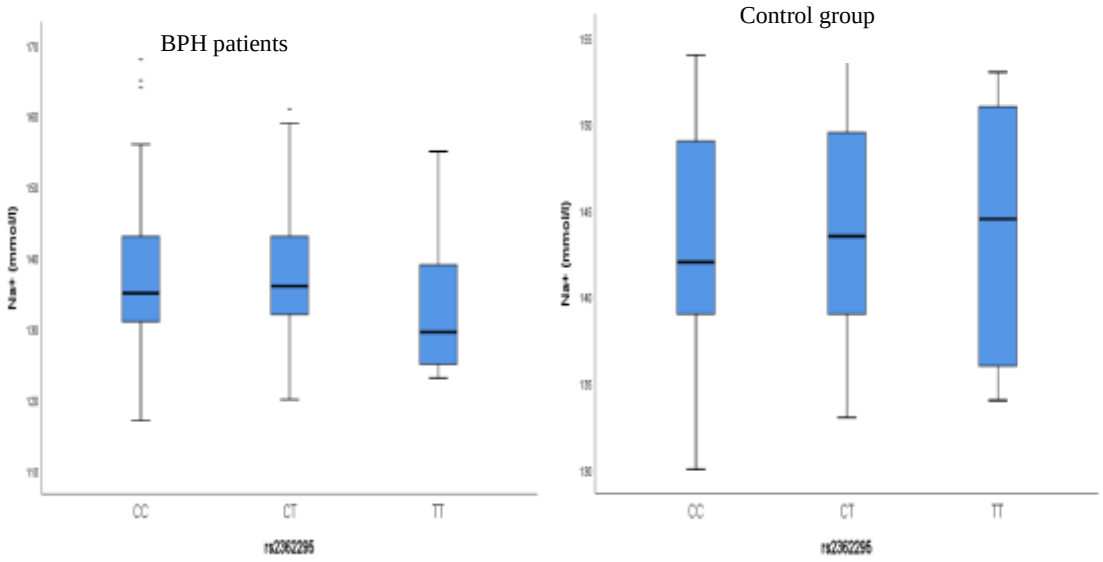

Figure 4.18: Serum Sodium levels across genotype carriers at SNP rs2362295 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.4.2 rs2362295 Genotypes and potassium levels

potassium levels in BPH patients are shown in Figure 4.18 the potassium levels were comparable across the different genotypes for SNP rs2362295 among BPH patients (p=0.067), CC carriers had the highest levels of potassium ions concentration in the serum compared to the CT carriers, (median, 4.1; IQR, 3.8 mmol/l vs median 3.9 IQR 3.2 mmol/l p=0.034), TT carriers had the lowest concentration of potassium ions compared to CT carriers (median 3.8; IQR 2.9 mmol/l vs median 3.9 IQR 3.2 mmol/l; p=0.016). Marked hypokalemia was observed among CC carriers (n=28, 14.51%) compared to CT carriers (n=20, 10.36%), TT had the least observations with hypokalemia (n=5, 2.59%). Hyperkalemia was higher in CC carriers compared to CT carriers (n=9, 4.66% vs n=5, 2.59%) only a single observation, was made for Hyperkalemia among the TT carriers as shown in Table 4.11.

The potassium serum level in the control group is shown in Figure 4.18. The levels were comparable across the different genotypes for SNPs rs23622895 among the control participants (p=0.061). The CC carriers showed the highest levels of potassium concentration compared to CT (median 4.5; IQR; 2.1 mmol/l, vs median 4.4; IQR 2.0 mmol/l), TT carriers showed the least levels of potassium concentration (median 4.4, IQR 2.3 mmol/l) Both CC and CT carriers had no observation made for hypokalemia, however, a single observation was made for hypokalemia among TT carriers (n=1, 0.52%, p=0.006), a low level of hyperkalemia (n=2, 1.04%) was observed among CC carriers, there was no observation for hyperkalemia made among the CT and TT carriers as shown in table 4.12.

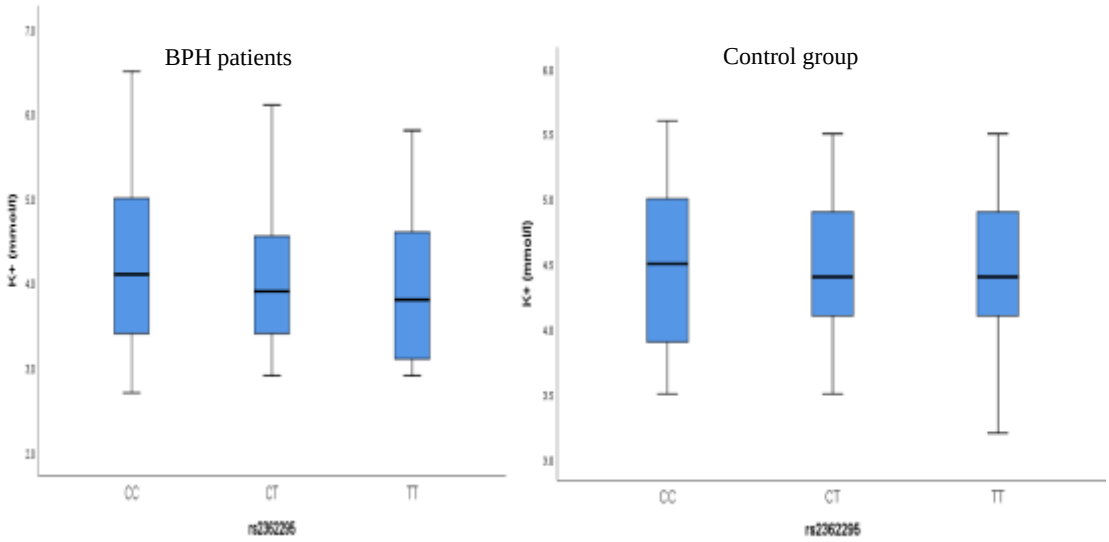

Figure 4.19: Serum Potassium levels across genotype carriers at SNP rs2362295 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.4.3 rs2362295 Genotypes and chloride levels

Chloride levels in BPH patients are shown in Figure 4.19 the serum chloride levels were comparable across the different genotypes for SNP rs2362295 among BPH patients (p=0.474), CC carriers had the highest levels of chloride ions concentration in

the serum compared to the CT carriers, (median, 100.1; IQR, 22.9 mmol/l vs median 100.0, IQR 19.0 mmol/l p=0.049), TT carriers had the lowest concentration of chloride ions compared to CT carriers (median 97.0; IQR 22.6 mmol/l vs median 100.0, IQR 19.0 mmol/l mmol/l; p=0.213). Marked hypochloremia was observed in CC carriers compared to CT carriers (n=33, 17.09% vs n=15, 7.77%), and it was observed that TT carriers had the lowest observation for hypochloremia (n=8, 4.14%). Hyperchloremia was observed in CC carriers more than in CT carriers while TT had a single observation for hyperchloremia (n=1, 0.52%) as shown in Table 4.11.

The chloride serum level among the Control group is shown in Figure 4.19, the serum chloride concentration was comparable among the different genotypes for SNP rs8042919 among the control group (p=0.742), The CC carriers showed the highest levels of chloride concentration compared to CT (median 101.5; IQR; 13.4 mmol/l, vs median 102.5; IQR 11.7 mmol/l), TT carriers showed lowest levels of chloride concentration compared to CT carriers (median 100.5 median; IQR, 8.9 mmol/l vs median 102.5; IQR 11.7 mmol/l p=0.231), Both CC and CT had an equal observation for Hypochloremia (n=4, 2.07%) a single observation for hypochloremia was made among the TT carriers (n=1,0.52%). Marked hyperchloremia was observed among the CC carriers (n=7, 3.67%) slightly more than for CT carriers (n=6, 3.11%) as shown in Table 4.12.

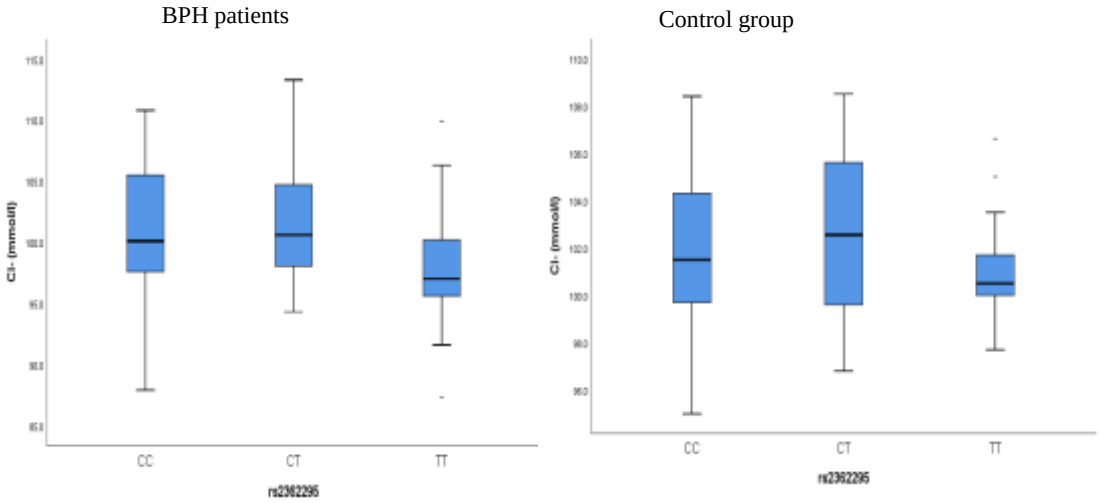

Figure 4.20: Serum Chloride levels across genotype carriers at SNP rs2362295 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

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4.5.4.4. rs2362295 Genotypes and Bicarbonate Levels

Bicarbonate levels in BPH patients are shown in Figure 4.20 the serum bicarbonate levels were comparable across the different genotypes for SNP rs2362295 among BPH patients (p=0.425), CC carriers had the highest levels of Bicarbonate ions concentration in the serum compared to the CT carriers, (median, 25.6; IQR, 11.2 mmol/l vs median 24.5, 12.00 mmol/l p=0.176), TT carriers had the lowest concentration of bicarbonate ions compared to CT carriers (median 24.75; IQR 10.0 mmol/l vs median 24.5, 12.0 mmol/l; p=0.156). Marked acidosis was observed among CT carriers (n=18, 9.33%), higher than the observation made among the CC carriers (n=15,7.78%), there was a single observation for acidosis among both the TT carriers (n=1, 0.52%). An equal observation of Alkalosis was made among CC and CT carriers (n=12, 6.22%) however, there was a low level of observation for Alkalosis among the TT carriers (n=3, 1.55%) as shown in Table 4.11.

The bicarbonate serum level among the Control group is shown in Figure 4.20 the serum bicarbonate concentration was comparable among the different genotypes for

SNP rs2362295 among the control group (p=0.132), The CC carriers showed the highest levels of bicarbonate concentration compared to CT (median 24.3; IQR; 7.60 mmol/l, vs median 24.5 IQR 8.50 mmol/l), TT carriers showed a minimal change in bicarbonate concentration (median 25.5; IQR, 7.70 mmol/l), CC carriers had a minimal observation for acidosis (n=2, 1.04%) less than observed in CT carriers (n=3, 1.55%), however, there was no observation for acidosis among the TT carriers. there was a single observation among both CT and TT carriers for alkalosis (n=1, 0.52%), CC carriers had no observation for acidosis as shown in Table 4.12.

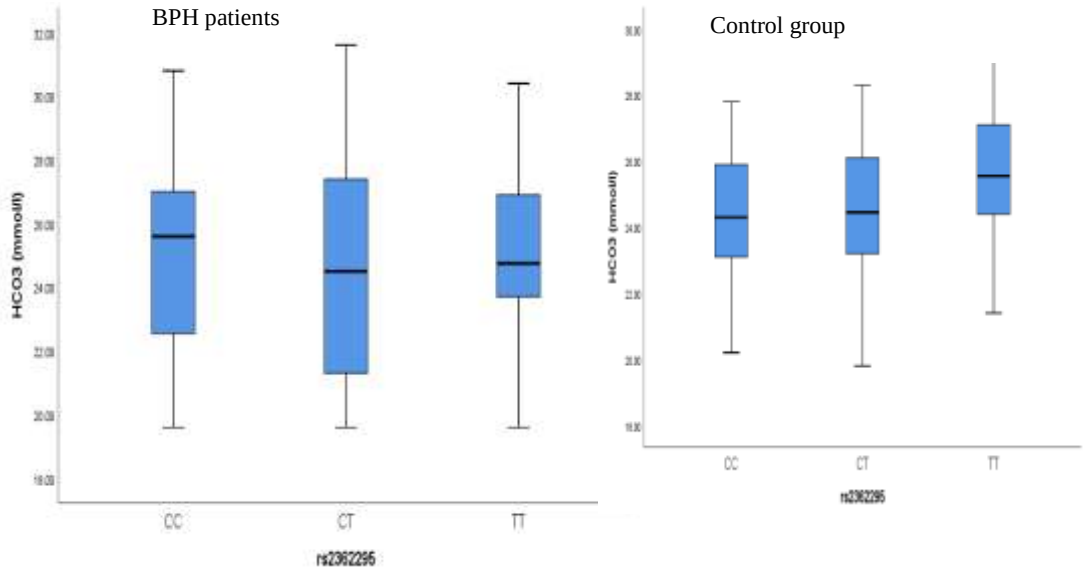

Figure 4.21: Serum Bicarbonate levels across genotype carriers at SNP rs2362295 among control participants.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.4.5 rs2362295 Genotypes and Magnesium Levels

Serum Magnesium levels in BPH patients are shown in Figure 4.21 the serum magnesium levels were comparable across the different genotypes for SNP rs2362295 among BPH patients (p=0.015), CC carriers had the highest levels of magnesium ions concentration in the serum compared to the CT carriers, (median,0.61; IQR, 0.12 mmol/l vs median 0.58, IQR 0.73 mmol/l p=0.017), TT carriers had the lowest

concentration of magnesium ions compared to CT carriers (median 0.63; IQR 0.8 mmol/l vs median 0.58, IQR 0.73 mmol/l p=0.141). Marked hypomagnesemia was observed among CC carriers (n=67, 34.71%) compared to CT carriers (n=53, 27.46%), AA carriers presented with the least number of participants with hypomagnesemia (n=8, 4.15%). Hypermagnesemia was highly observed among the CC (n=5, 2.59%) carriers than in single observation among the TT carriers (n=1, 0.52%), there was no observation of hypermagnesemia among the CT carriers as shown in Table 4.11.

The magnesium serum level among the Control group is shown in Figure 4.21, the serum magnesium concentration was comparable among the different genotypes for SNP rsrs2362295 of the control group (p=0.603), The CC carriers showed the highest levels of magnesium concentration compared to CT carriers (median 0.84; IQR; 1.54 mmol/l, vs median 0.88; IQR 1.65mmol/l), TT carriers showed the lowest levels of magnesium concentration (median 0.80; IQR, 0.38 mmol/l, p=0.137), CC carriers (n=4, 2.07%) had a higher observation of hypomagnesemia compared to CT carriers (n=2, 1.04%) however, TT carriers did not have an observation for both hypomagnesemia and hypermagnesemia. CC and CT carriers had an equal observation for hypermagnesemia (n=2, 1.04%) as shown in Table 4.12.

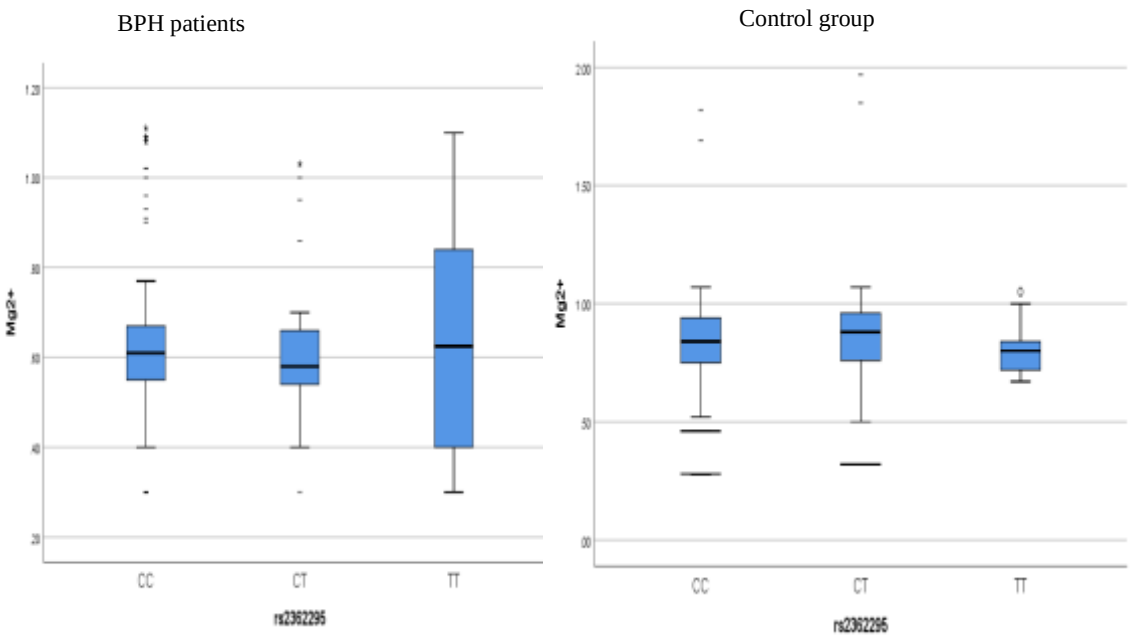

Figure 4.22: Serum bicarbonate levels across genotype carriers at SNP rs2362295 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.5 Association between TRPM8 rs10490018 and Electrolytes

4.5.5.1 rs10490018 Genotypes and Sodium Levels

Sodium levels in BPH are shown in Figure 4.22, The serum sodium level was comparable across the different genotypes for SNPS rs10490018 among BPH patients (P=0.288), CC carriers had higher levels of sodium compared to CT carriers (Median, 135.0; IQR, 51 mmol/l vs Median 136.0; IQR, 45 mmol/l; p=0.316). Similarly, CT carriers had higher levels of sodium compared to TT carriers (Median 136.0; IQR, 45 mmol/l; vs Median 129.5; IQR, 32 mmol/l p=0.559). Marked Hyponatremia (n=44, 22.91%) was observed in CC carriers higher than it was observed among the CT carriers (n=38, 19.79%), TT carriers had the least occurrence for hyponatremia (n=7, 3.65%) CC carriers had a higher occurrence of hypernatremia (n=6, 3.12%) compared to CT carriers (n=2, 1.04%) TT carriers did not depict hypernatremia, as shown in Table 4.11.

The serum level of sodium in the control group is shown in Figure 4.22, the circulating sodium level was proportionate across different genotypes for SNPs rs10490018 among the control group (p=0.523), CC carriers had higher levels of sodium compared to CT carriers (median, 142.0; IQR, 25 mmol/l vs median, 143.0; IQR, 24 mmol/l) TT carriers had the lowest levels of sodium in serum (median 142.0 IQR 17mmol/l). Low levels of hyponatremia were observed in both CC carriers (n=4, 2.08%) and CT carriers (n=2, 1.04%) however, no observation was made among TT carriers in addition there was no occurrence of hypernatremia among all the genotype carriers as shown in table 4.12.

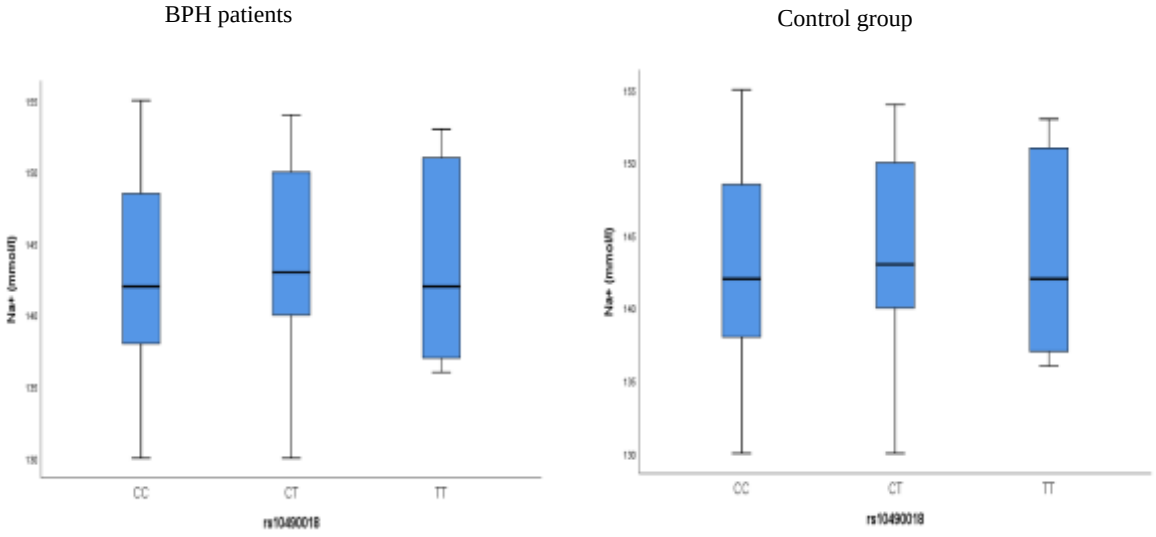

Figure 4.23: Serum Sodium levels across genotype carriers at SNP rs10490018 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.5.2 rs10490018 Genotypes and Potassium Levels

Potassium levels in BPH patients are shown in Figure 4.23 the potassium levels were comparable across the different genotypes for SNP rs10490018 among BPH patients (p=0.054), CC carriers had the highest levels of potassium ions concentration in the serum compared to the CT carriers,(median 3.9; IQR, 3.2 mmol/l vs median 4.1 IQR

3.8 mmol/l p=0.027), TT carriers had the lowest concentration of potassium ions compared to CT carriers (median 3.7; IQR 2.9 mmol/l vs median 4.1 IQR 3.8 mmol/l; p=0.048). Marked hypokalemia was observed among CC carriers (n=27, 14.06%) compared to CT carriers (n=19, 9.89%), TT had the least observations with hypokalemia (n=6, 3.13%). Hyperkalemia was higher in CT carriers compared to CC carriers (n=11, 5.73% vs n=3, 1.56%) only a single observation, was made for Hyperkalemia among the TT carriers as shown in Table 4.11.

The potassium serum level in the control group is shown in Figure 4.23. The levels were comparable across the different genotypes for SNPs rs10490018 among the control participants (p=0.059). The CC carriers showed the highest levels of potassium concentration compared to CT (median 4.4; IQR; 2.4 mmol/l, vs median 4.5; IQR 2.0 mmol/l), TT carriers showed the lowest levels of potassium concentration (median 4.2, IQR 2.1 mmol/l) Both CT and TT carriers had no observation made for hypokalemia, however, a single observation was made for hypokalemia among CC carriers (n=1, 0.52%, p=0.179), a single occurrence for hyperkalemia (n=1, 0.52% p=0.285) was observed among both CC and TT carriers, there was no observation for hyperkalemia made among the CT carriers as shown in table 4.12.

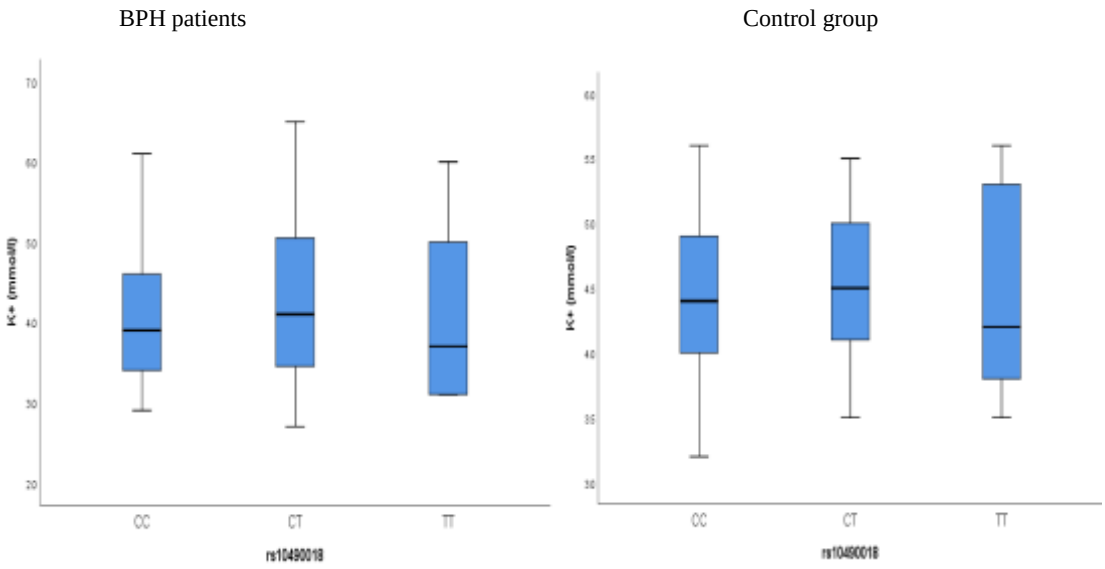

Figure 4.24: Serum Potassium levels across genotype carriers at SNP rs10490018 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles

4.5.5.3 rs10490018 Genotypes and chloride levels

Chloride levels in BPH patients are shown in Figure 4.24 the serum chloride levels were comparable across the different genotypes for SNP rs10490018 among BPH patients (p=0.152), CC carriers had the highest levels of chloride ions concentration in the serum compared to the CT carriers, (median, 100.1; IQR, 26.0 mmol/l vs median 100.1, IQR 22.7 mmol/l p=0.038), TT carriers had the lowest concentration of chloride ions compared to CT carriers (median 99.0; IQR 11.2 mmol/l vs median 100.1, IQR 22.7 mmol/l mmol/l; p=0.277). Marked hypochloremia was observed in CC carriers compared to CT carriers (n=26, 13.47% vs n=24, 12.5%), it was observed that TT carriers had the lowest observation for hypochloremia (n=5, 2.60%). Hyperchloremia was observed among CT carriers (n=9, 4.66%) more than in CC carriers (n=8, 4.17%) while TT had no occurrence for hyperchloremia as shown in Table 4.11.

The chloride serum level among the Control group is shown in Figure 4.24, the serum chloride concentration was comparable among the different genotypes for SNP rs10490018 among the control group (p=0.393), CC carriers showed the highest levels of chloride concentration compared to CT (median 102.1; IQR; 11.7 mmol/l, vs median 101.4; IQR 13.1 mmol/l), TT carriers showed lowest levels of chloride concentration compared to CT carriers (median 100.3 median; IQR, 7.9 mmol/l vs median 101.4; IQR 13.1 mmol/l p=0.274),

Both CC and CT carriers had an equal occurrence (n=4, 2.07%) for hypochloremia however, TT carriers and a single occurrence (n=1, 0.52%), CC carriers had higher occurrence for hyperchloremia compared to CT carriers, individuals with TT carriers showed no occurrence for hyperchloremia, marked hyperchloremia was observed among the CC carriers (n=7, 3.67%) slightly more than for CT carriers (n=6, 3.11%) as shown in table 4.12.

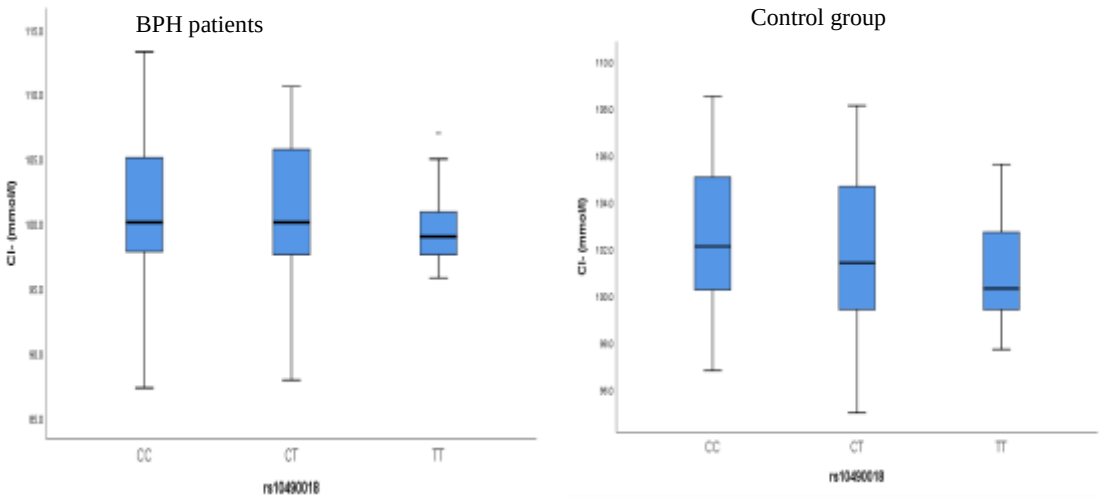

Figure 4.25:Serum Chloride levels across genotype carriers at SNP rs10490018 among BPH patients.

The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers shows the 10th and 90th percentiles

4.5.5.4 rs10490018 Genotypes and Bicarbonate Levels

Bicarbonate levels in BPH patients is shown in figure 4.25 the serum bicarbonate levels were comparable across the different genotype for SNP rs10490018 among BPH patients (p=0.645), CC carriers had the highest levels of Bicarbonate ions concentartion in the serum compared to the CT carriers, (median, 24.9; IQR, 12.0 mmol/l vs median 25.8, 11.2 mmol/l p=0.213), TT carriers had the lowest concentration of bicarbonate ions compared to CT carriers (median 24.05; IQR 8.0 mmol/l vs median 25.8, 11.2 mmol/l; p=0.237). Marked acidosis was observed among CC carriers (n=20, 10.36%), higher than in the CT carriers (n=10, 5.21%), there was a lower observation for acidosis among the TT carriers (n=4, 0.52%). An equal observation of Alkalosis was made among CC and CT carriers (n=12, 6.22%) however, there was as low level of observation for Alkalosis among the TT carriers (n=4, 2.08%) as shown in table 4.11.

The bicarbonate serum level among the Control group is shown in figure 4.25, the serum bicarbonate concentration was comparable among the different genotypes for SNP rs rs10490018 among the control group (p=0.183), The CC carriers showed the highest levels of bicarbonate concentration compared to CT (median 24.0; IQR; 9.30 mmol/l, vs median 24.5 IQR 7.40mmol/l), TT carriers showed a minimal change in bicarbonate concentration (median 23.5; IQR, 7.00 mmol/l), CC carriers had a minimal observation for acidosis (n=3, 1.56%) less than observerd in CT carriers (n=2, 1.04%), however there was no observation for acidosis among the TT carriers. there was a single observation among both CC and TT carriers for alkalosis (n=1, 0.52%) as shown in table 4.12.

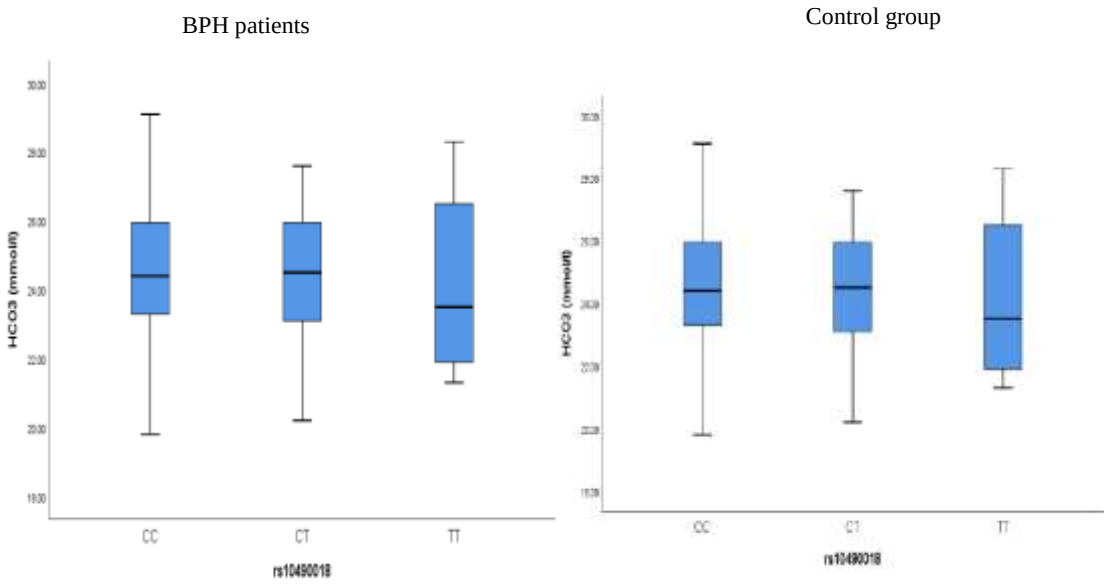

Figure 4.26: Serum Bicarbonate levels across genotype carriers at SNP rs10490018 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers shows the 10th and 90th percentiles

4.5.5.5 rs10490018 Genotypes and Magnesium Levels

Serum Magnesium levels in BPH patients is shown in figure 4.26 the serum magnesium levels were comparable across the different genotype for SNP rs rs10490018 among BPH patients (p=0.098), CC carriers had the highest levels of magnesium ions concentration in the serum compared to the CT carriers, (median,0.60; IQR, 0.12 mmol/l vs median 0.61, IQR 0.79 mmol/l p=0.017), TT carriers had the lowest concentration of magnesium ions compared to CT carriers (median 0.58; IQR 0.78 mmol/l vs median 0.61, IQR 0.79 mmol/l p=0.597). Marked hypomagnesemia was observed among CC carriers (n=71, 3.64%) compared to CT carriers (n=45, 23.43%), marked hypomagnesemia was observed among the CC carriers (n=71, 36.97%) more than in CT carriers (n=45, 23.44%) and TT carriers (n=11, 5.73%) as shown in table 4.11.

The magnesium serum level among the Control group is shown in Figure 4.26; the serum magnesium concentration was comparable among the different genotypes for SNP rs10490018 of the control group (p=0.055); the CC carriers showed the highest levels of magnesium concentration compared to CT carriers (median 0.85; IQR; 1.69 mmol/l, vs median 0.82; IQR 1.17mmol/l), TT carriers showed the lowest levels of magnesium concentration (median 0.92; IQR, 0.55 mmol/l, p=0.173), CC carriers (n=3,1.56%) had a higher observation of hypomagnesemia compared to CT carriers (n=2, 1.04%) however, TT carriers had a single observation for hypomagnesemia. TT carriers did not have an observation for hypermagnesemia; however, CT (n=1,0.52%) carriers had a single observation less than CC (n=3, 1.56%) for hypermagnesemia (n=2, 1.04%), as shown in Table 4.12.

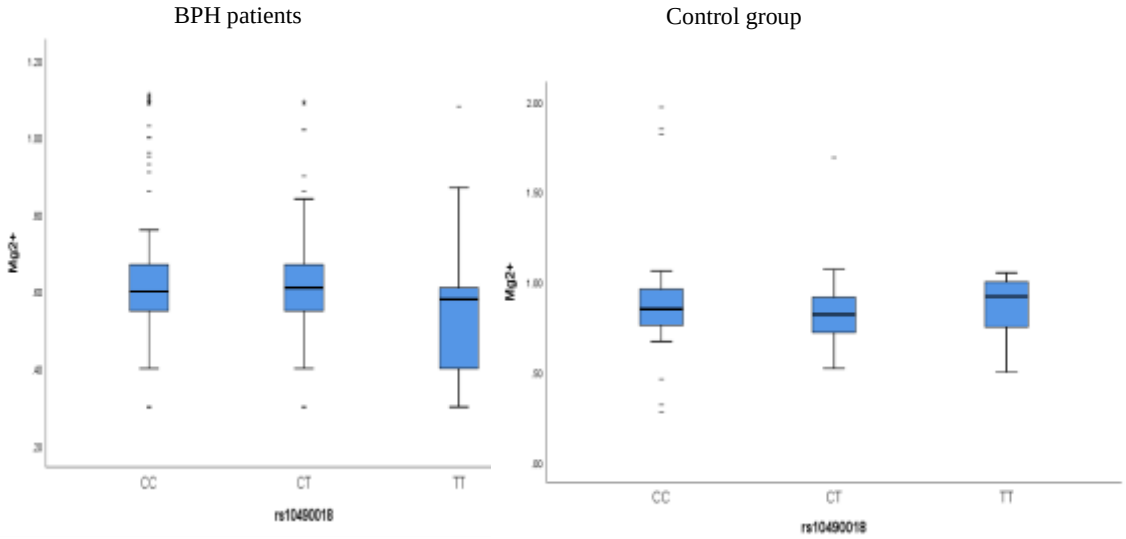

Figure 4.27: Serum Magnesium levels across genotype carriers at SNP rs10490018 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles.

4.5.6 Association between TRPM8 rs1016062 and Electrolytes

4.5.6.1 rs1016062 Genotypes and Sodium Levels

Sodium levels in BPH are shown in Figure 4.27. The serum sodium level was comparable across the different genotypes for SNPS rs1016062 among BPH patients (P=0.054); GG carriers had higher levels of sodium compared to GA carriers (Median, 134.0; IQR, 39 mmol/l vs Median 136.5; IQR, 45 mmol/l; p=0.071). Similarly, GA carriers had higher sodium levels than AA carriers (Median 136.5; IQR, 45 mmol/l; vs Median 141.0; IQR, 42 mmol/l p=0.029). Marked Hyponatremia (n=49, 25.26%) was observed among GG carriers higher than among the GA carriers (n=37, 19.07%), AA carriers had the minor occurrence for hyponatremia (n=4, 2.06%) GG carriers had higher occurrence of hypernatremia (n=45, 23.19%) compared to GA carriers (n=37, 19.07%) AA carriers had the most minor occurrence of hypernatremia (n=2, 1.04%) as shown in table 4.11.

The serum level of sodium in the control group is shown in Figure 4.27; the circulating sodium level was proportionate across different genotypes for SNPs rs1016062 among the control group (p=099), GG carriers had higher levels of sodium compared to GA carriers (median, 142.0; IQR, 24 mmol/l vs median, 143.0; IQR, 22 mmol/l) AA carriers had the lowest levels of sodium in serum (median 144.0 IQR 19 mmol/l). Low levels of hyponatremia were observed among the GG carriers (n=4, 2.08%); however, both the GA and AA carriers had a single observation for hyponatremia (n=1, 0.52%). There was no hypernatremia among all the genotype carriers, as shown in Table 4.12.

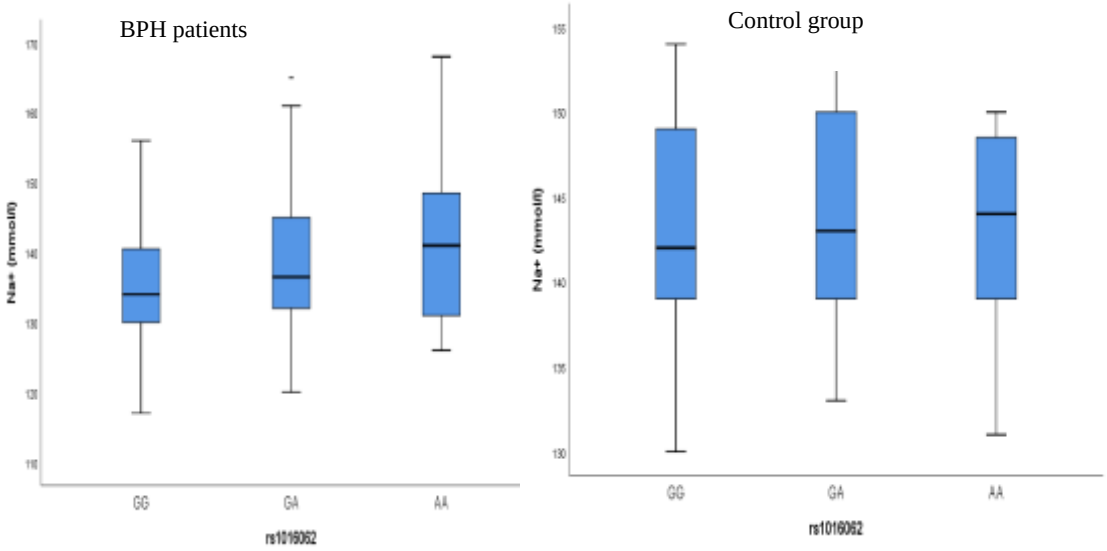

Figure 4.28:Serum Sodium levels across genotype carriers at SNP rs1016062 among control participants.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles.

4.5.6.2 rs1016062 Genotypes and Potassium Levels

potassium levels in BPH patients are shown in Figure 4.28. The potassium levels were comparable across the different genotypes for SNP rs1016062 among BPH patients (p=0.086); GG carriers had the highest levels of potassium ions concentration in the serum compared to the GA carriers (median, 4.0; IQR, 3.8 mmol/l vs. median 3.9 IQR 3.2 mmol/l p=0.054), AA carriers had the lowest concentration of potassium ions compared to GA carriers (median 4.0; IQR 3.0 mmol/l vs. median 3.9 IQR 3.2 mmol/l; p=0.016). Marked hypokalemia was observed among GG carriers (n=22, 14.51%) compared to GA carriers (n=27, 13.92%); AA had the most minor observations with hypokalemia (n=4, 2.06%). Hyperkalemia was higher in GG carriers compared to GA carriers (n=9, 4.64% vs. n=6, 3.09%). Only a single observation was made for Hyperkalemia among the AA carriers (n=1,0.52%), as shown in Table 4.11.

The potassium serum level in the control group is shown in Figure 4.28. The levels were comparable across the different genotypes for SNPs rs1016062 among the control participants (p=0.372). The GG carriers showed the highest levels of potassium concentration compared to GA (median 4.5; IQR; 2.3 mmol/l, vs. median 4.4; IQR 2.0 mmol/l), AA carriers showed the most minor levels of potassium concentration (median 2.0, IQR 1.0 mmol/l) Both GA and AA carriers had a no observation made for hypokalemia. However, a single observation was made for hypokalemia among GG carriers (n=1, 0.52%, p=0.081), a single observation of hyperkalemia (n=1, 0.52% p=0.021) was observed among both AA and GA carriers, there was no observation for hyperkalemia made among the GG carriers as shown in table 4.12.

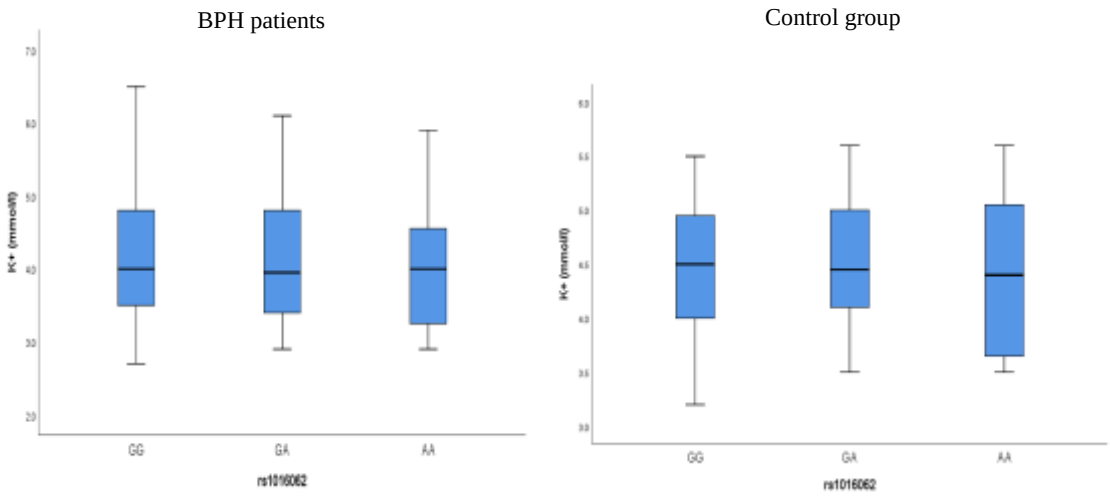

Figure 4.29:Serum Potassium levels across genotype carriers at SNP rs1016062 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles.

4.5.6.3 rs1016062 Genotypes and Chloride Levels

Chloride levels in BPH patients are shown in Figure 4.29. The serum chloride levels were comparable across the different genotypes for SNP rs1016062 among BPH patients (p=0.217); GG carriers had the highest levels of chloride ions concentration in the serum compared to the GA carriers (median, 99.85; IQR, 23.3 mmol/l vs

median 100.6, IQR 23.0 mmol/l p=0.514), AA carriers had the lowest concentration of chloride ions compared to GA carriers (median 100.1, IQR 13.8 mmol/l vs median 100.6, IQR 23.0 mmol/l; p=0.277). Marked hypochloremia was observed in GG carriers compared to GA carriers (n=31, 15.98% vs. n=24, 12.50%), and it was observed that AA carriers had a singular occurrence for hypochloremia (n=1, 0.52%). Hyperchloremia was observed among GG carriers (n=11, 5.67%) more than in GA carriers (n=6, 3.09%), while AA had a singular occurrence for hyperchloremia, as shown in Table 4.11.

The chloride serum level among the Control group is shown in Figure 4.29; the serum chloride concentration was comparable among the different genotypes for SNP rs1016062 among the control group (p=0.469), GG carriers showed the highest levels of chloride concentration compared to GA (median 101.6; IQR; 12.7 mmol/l, vs. median 102.4; IQR 11.7 mmol/l), GA carriers showed lowest levels of chloride concentration compared to AA carriers (median 102.4; IQR 11.7 mmol/l vs. median 100.8 IQR 8.0 mmol/l p=0.637),

Both GA and AA carriers had no observation for hypochloremia; however, GG carriers had a single occurrence (n=1, 0.52%). GG carriers had no occurrence for hyperchloremia; however, both GA and AA carriers had a singular occurrence (n=1, 0.52%), as shown in Table 4.12.

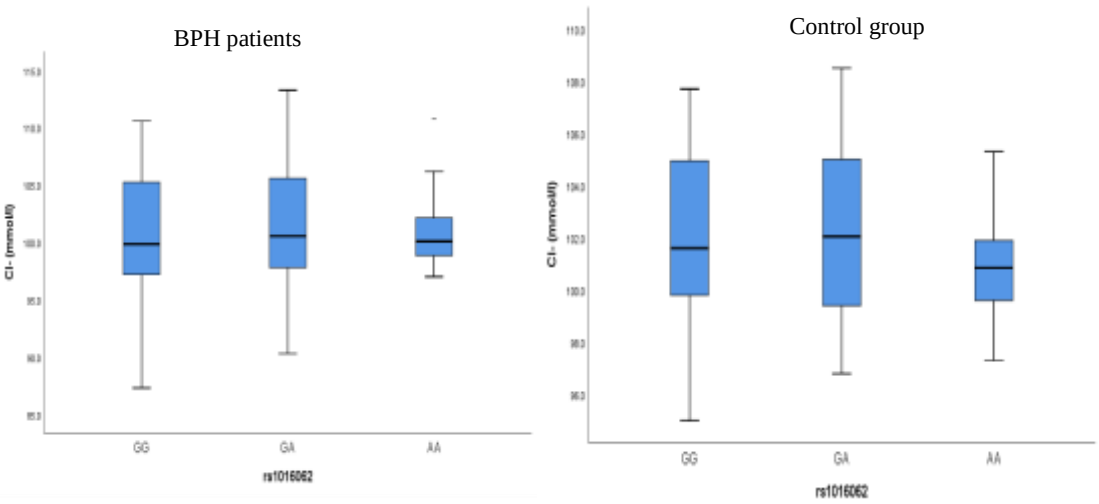

Figure 4.30: Serum Chloride levels across genotype carriers at SNP rs1016062 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles.

4.5.6.4 rs1016062 Genotypes and Bicarbonate Levels

Bicarbonate levels in BPH patients are shown in Figure 4.30. The serum bicarbonate levels were comparable across the different genotypes for SNP rs1016062 among BPH patients (p=0.645); GG carriers had the highest levels of Bicarbonate ions concentration in the serum compared to the GA carriers (median, 25.7; IQR, 11.6 mmol/l vs median 24.4, 12.0mmol/l p=0.112), AA carriers had the lowest concentration of bicarbonate ions compared to CT carriers (median 24.9; IQR 8.20 mmol/l vs median 24.4, 12.0mmol/l; p=0.514). Marked acidosis was observed among GA carriers (n=17, 8.76%), higher than in the GG carriers (n=15, 7.73%), and there was a lower observation for acidosis among the AA carriers (n=2, 1.04%). Marked Alkalosis was observed among GG carriers (n=17, 8.76%), higher than the observation made among the GA carriers (n=9, 4.64%); however, there was a low level of observation for Alkalosis among the AA carriers (n=2, 1.04%) as shown in table 4.11.

The bicarbonate serum level among the Control group is shown in Figure 4.30; the serum bicarbonate concentration was comparable among the different genotypes for SNP rs1016062 among the control group (p=0.250); the GG carriers showed the highest levels of bicarbonate concentration compared to GA (median 24.2; IQR; 8.90 mmol/l, vs median 24.7 IQR 8.50 mmol/l), AA carriers had a lower concentration of bicarbonate (median 24.2; IQR,4.10). GA carriers had a minimal observation for acidosis (n=4, 2.06%), and GG carriers had a single observation (n=1, 0.52%) for acidosis; however, there was no acidosis among the AA carriers, as shown in Table 4.12.

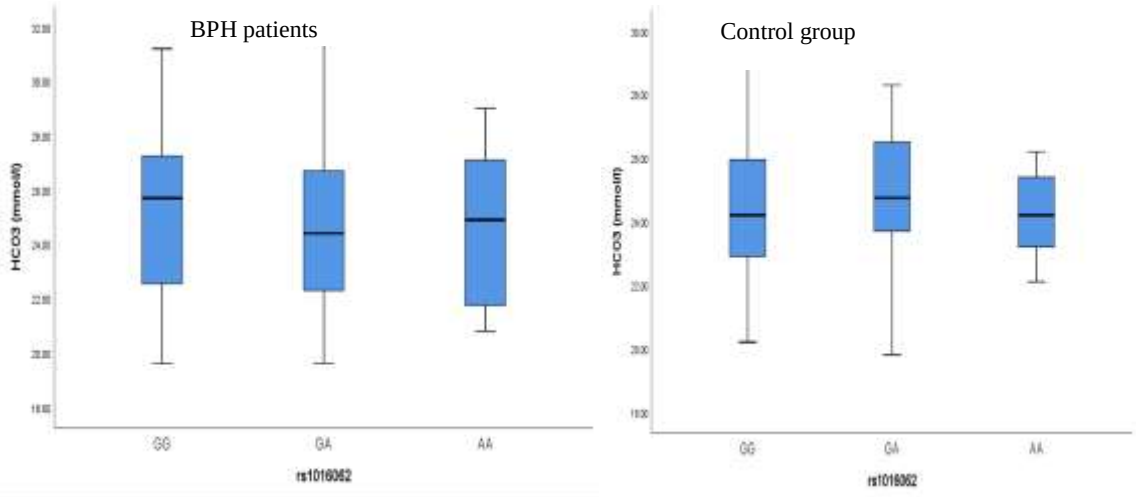

Figure 4.31:Serum Bicarbonate levels across genotype carriers at SNP rs1016062 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles.

4.5.6.5 rs1016062 Genotypes and Magnesium Levels

Serum Magnesium levels in BPH patients are shown in Figure 4.31. The serum magnesium levels were comparable across the different genotypes for SNP rs1016062 among BPH patients (p=0.010); GG carriers had the highest levels of magnesium ions concentration in the serum compared to the GA carriers, (median,0.60; IQR, 081 mmol/l vs. median 0.58, IQR 0.78 mmol/l p=0.026), AA carriers had the lowest

concentration of magnesium ions compared to GA carriers (median 0.68; IQR 0.62 mmol/l vs median 0.61, IQR 0.58 mmol/l p=0.152). Marked hypomagnesemia was observed among GA carriers (n=59, 34.02%) compared to GG carriers (n=59, 30.41%), low occurrence of hypomagnesemia was observed among the AA carriers (n=4, 2.06%) there was no observation made for hypermagnesemia among the AA carriers however low occurrence was seen among GG carriers (n=5, 2.52%), as shown in table 4.11.

The magnesium serum level among the Control group is shown in Figure 4.31; the serum magnesium concentration was comparable among the different genotypes for SNP rs1016062 of the control group (p=0.089); the GG carriers showed the highest levels of magnesium concentration compared to GA carriers (median 0.83; IQR; 1.50 mmol/l, vs median 0.84; IQR 1.69mmol/l), AA carriers showed the lowest levels of magnesium concentration (median 0.89; IQR, 0.59 mmol/l, p=0.232), there was an equal observation of the occurrence for hypomagnesemia across all the carriers (n=2,1.04%), Both GG and GA carriers had an equal observation for hypermagnesemia (n=2, 1.04%) however, AA carriers had no observation for hypermagnesemia as shown in table 4.12.

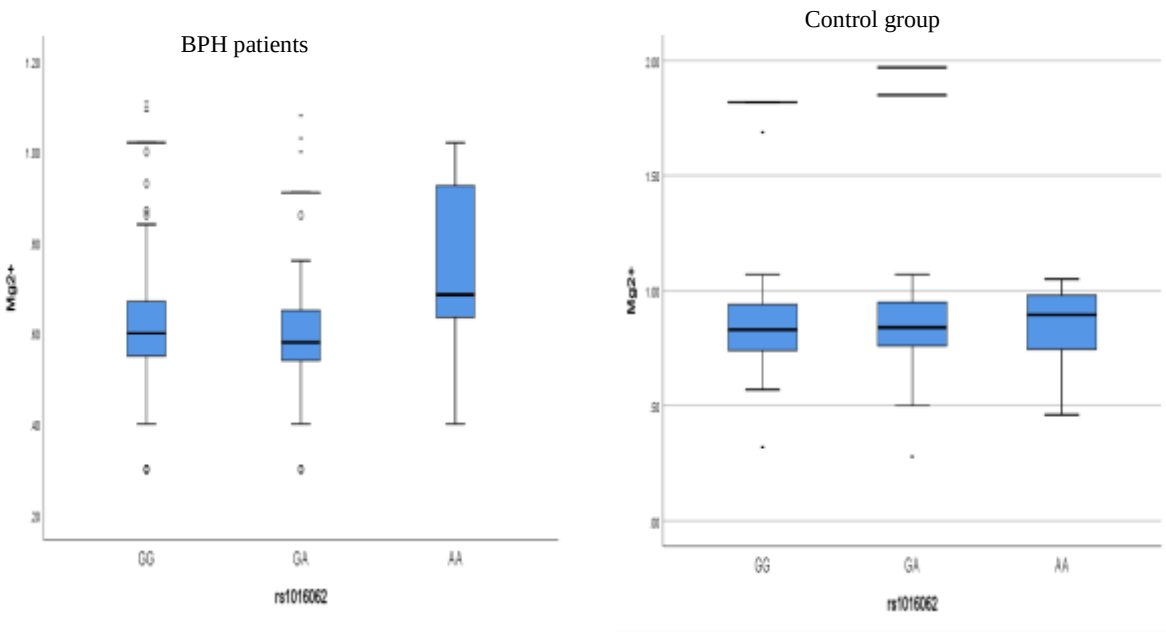

Figure 4.32: Serum Magnesium levels across genotype carriers at SNP rs1016062 among BPH patients.
The data are displayed using box plots, where the box illustrates the interquartile range, the line inside the box represents the median, and the whiskers show the 10th and 90th percentiles.

4.6 Haplotype frequencies

The haplotype distribution frequency is shown in Table 4.13. Significant associations were identified in the haplotype distribution of TRPM2, in which the combination of the rs168355 genotypes showed a notable distinction between the BPH patients and the control group ($p<0.0001$). The haplotypes GATT, CTT, GGCC, and CCA were seen less in BPH patients than in the control group.

Table 4.15: Distribution of haplotype frequency in BPH patients and controls

Gene	SNP Combination	Haplotype Distribution (Patients/Controls)	p- Value
TRPM2	rs 168355 (TT/TG/GG)	TT: 178/119, TG: 11/51, GG: 3/23	<0.001**
TRPM6	rs 4745363 (TT/TA/AA)	TT: 176/180, TA: 11/11, AA: 4/3	0.035**
TRPM7	Rs8042919, rs2362295	GGCC: 130/139, GCTT: 58/42, GATT: 5/11	0.445
TRPM8	Rs10490018, rs1016062	CCTT: 110/103, CTT: 52/75, CCA: 18/41	0.081

* Hardy Weinberg equilibrium was calculated using the genome studio genotyping module of the Illumina Infinium assay.
**statistically significant

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

5.1.1 Socio-demographic, Clinical Characteristics and laboratory profiles in BPH patients and controls participants

5.1.1.1 Age:

In this study, the average age of individuals with benign prostatic hyperplasia (BPH) was 65.47 years with a standard deviation of 12.55, ranging from 38 to 92 years. The healthy control group had an average age of 64.52 years with a standard deviation of 12.19, ranging from 39 to 91 years. Among patients with benign prostatic hyperplasia (BPH) in Iraq, the majority were over 66 years old, with the lowest occurrence in the age group of 45-55 years,(Radhi *et al*., 2023). According to (Lv *et al*., (2023), the median age of Chinese patients with benign prostatic hyperplasia (BPH) was 69 years, with an interquartile range (IQR) of 63 to 73 years. A Hospital-Based study conducted in Nigeria to determine the prevalence and distribution of post-void residual urine volume in men with symptomatic benign prostatic hyperplasia found that the average age of the participants was 63.70 ± 9.92 years, ranging from 42 to 88 years, (Anyimba *et al*., 2023). In Nigeria, recent data on open prostatectomy for benign prostatic hyperplasia regarding patient presentation and surgical outcomes indicated that the mean age of the 148 patients studied was 66.2 (±7.9) years, (Obi *et al*., 2023). Affusim *et al*., (2023), indicated that the average age of patients with Benign Prostatic Enlargement in Nigeria was 63.53 ± 9.84 years. In keeping with this study's findings regarding age distribution among BPH patients, (Lim, (2017), noted that the prevalence of BPH significantly increases with the advancing of age, (Munien *et al*., (2024), found that the frequency of histological BPH during autopsies is notably high,

ranging from 50% to 60% in males in their 60s, and increasing to 80% to 90% in those aged over 70 years. The current study findings on the mean age of BPH patients were consistent with previous studies pointing out that BPH-related indicators and risk of BPH progression were positively correlated with age after 61 years among male patients in Kisumu County. As the prostate gland enlarges with an increase in age, depending on other risk factors such as family history, lifestyle, diabetes and heart disease, it is inevitable that BPH will be more prevalent and pose a public health concern in health care settings in Kenya.

5.1.1.2 LUTS in BPH patients

The study revealed a significant variation in symptom severity among BPH patients, the majority exhibited severe LUTS n=123, (63.4%), with a smaller proportion showing moderate n=44, (22.7%) or mild symptoms n=27, (13.9%). The control participants did not exhibit such symptoms. This pattern aligns with findings from other regions, for instance a community survey in the United Kingdom evaluating the BPH symptoms among the community population 45% of participants showed moderate to severe LUTS, (Radhi *et al*., 2023). Similarly, a study in Indonesia reported a high prevalence of severe LUTS (54.8%), (Raharjo, 2016). consistent with the current study. Xiong *et al*., (2020) recorded that LUTS among BPH patients was 11.97%, In another study that aimed to test metabolic syndrome linkages to LUTS among Korean men established a 41.2% mild LUTS, 39.6% moderate LUTS and 19.2% LUTS among the BPH patients, (Park *et al*., 2019), these studies showed a lower percentage as compared to our study. The variation in LUTS severity across populations underscores the need for tailored approaches to diagnosis and management. LUTS in ageing men are usually attributed to bladder outlet obstruction from BPH and the resistance within the enlarged gland that increases the smooth

muscle tone, The findings also underscore the importance of early detection and management of LUTS, as its severity is a key indicator of BPH progression and can significantly impact the quality of life in ageing men.

5.1.1.3 PSA levels in BPH patients

In this study, it was found that the mean PSA levels were significantly higher among BPH patients, with a mean of 135.76 ± 578.03, compared to the control group, which had a mean of 2.01 ± 1.09. This is in contrast to another study in Nigeria, where the mean PSA level was found to be 76.59, (Udoh *et al*., 2020). A study conducted among men in Australia showed that the average PSA levels in patients with BPH were 109.02 (Ang *et al*., 2016). According to, (Abotsi *et al*., (2022), the mean PSA levels among the BPH patients in South Africa was 21.59 ± 3.78, a level which is quite lower compared to this study. In Iraq study on the PSA antigen ratio in BPH and Pca patients revealed a mean PSA level of 124.12 ± 21 which corroborated with this study, (Hussein *et al*., 2020). Inflammation, a crucial symptom of BPH, is widely recognized for its significant role in the development and pathogenesis of the condition. The growth of the prostate is affected by an imbalance between the growth of prostatic cells and their natural cell death, (Ng *et al*., 2024). Patients with inflamed prostate glands present with higher levels of PSA due to enhanced growth and multiplication of the stromal cells which leads to higher secretion of the PSA marker, (Nepal *et al*., 2023).

5.1.1.4 Education, residency and occupation

In this study education background, residency and occupation were comparable among the participants. Occupation distribution showed Variation with a higher percentage of salaried individuals in the control group and more farmers and businesses among the BPH patients. In a study conducted in Poland by Prajsner *et al*.,

(2015), it was found that BPH patients were more inclined to have been employed in white-collar jobs, in the same study it indicated that the majority of the patients had a formal education and lived in Rural areas. While another study in North Carolina USA indicated that a higher percentage of the BPH patients were farmers and resided in rural areas as compared to the control participants, (Kaplan *et al*., 2024). According to a study done by Bhat & Rather, (2021), that was looking into the risk of BPH concerning socio-economic status indicated that the majority of the patients were unemployed (31.65%) and 53% of them lived in rural areas. A different study among BPH patients in South Korea population showed that 6.1% of the participants were salaried and 21.6% were unemployed majority of the participants had formal education (Jo *et al*., 2021), Mohamed *et al*., (2018), in Egypt recorded that 12.5% of BPH patients were salaried while 52.5% were unemployed in addition most of the participants had informal education and lived in rural setup. The findings of these studies align with the results of this study, which showed no significant difference in education level or residential status among the participants. The rural-urban residence difference may be related to environmental or lifestyle factors influencing BPH development, (Pettaway *et al*., 2011), and the duration of the symptoms may suggest the progressive nature of BP, however regarding occupation the present study revealed that almost half of the participants were unemployed in disagreement with the aforementioned studies, the nature of occupation may not necessarily affect the severity of BPH and does not potentially increase the risk.

5.1.2 Association between TRPM2, TRPM6, TRPM7 & TRPM8 and BPH

The results indicated a significant link between TRPM2 (rs168355) and TRPM7 (rs2362295) polymorphisms and the risk of BPH. This aligns with a study by Zeng *et al*., (2010) that found TRPM2 channels expressed on prostate cell membranes. Their

research demonstrated that TRMP2 plays a distinctive role in promoting stromal cell proliferation in the prostate glands, highlighting the unique roles of TRPM2 and TRPM8 in this process., it showed a unique role of TRPM2 and TRPM8 in the facilitation of proliferation of the cells in the prostate glands. In another study by Di Donato *et al*., (2021), in characterizing and investigating the effect of TRPM modulators in the prostate, they demonstrated that androgen-stimulated prostate hyperplasia is inhibited by TRPM8 antagonists. In another study carried out by Ciaglia *et al*., (2023), they demonstrated higher expression of TRPM2 in BPH cell lines compared to the healthy prostatic epithelium cells they concluded the cells with higher expression had a higher chance of developing the ROS cascade responsible for the progression of BPH. The TRPM8 gene was identified as an indicator of androgen receptor transcriptional activity in prostatic cells and labelled as a biomarker for prostatic hyperplasia, (Chinigò *et al*., 2022). The androgen-androgen receptor signalling pathway plays a significant role in promoting cell growth in both stromal and epithelial cells, thereby contributing to the progression of BPH, (Izumi *et al*., 2013). In a study by Yang *et al*., (2020), TRPM7 was shown to function as a channel permeable to Mg^{2+} and Ca^{2+}, as well as an enzyme kinase that regulates cell adhesion and endurance. TRPM7 is heavily expressed in various tissues including the heart, adipose tissues and stromal cells, (Zhong *et al*., 2023). Excess expression of the TRPM7 in the cell leads to maintenance and acceleration of cell growth thus increasing the epithelial mesenchymal transition cells leading to BPH. TRPM6 was not found to be statistically significant among the BPH patients.

5.1.3 Levels of the variant of TRPM2, TRPM6, TRPM7 & TRPM8 in BPH patients and healthy individuals

In the TRPM2 polymorphism there was an increase in the TT genotype (92.7%) and the T allele frequency (95.6%) in BPH patients when compared to the control group of the healthy participants (TT, 61.6%, T, 74.9%) $p<0.001$). There was a lower frequency of GG genotypes observed in BPH patients (1.6%) compared to the control group (11.9%) $p<0.001$. The TT/TG/GG genotypes and T/G alleles exhibited a marked difference between patients and controls. There are limited studies on the polymorphic variants of TRPM in BPH patients, however, several studies have been carried out on other conditions, Jimenez *et al*., (2020), recorded no statistical significance in TRPM2 among patients with systemic sclerosis. A different study by Mahmuda *et al*., (2020) established that SNP rs1618355 in TRPM2 with C-T-A haplotypes were significantly associated with early onset of bipolar disorder. In a study evaluating the Mg^{2+} permeability of TRPM and the development of meningomyelocele Saraç *et al*., (2016), established that a decrease in AA and GG genotypes was not significant, however, the low expression of the GG genotype of rs3750425 compared to GA and AA genotypes were statistically significant in patients with hypomagnesemia. Shen *et al*., (2014), recorded an increased risk of GG genotype and G allele of rs7173321 and breast cancer among a population in China. In this study, TT variants in TRPM2 rs 168355 showed a higher occurrence compared to TG and GG variants, and CC variants in TRPM7 showed a higher occurrence compared to CT and TT variants. Thus, the study postulates that the TRPM2 and TRPM7 variants are associated with BPH and the symptoms associated with the condition. (Ali *et al*., 2023; Chen *et al*., 2013; Jiang, 2017). Chronic prostitis is one of the major symptoms of BPH and it has recently been discovered that it has the

capability of directly stimulating the proliferation of stromal and glandular cells within the prostate by generating reactive oxygen species (ROS). The consequential prostatic tissue damage and vascular injury resulting from ROS production in BPH contribute to a spectrum of effects beyond structural and functional impact on proteins, (Naiyila *et al*., 2023; Oseni *et al*., 2023; Pérez-Gómez *et al*., 2023).

TRPM7 CT and TT genotypes were linked to increased risk of severe LUTS providing further evidence of the involvement of the TRPM channel genes in BPH manifestation. TRPM7 enables magnesium and calcium ions binding, and kinase activity. Prostatic Tyrosine kinase enzyme has been evidenced in human hyperplastic prostate and prostatic carcinoma lines and is known to rely on the presence of the Magnesium ions (M^{g2+}), (Zou *et al*., 2019). The TRPM7-dependant Mg^{2+} influx is required for smooth muscle proliferation; therefore, it is believed to be involved in magnesium ion transport, cell-dependent matrix adhesion, and protein phosphorylation, (Sun *et al*., 2020). The gradual prostatic injury involves pertinacious cell damage and apoptosis resulting in intimal thickening, delamation and obliteration of the prostate blood vessels ultimately leading to tissue ischemia and severe clinical manifestation. Studies have shown that TRPM7 expression increases in androgen-independent prostate disease in comparison to normal prostate cells. Additionally, it is also known to play a role in the development of prostatic disease, (Chen *et al*., 2017; Yang *et al*., 2020).

The study investigated the frequencies of haplotypes in genes linked to transient receptor potential melastatin (TRPM) channels thus offering a distinct understanding of their potential roles in the genetic susceptibility to Benign Prostatic Hyperplasia (BPH). In the investigation into TRPM2 gene haplotypes, particularly focusing on the rs168355 genotypes, there was a notable variation in haplotype distribution between

BPH patients and the control group, with statistical significance ($p<0.001$). The less prevalent haplotypes GATT, CTT, GGCC, and CCA in BPH patients imply a potential protective role against the path progression and development of BPH. The TRPM2 gene encodes a cation channel involved in oxidative stress response and various physiological processes, making it a promising candidate for understanding genetic predisposition to BPH, (Sita *et al*., 2018). Further functional investigations into the specific roles of these haplotypes are warranted to outline their role in disease susceptibility.

The haplotype distributions in TRPM6 rs4745363 and TRPM7 rs8042919/rs2362295 did not show significant associations with BPH patients and controls. These genes, implicated in magnesium homeostasis (TRPM6) and cell proliferation (TRPM7), (Kampuang & Thongon, 2022; Luongo *et al*., 2018), may not be major contributors to BPH susceptibility based on the observed haplotype frequencies. However, the absence of significant associations does not preclude potential subtle effects or interactions with other genetic factors. Further investigations, possibly with larger sample sizes, are necessary to elucidate their role in BPH development and progression. While the haplotype distribution in TRPM8 rs10490018/rs1016062 did not demonstrate a statistically significant association with BPH, the observed trends warrant further investigation. TRPM8, associated with cold sensation, (Kasuga *et al*., 2022), and implicated in prostate cancer, may have subtle genetic variations that could play a role in BPH. Larger sample sizes and more in-depth analyses are necessary to determine the extent of TRPM8's involvement in BPH susceptibility.

Understanding how different combinations of genetic variants impact individual vulnerability to BPH emphasizes the significance of considering multiple single nucleotide polymorphisms (SNPs) collectively. The intricate interplay of genes and

their impact of environmental factors on these interactions are key considerations in unravelling the genetic basis of BPH, (Virolainen *et al*., 2023). The interplay of genetics and environment adds layers of complexity to disease susceptibility and necessitates a holistic approach to explaining the underlying mechanisms.

Prostate surface antigen (PSA) levels are a vital marker in evaluating the health of the prostate, (Filella & Foj, 2016), this study established an association between TRMP2 (rs168355) and TRMP7 (rs2362295) genotypes and PSA levels. The TRPM2 GG genotype was associated with reduced odds of severe levels of PSA while the TRPM7 CC genotype showed an increased odds ratio for severe PSA levels. This suggests a potential link between the TRPM gene variants and the severity of prostatic changes reflected in PSA secretions. It has been observed that overexpression of TRPM2 is induced by tissues undergoing remodelling, apoptosis and ROS. The High oxidative stress in the prostate may activate TRPM2 interactions with other cellular pathways leading to an influx of cations, this influx could contribute to cellular response including secretion of (PSA), (Maliougina & El Hiani, 2023).

5.1.4 Electrolytes levels and TRPM2, TRPM6, TRPM7 & TRPM8 expression in BPH patients and healthy individuals

The results in this study showed a statistical significance for TRPM2 rs168355 in all the electrolytes included in the study, TRPM6 rs4745363 for potassium ions, TRPM7 rs8042919 for all the electrolytes except sodium, and TRPM8 rs1016062 for magnesium ions. According to Du *et al.,* (2022), TRPM2 exhibited a permeability both to divalent ions especially $Mg^{2+,}$ in addition to monovalent ions including Na^{+} and K^{+}, In a study by (Liu *et al*., (2018) they demonstrated a linear current-voltage relationship permitting increased flow of Na^{+}, K^{+}, $Mg^{2+,}$ and $Zn^{2+.}$ A similar study by Souza et al., (2024) on proton pump inhibition among patients attending renal clinic

in Brazil attributed hypomagnesemia to reduced intracellular transportation via TRPM6 and TRPM7. Liamis *et al*., (2021), showed that reduced TRPM6 and TRPM7 expression in colon and DCT induces hypomagnesemia. Jimenez *et al*., (2020) on the contrary demonstrated that the permeability of Mg^{2+} in TRPM8 is dependent on temperature and partially actuated by voltage. In concurrence with the above studies, the current study showed that TRPM channels are significantly involved in electrolyte homoeostasis especially the magnesium and bicarbonate ions and their dysregulation could contribute to the risk of developing BPH, the hypothesis of their possible involvement arises from their expression in epithelial and vascular muscles, (Zholos *et al*., 2011). Hyponatremia and hypokalemia were identified as secondary electrolyte imbalances in our study. These imbalances could be linked to reduced reabsorption, potentially stemming from kidney injury or damage caused by urinary tract infections, (Puckett, 2023). Chronic kidney injury has also been associated with hypochloraemia, (Kee *et al*., 2020), which could result from excessive chloride loss in the urine due to tubular failure in the kidneys, (Ding *et al*., 2017). BPH heightens the risk of UTI by causing LUTS, which can lead to incomplete bladder emptying, creating an environment conducive to bacterial growth, (Madersbacher *et al*., 2019).

The study noted elevated levels of PSA secretion in BPH patients. PSA is produced by the stromal and epithelial cells of enlarged prostate glands, where it cleaves semenogelin I and II. When PSA enters the bloodstream, most of it forms complexes with alpha-1 anti-chymotrypsin, while some bind with alpha-2-macroglobulin, (Muazzam *et al*., 2021; Oto *et al*., 2020). The increase in PSA levels can be linked to the excessive growth of prostate tissues, along with constriction in the prostatic ducts. This condition leads to inflammation and trauma, resulting in the release of a higher amount of PSA, (Cai *et al*., 2019; Stewart & Lephart, 2023). The impaired clearance

of PSA from circulation has been suggested as a factor contributing to elevated PSA levels in BPH patients. This may be due to urine obstruction caused by inflammation of the prostatic tissues, (Cakir *et al*., 2018).

The current study revealed significant differences in electrolyte profiles between BPH patients and the control group. This electrolyte imbalance is believed to contribute to the progression of BPH, (Pourfridoni *et al*., 2021).

In BPH patients, low serum magnesium levels, known as hypomagnesemia, were the most common electrolyte abnormality found in this study. This finding aligns with a study by Asare *et al*., (2017), which also reported a high prevalence of hypomagnesemia among BPH patients at the Ghana Police Hospital. Magnesium ions play a role in various physiological, cellular, and molecular processes in the body, including cell proliferation and signalling, (Liu *et al*., 2019). Magnesium deficiency can occur due to various factors, including reduced intake, changes in reabsorption and excretion influenced by tissue alterations, and physiological changes caused by BPH, (Chubanov *et al*., 2018). Severe cases of BPH often lead to hydronephrosis, a condition where the kidneys swell due to urinary flow obstruction, which is common in BPH. The obstruction caused by enlarged prostate glands can result in backed-up urine reaching the kidneys, leading to inflammation. If not treated, this inflammation can damage renal tissues, affecting the kidney's ability to regulate electrolytes, (Iqbal *et al*., 2017).

5.2 Conclusion

1. The demographic characteristics of participants in this study revealed key patterns related to age, marital status, education, and occupation among men with benign prostate hyperplasia (BPH) in Kisumu County. The prevalence of BPH increased with age, particularly among men aged 60-79, who also had the highest PSA levels in the

severe range (>20 mmol/L), reinforcing the link between age and BPH. Other demographic factors such as marital status, education, and occupation were also notable. Married men, those with lower education levels, and individuals engaged in manual labour had a higher prevalence of BPH, suggesting that these factors may influence health-seeking behaviour, awareness, and access to healthcare. These findings underscore the need for targeted screening and public health strategies to address prostate health in older, less educated, and working-class men.

2. The study found a significant association between the TRPM2 (rs168355) and TRPM7 (rs2362295) polymorphisms and benign prostatic hyperplasia (BPH) in men from Kisumu County, Kenya. Furthermore, the TRPM2 GG genotype and TRPM7 CT and TT genotypes were linked to a higher risk of severe lower urinary tract symptoms demonstrating the involvement of TRPM channel genes in the manifestation of BPH.

3. Compared to the control participants in Kisumu County, Kenya, there was a statistically significant Genotype and allele distribution of possible haplotype in TRPM2 (TT/TG/GG), rs168355 (T/G) and TRPM7 (CC/CT/TT) among BPH.

4. Among the BPH patients in Kisumu County, there were statistically significant association between TRPM2 & sodium, potassium, chloride, bicarbonates and magnesium levels, TRPM6 & potassium levels, TRPM7 (rs8042919) and magnesium levels, TRPM7 (rs2362295) & potassium, chloride and magnesium levels, TRPM8 (rs10490018) & potassium levels, TRPM8 (RS1016062) and magnesium levels compared to control group that had statistically significant association between TRPM2 (RS168355) & potassium levels and TRPM7 (RS2362295) & potassium, bicarbonates and magnesium levels.

5.3 Recommendations

1. Targeted public health interventions be developed to address the increased risk of benign prostate hyperplasia (BPH) among men, particularly those aged 40 years and above in Kisumu County. Regular screening programs for prostate-specific antigen (PSA) levels should be prioritized for early detection, especially for men in higher-risk groups such as those with lower education levels, manual labour occupations, and limited access to healthcare. Educational campaigns to raise awareness of BPH symptoms and the importance of early medical intervention are also essential. Additionally, healthcare systems should aim to improve access to preventive care for all men, regardless of socio-economic status, to help mitigate the progression of BPH and its associated complications.

2. Early detection of genetic predispositions to institute prompt and personalized treatment plans and routine monitoring of electrolyte profile among men with Benign Prostate Hyperplasia in Kisumu County, Kenya.

3. Considering the potential for gene-based therapy targeting Genotype and allele distribution of possible haplotype in TRPM2 (TT/TG/GG), rs168355 (T/G) and TRPM7 (CC/CT/TT) among men with Benign Prostate Hyperplasia in Kisumu County, Kenya.

4. Develop and implement a Guideline that will offer valuable insights into the evidence-based management of Lower Urinary Tract Symptoms caused by Benign Prostatic Hyperplasia in male patients in Kisumu County and those that are targeting TRPM2 & sodium, potassium, chloride, bicarbonates, and magnesium levels, TRPM6 & potassium levels, TRPM7 (rs8042919) and magnesium levels, TRPM7 (rs2362295) & potassium, chloride and magnesium levels, TRPM8 (rs10490018) & potassium levels, TRPM8 (RS1016062) and magnesium levels.

5.3.1 Recommendations for future studies

The study suggests the use of computational biology and bioinformatics to analyse the protein-protein interaction network of differentially expressed genes and distinct subtypes of benign prostatic hyperplasia in Kisumu.

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APPENDICES

Appendix I: a) Informed Consent (English)

INFORMED CONSENT

TITLE OF THE STUDY: GENETIC POLYMORPHISM OF ION CHANNEL TRANSIENT RECEPTOR POTENTIAL MEMBRANE MELASTATIN AND RISK OF BENIGN PROSTATE HYPERPLASIA AMONG MEN IN KISUMU COUNTY.

ROLE	NAME	AFFILIATION
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	DR. RODGERS NORMAN DEMBA	MASENO UNIVERSITY

Study Location: Kisumu County

Funding Source: Principal Investigator; Royronald Ochieng Ongong'a**.**

Introduction and Purpose of The Study: You are being requested to volunteer for this research study and it is upon you to decide whether to participate or not. There are no penalties if you choose not to participate in or withdraw from the study. The purpose of this study is to determine the genetic polymorphism of the transient receptor potential membrane ion channels as a risk for BPH among men in Kisumu County. The information collected will allow scrutiny and understanding of the effect of polymorphism on the vulnerability to the disease and development of appropriate drugs, in addition completion of this study will allow the principal investigator, who is a PHD student at Kenyatta University complete his studies.

Duration Of the Study: This study will take approximately one year your part will take a three minutes verbal questionnaire about your demographics and two minutes for drawing the blood.

Subject participation: a) I estimate that 193 male participants who have been diagnosed of having BPH and are aged forty years and above will be enrolled in this study. Your clinical and demographic information will be collected including

abstracted medical records in addition blood will be drawn from your vein, the blood will be used to analyze for PSA, Mg^{2+}, Na^{+} and Ca^{2+}.

b) For control participants: Your donor and demographic information will be collected in addition 3mls of blood will be taken from your donated pint. The blood will be used to analyze for PSA, $Mg^{2+,}$ $Na^{+.}$

Potential Risk and Discomfort: During this study you will be asked questions or your clinical information may be accessed. There are minimal risks associated with this study. The possible risk of participating in this study is that you will feel pain during the time of drawing the blood. To protect your information from people outside this study, all the information will be coded with a unique number rather than using your name.

Potential Benefit: There will be no payment for participating on the study however, Individuals who participate in the study may have a better understanding of the genetic polymorphism of ion channel potential membrane and risk of developing BPH.

Confidentiality: All the information collected during the study will be coded to protect the participant's name. No name or any other identifiers will be used when discussing or reporting the data. Furthermore, if we publish this study, we will not use your name or identify you personally. The principal investigator will safely keep all files and data collected in a locked cabinet and password protected documents in the computer. Once the data has been fully analyzed they will be destroyed.

Voluntary participation and Authorization: Your participation in this study is entirely voluntary. If you choose not to take part in this study, it will have no effect on the care service, or benefits to which you are entitled.

Withdrawal from the study and/or withdrawal of Authorization: If you decide to participate in this study, you may withdraw from your participation at any time without penalty.

Authorization: By signing this form, I authorize the use of my records, any observations and findings found during the course of this study for education, publication and/or presentation.

In case you have a question or problem: If you have any question or concern about this study, or in event of a study related injury you can contact Royronald Ochieng Ongong'a at **0721314008** OR **rongonga@yahoo.com**

I voluntarily agree to take part in this study.

☐ **YES**
☐ **NO**

SIGNATURE OF PARTICIPANT:

Printed Name of the Participant

_____

_____

Signature: _____ Date: _____

SIGNATURE OF WITNESS TO CONSENT (THIS MAY BE A RELATIVE OF THE PARTICIPANT BUT NOT AN INDIVIDUAL INVOLVED IN THE RESEARCH STUDY)

_____

_____

Signature: _____ Date: _____

SIGNATURE OF THE INDIVIDUAL ADMINISTERING CONSENT: (CAN ONLY BE SIGNED BY THE PI OR STAFF APPROVED TO ADMINISTER CONSENT

Printed Name of Administering Individual:

_____

_____

Signature: _____ Date: _____

Appendix I b). Informed Consent (Kiswahili)

INFORMED CONSENT (Kiswahili)

TITLE OF THE STUDY: GENETIC POLYMORPHISM OF ION CHANNEL TRANSIENT RECEPTOR POTENTIAL MEMBRANE MELASTATIN AND RISK OF BENIGN PROSTATE HYPERPLASIA AMONG MEN IN KISUMU COUNTY.

JUKUMU	JINA	USHIRIKA
MPELELEZI MKUU	ROYRONALD OCHIENG ONGONG'A	KENYATTA UNIVERSITY TEL: 0721314008 E-Mail: rongonga@yahoo.com
WASIMAMIZI	DR. NELSON MENZA	KENYATTA UNIVERSITY
	DR. RODGERS NORMAN DEMBA	MASENO UNIVERSITY

Pahali Pa Utafiti: Kata Ya Kisumu

Chanzo Cha Fedha : Mpelelezi Mkuu;Royronald Ochieng' Ongong'a

Utangulizi na Madhumuni ya Utafiti: Unaombwa kujitolea kwa ajili ya utafiti huu na ni juu yako kuamua kama utashiriki au la. Hakuna adhabu ikiwa utachagua kutoshiriki au kujiondoa kwenye utafiti. Madhumuni ya utafiti huu ni kubainisha upolimishaji wa kijeni wa chaneli za ioni za utando wa vipokezi vya muda kama hatari ya BPH miongoni mwa wanaume katika Kaunti ya Kisumu. Taarifa iliyokusanywa itaruhusu uchunguzi na uelewa wa athari za polymorphism kwenye hatari ya ugonjwa huo na maendeleo ya dawa zinazofaa, pamoja na kukamilika kwa utafiti huu kutamruhusu mchunguzi mkuu, ambaye ni mwanafunzi wa PHD katika Chuo Kikuu cha Kenyatta kukamilisha masomo yake.

Muda wa Utafiti: Utafiti huu utachukua takriban mwaka mmoja sehemu yako itachukua dodoso la maneno la dakika tatu kuhusu demografia yako na dakika mbili za kuchora damu.

Ushiriki wa somo: a) Ninakadiria kuwa washiriki wanaume 193 ambao wamegunduliwa kuwa na BPH na wana umri wa miaka arobaini na kuendelea

wataandikishwa katika utafiti huu. Taarifa zako za kiafya na kidemografia zitakusanywa ikijumuisha rekodi za matibabu zilizotolewa pamoja na damu itatolewa kutoka kwenye mshipa wako, damu itatumika kuchanganua PSA, Mg2+, Na+ na Ca2+.

b) **Kwa washiriki wa udhibiti:** Taarifa za mfadhili wako na demografia zitakusanywa pamoja na 3mls za damu zitachukuliwa kutoka kwa panti yako uliyochanga. Damu itatumika kuchanganua kwa PSA, Mg2+, Na+.

Hatari Inayowezekana na Usumbufu: Wakati wa utafiti huu utaulizwa maswali au maelezo yako ya kliniki yanaweza kufikiwa. Kuna hatari ndogo zinazohusiana na utafiti huu. Hatari inayowezekana ya kushiriki katika utafiti huu ni kwamba utasikia maumivu wakati wa kuchora damu. Ili kulinda taarifa zako kutoka kwa watu walio nje ya utafiti huu, taarifa zote zitawekwa nambari maalum badala ya kutumia jina lako.

Manufaa Yanayowezekana: Hakutakuwa na malipo ya kushiriki kwenye utafiti hata hivyo, Watu Binafsi wanaoshiriki katika utafiti wanaweza kuwa na uelewa bora wa upolimishaji kijeni wa utando unaowezekana wa ioni na hatari ya kupata BPH.

Usiri: Taarifa zote zilizokusanywa wakati wa utafiti zitawekwa geresho ili kulinda jina la mshiriki. Hakuna jina au vitambulishi vingine vyovyote vitatumika wakati wa kujadili au kuripoti data. Zaidi ya hayo, ikiwa tutachapisha utafiti huu, hatutatumia jina lako au kukutambulisha kibinafsi. Mpelelezi mkuu ataweka kwa usalama faili na data zote zilizokusanywa kwenye kabati iliyofungwa na hati zilizolindwa na nenosiri kwenye kompyuta. Mara data imechanganuliwa kikamilifu itaharibiwa.

Ushiriki wa Hiari na Uidhinishaji: Kushiriki kwako katika utafiti huu ni kwa hiari kabisa. Ukichagua kutoshiriki katika utafiti huu, hautakuwa na athari kwa huduma ya matunzo, au manufaa ambayo unastahiki.

Kujiondoa kwenye utafiti na/au kuondolewa kwa Uidhinishaji: Ukiamua kushiriki katika utafiti huu, unaweza kujiondoa katika ushiriki wako wakati wowote bila adhabu.

Uidhinishaji: Kwa kusaini fomu hii, ninaidhinisha matumizi ya rekodi zangu, uchunguzi na matokeo yoyote yaliyopatikana wakati wa utafiti huu kwa elimu, uchapishaji na/au uwasilishaji.

Ikiwa una swali au tatizo: Ikiwa una swali au wasiwasi wowote kuhusu utafiti huu, au ikitokea jeraha linalohusiana na utafiti unaweza kuwasiliana na **Royronald Ochieng Ongong'a** kwa **0721314008** AU rongonga@yahoo.com

Ninakubali kwa hiari kushiriki katika utafiti huu.

☐ **NDIO**
☐ **LA**

SAINI YA MSHIRIKI:

Jina Lililochapishwa la Mshiriki: _____
_____

Sahihi:
_____Tarehe:_____

SAINI YA SHAHIDI KURIDHIA (HUYU ANAWEZA KUWA NI JAMAA WA MSHIRIKI LAKINI SI MTU ALIYEHUSIKA KATIKA UTAFITI HUU)

Jina lililochapishwa la shahidi:
_____
_____

Sahihi: _____Tarehe _____

SAINI YA RIDHAA YA USIMAMIZI WA MTU BINAFSI: (INAWEZA KUTIWA SAINI TU NA PI AU WAFANYAKAZI WALIOIDHINISHWA ILI RIDHAA YA USIMAMIZI.

_____
_____

Sahihi: _____ Tarehe: _____

Appendix I c). Informed Consent (Dholuo)

INFORMED CONSENT

TITLE OF THE STUDY: GENETIC POLYMORPHISM OF ION CHANNEL TRANSIENT RECEPTOR POTENTIAL MEMBRANE MELASTATIN AND RISK OF BENIGN PROSTATE HYPERPLASIA AMONG MEN IN KISUMU COUNTY.

MIGAO	NYING	USHIRIKA
JANONRO MADUO'NG	ROYRONALD OCHIENG ONGONG'A	KENYATTA UNIVERSITY TEL: 0721314008 E-Mail: rongonga@yahoo.com
JOCHU'NG	DR. NELSON MENZA	KENYATTA UNIVERSITY
	DR. RODGERS NORMAN DEMBA	MASENO UNIVERSITY

Kama Itime Nonro: kaunti ma Kisumo

Migao Mochiwo Omenda Mar Timo Nonro Ni: Janonro Maduo'ng; RoyRonald Ochie'ng

Weche Motelo Kod Tiend Timo Nonro: Ikwayi mondo ichiwri e nononrni. En yiero mari koda idhi yiero chiwori kata ibiro dagi. Onge kum kendo okibilalo gimoramora ka ing'ado ni okidonj e nonoro kata ka bang'donjo e nonro,I yiero mar weyo. Dwaro mar timo nonroni en mar yango lokruok mag ngi'e ngi'e mag del kod tuo mar duong mar dichuo e kind chuo manie kaunti ma Kisumo. Weche ma ochoki kokalo kuom nyanonroni biro chiwo thuolo mar yango kod ng'eyo chandruok ma lokruok mag ngi'e ngi'e mag del kod tuo mar duong mar dichuo kod dongruok mar yudo yedhe mowinjore,e wi mano giko mar nyanonroni biro konyo janonro maduo'ng ma en japuonjre e mbalariany ma Kenyatta tieko sombe mar PhD.

Thuolo Timo Nonro: Nonroni biro kawo thuolo ma dirom higa achiel, ibiro bedo gi thuolo mar dakika adek mar duoko penjo kuom ngeyo chal mar dakmari kod dakika ariyo mar golo remo.

Jochiwre Mochiknegi Klinik: a) Nonroni biro kawo ji 193 ma dichuo ma oseyud kod tuo mar kar nyuol kendo gin kod higni piero ang'wen kadhimalo.Wecheni duto mag clinic kod wecheni mogul e findi ibiro kaw, remo matin mar pimo nyiriri mag del.

Jochiwre Ma Ok Ochiknegi Klinik: b) Wechegi duto mane indiko kaidhi golo remo ibiro kaw kendo remo matin maichiwo ibiro kaw mondo opim godo nyiriri mag del.

Richo Kod Thagruok Manyalo Bedo: E kinde mar nonoroni ibiropenji penjo kuom tuo mari kata findi mar klinik inyalo som .Nitirie rach matin manyolo bedo kaluore kod nonro ni .Rach manyalo bedoe ka I riwpori e nonro ni en win lit matin sama I goloe remo kuomi.Mondo warit wechegi kik jomanioko mar nonroni ong'e ,en ni wechemokaw kuomi ibiro mi namba ma onge kato manyalo ngeyo kendo nyingi okbitigo e nonroni.

Ber Minyalo Yudo: Onge chudo moramora ma ibiro miyi kuom riwori e somoni, kata kamano jogo moriwore e somoni biro bedo kod lony makende e ngeyo kaka lokruok mar ngie ngie mag delo kelo bedo kod tuo mar kuot mar dak mar gik nyuol e chou.

Rito Weche Maling Ling: Wecheduto ma ibiro choki e nonro ni ibiro mi number maonge ngama nyalo ng'eyo kendo nying jogo mochiwore ok bitigo. Janonro madison biro keto weche meki malingling e findi ma iloronegi kuma Opondo kendo ibiro ketgi e computer man kod number mawendo.Finde go ibiro wang bandg ka nonro ni oserumo. Kaponi weche mabiro yudore bang nonro ni ibiro go chapa okbiti kod nyingi kata gino manyalo miyo ng'ato difweny ni ne en in.

Yie Maonge Achune: Mondo idonje e nonroni en in iwuon ema itimo yiero maokonyuni godo. En yiero marikoda idhi yiero chiwori kata ibiro dagi.Onge kum

kendo ok ibilalo gimoro amora king'ado ni ok idonji e nonro kata ka bang donjo e nonoro iyiero weyo.

Wuok Kuom Nonro: Kaponi iyiero mar wuok e nonro ni ,inyalo wuok saaya saaya maonge kum mora mora.

Yie Mondo Adonje Nonroni: Ketona seyii e formni ,Ayie mondo wechega manie finda mar klinik, kod duoko moro a mora madyudre e kinde mar nonroni kata e goyo chapa mar duoko mar nonroni

Ka In Gi Penjo Kata Chandruok Moro Amoro: Ka poni in kod chandruok kata penjo e wi nonroni ,kata kaponi iyudo hinyrok motenore kod nonroni ,inyalo tudori kod Royronald Ochieng Ongong'a kokalo kuom namba mar sime 0721314008 kata address mar mbui **rongonga@yahoo.com**.

Ayie mondo adonje e nonroni .

☐ **Ayie**
☐ **Adagi**

SEYI MAR JACHIWRE:

Nying' jachiwre e andika maduongo.

_____

_____

Seyi: _____ Tarik: _____

SEYI MAR JAJACHUNG'NE JACHIWRE (JALNI NYALO BEDO WAT JACHIWRE TO OKNYAL BEDO JALNO MATIYONE NONRO)

_____

_____

Seyi: _____ Tarik: _____

SEYI MAR JACHIW AYIE: (KAE INYAKLO MANA KET SEYI KOD JATEND NONORO KATA JATIJ NONRO MOYIENE CHIWO AYIE)

_____

Seyi: _____ Tarik: _____

APPENDIX II: KENYATTA UNIVERSITY GRADUATE SCHOOL APPROVAL

KENYATTA UNIVERSITY
GRADUATE SCHOOL

E-mail: kubps@yahoo.com
dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 8710901 Ext. 57530

Our Ref: P97/28771/19 Date: 31st August, 2021

The Director General,
National Commission for Science, Technology & Innovation,
P.O. Box 30623-00100,
NAIROBI

Dear Sir/Madam,

RE: RESEARCH AUTHORIZATION FOR MR.OCHIENG R. ONGONG'O - REG. NO. P97/28771/19

I write to introduce Mr. Ongong'a who is a Postgraduate Student of this University. He is registered for a Ph.D. degree programme in the **Department of Medical Laboratory Sciences in the School of Medicine.**

Mr. Ongong'a intends to conduct research for Ph.D. thesis entitled, **"Genetic Polymorphism of Ion Channel Transient Receptor Potential Membrane Melastatin and Risk of Benign Prostate Hyperplasia among Men in Kisumu County".**

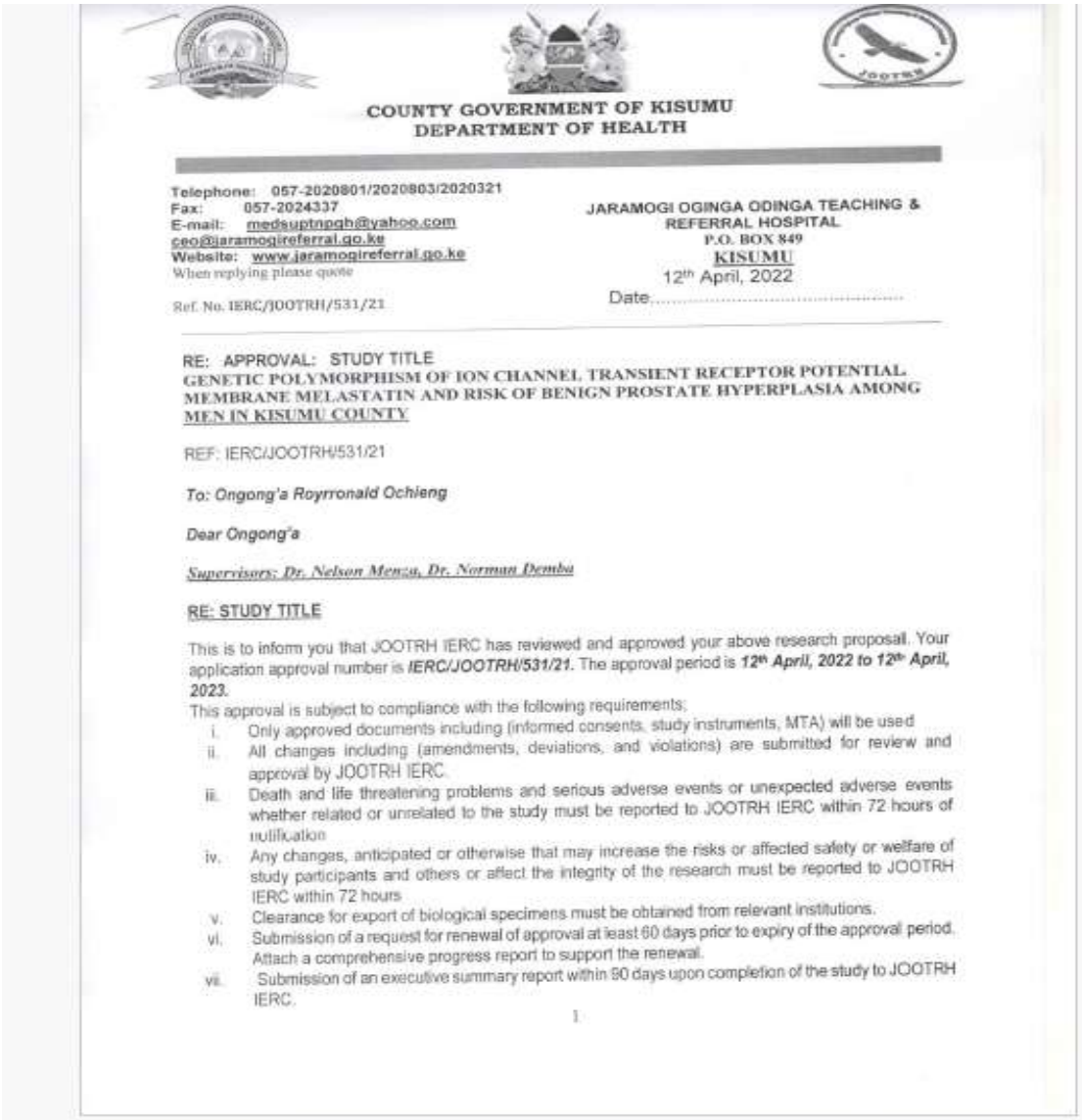
Any assistance given will be highly appreciated.

Yours faithfully,

PROF. ELISHIBA KIMANI
DEAN, GRADUATE SCHOOL

RM/cao

APPENDIX III: JARAMOGI OGINGA ODINGA TEACHING AND REFERRAL HOSPITAL-ERC APPROVAL



COUNTY GOVERNMENT OF KISUMU
DEPARTMENT OF HEALTH

Telephone: 057-2020801/2020803/2020321
Fax: 057-2024337
E-mail: medsuptnpgh@yahoo.com
ceo@jaramogireferral.go.ke
Website: www.jaramogireferral.go.ke
When replying please quote

JARAMOGI OGINGA ODINGA TEACHING & REFERRAL HOSPITAL
P.O. BOX 849
KISUMU

Ref. No. IERC/JOOTRH/531/21

Date 12th April, 2022

RE: APPROVAL: STUDY TITLE
GENETIC POLYMORPHISM OF ION CHANNEL TRANSIENT RECEPTOR POTENTIAL MEMBRANE MELASTATIN AND RISK OF BENIGN PROSTATE HYPERPLASIA AMONG MEN IN KISUMU COUNTY

REF: IERC/JOOTRH/531/21

To: Ongong'a Royrronald Ochieng

Dear Ongong'a

Supervisors: Dr. Nelson Menza, Dr. Norman Demba

RE: STUDY TITLE

This is to inform you that JOOTRH IERC has reviewed and approved your above research proposal. Your application approval number is ***IERC/JOOTRH/531/21.*** The approval period is ***12th April, 2022 to 12th April, 2023.***

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by JOOTRH IERC.
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 JOOTRH IERC 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 JOOTRH IERC within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
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 JOOTRH IERC.

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viii. In case the study site is JOOTRH, kindly report to Chief Executive Officer before commencement of data collection.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.keand also obtain other clearances needed.

Yours sincerely,

ANTONY AYORA
SECRETARY – ISERC
JOOTRH - KISUMU

JOOTRH ETHICS & REVIEW COMMITTEE
P. O. Box 849 - 40100
KISUMU

APPENDIX IV: RESEARCH PERMIT FROM NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 697051

Date of Issue: 22/April/2022

RESEARCH LICENSE

This is to Certify that Mr.. Royronald Ochieng Ongong'a of Kenyatta University, has been licensed to conduct research in Kisumu on the topic: GENETIC POLYMORPHISM OF ION CHANNEL TRANSIENT RECEPTOR POTENTIAL MEMBRANE MELASTATIN AND RISK OF BENIGN PROSTATE HYPERPLASIA AMONG MEN IN KISUMU COUNTY for the period ending : 22/April/2023.

License No: NACOSTI/P/22/17031

697051

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

APPENDIX V: STUDY SITE