ASSOCIATION OF XRCC1 AND XPD POLYMORPHISM WITH RISK OF PROSTATE CANCER IN BANGLADESH POPULATION

By

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
B.Sc. in Biotechnology

Mathematics and Natural Sciences
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Declaration

It is hereby declared that

1. The thesis submitted is my/our own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I/We have acknowledged all main sources of help.

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Approval

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of Spring, 2023 has been accepted as satisfactory in partial fulfillment of the requirement for the degree of B.Sc. in Biotechnology on February 9th, 2023.

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Ethics Statement

The study was approved by the Ethical Review Committees of the Department of Biochemistry and Molecular Biology, University of Dhaka.

Abstract

This study examined the effects of the X-ray cross-complementing gene 1 (XRCC1) Arg194Trp and Xeroderma pigmentosum group D (XPD) Lys751Gln polymorphisms on the risk, severity, and clinical parameter of prostate cancer in Bangladeshi males. The blood samples and data of 132 prostate cancer patients and 135 healthy controls were obtained. PCR-RFLP was used to conduct a genotype analysis. Compared to the Arg/Arg genotype of XRCC1, the homozygous mutant Trp/Trp genotype was associated with prostate cancer with an Odd Ratio (OR) of 7.50 (95% CI, 1.227-85.12; p=0.04). In comparison to Arg/Arg + Arg/Trp genotypes, the Trp/Trp genotype increased risk by 7.5 folds (OR=7.50; 95% CI=1.23-85.12; p=0.04). XPD variants did not increase prostate cancer risk. Among prostate cancer patients, the XRCC1 Trp/Trp variant was associated with hematuria risk, higher mean serum creatinine, and mean prostate-specific antigen (PSA) levels. Only higher mean serum creatinine levels were associated with the XPD Gln/Gln variant. Tumor grade, tumor aggressiveness, prostate abnormalities, or UTIs were not affected by either XRCC1 or XPD variations (p>0.05).

Keywords: DNA repair genes; Polymorphism; XRCC1; XPD; Prostate Cancer, Biomarker

Dedication

Dedicated to the advancement of cancer research and treatment

Acknowledgement

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List of Acronyms

HDI	Human Development Index
SNPs	Single Nucleotide Polymorphisms
DRE	Digital Rectal Examination
PSA	Prostate-specific Antigen
MRI	Magnetic Resonance Imaging
PSMA PET	Prostate-specific Membrane Antigen positron emission tomography
TRUS	Transrectal Ultrasound
SSB	Single-Stranded Break
DSB	Double-Stranded Break
MMR	Mismatch repair
BER	Base-Excision Repair
NER	Nucleotide-Excision Repair
AP-site	Apurinic/Apyrimidinic site
SSR	Simple Sequence Repeats
GGR	Global Genomic NER
TCR	Tanscription Coupled NER
CSA	Cockayne Syndrome complementation group A
CSB	Cockayne syndrome complementation group B
BRCT	BRCA1 C-terminus
PCR	Polymerase Chain Reaction
RFLP	Restriction Fragment Length Polymorphism
SCE	Sister Chromatid Exchange

Chapter 1

Introduction

1.1 Prostate Gland and Pathophysiology of Prostate Cancer

The prostate is a small gland located immediately inferior to the bladder and anterior to the rectum, surrounding the top portion of the urethra (Drake et al., 2019). It secretes a fluid that is 20-30% of the total volume of the seminal fluid into the urethra and ejaculatory ducts.

Prostate cancer develops when normal prostate gland cells evolve from the benign to the malignant phenotype (Mustafa et al., 2016) (Figure 1). Initially, these neoplastic epithelial cells remain confined to normal prostate glands, a condition referred to as carcinoma in situ or prostate intraepithelial neoplasia (PIN) (Nelson & Matzo, 2003). These cancer cells then proliferate and spread to the surrounding prostate tissue (the stroma) over time, developing into tumors (Mustafa et al., 2016). Eventually, the cancer cells may invade nearby organs or metastasize. Due to its position, prostate disease typically impairs urination, ejaculation, and sometimes defecation (Mustafa et al., 2016) (Figure 2).

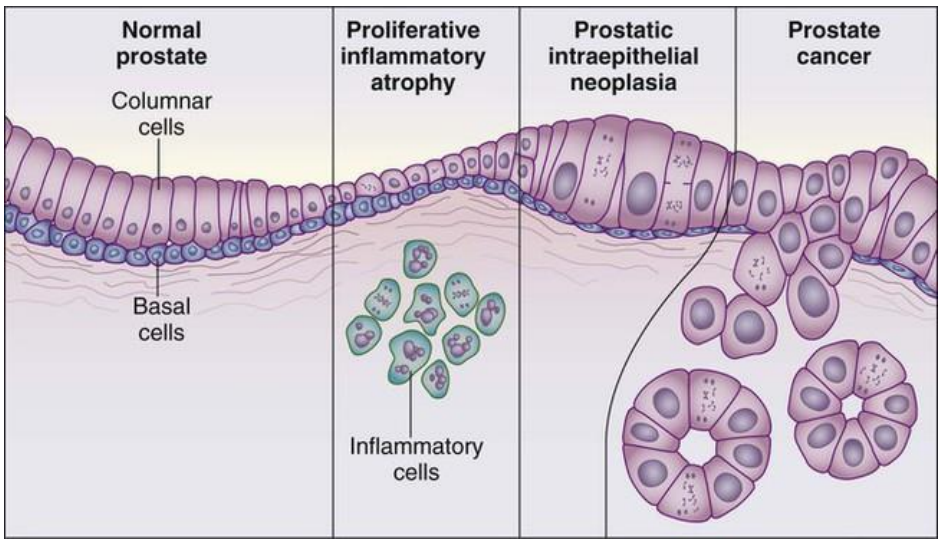

Figure 1: Pathophysiology of prostate Cancer

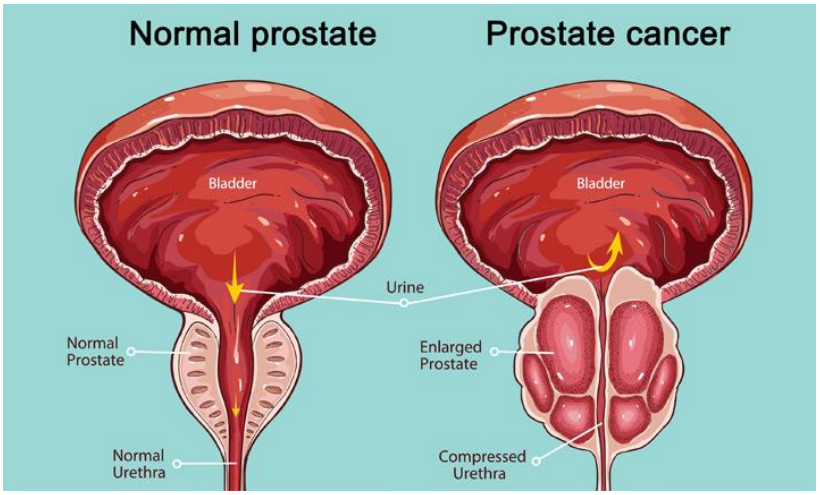

Figure 2: Normal prostate and prostate cancer

1.2 Epidemiology of Prostate Cancer

Prostate cancer is the second most common carcinoma and the fifth largest cause of cancer deaths in men, with 1.4 million new cases and 375,000 deaths worldwide (Sung et al., 2021). Race, ethnicity, and geography influence prostate cancer rates (Figure 3). Developed nations such as Australia, North America, and Western and Northern Europe, and Australia have the highest prostate cancer incidence (Rebbeck, 2017; Taitt, 2018). In terms of race and ethnicity, Afro-Caribbean and Sub-Saharan African men have the greatest mortality rates, with 18.7 to 29.3 fatalities per 100,000 (GLOBOCAN, 2012) and 43 per 100,000 in the years 2008–2011, respectively. The rates are significantly lower among Caucasians, Hispanics, and Asian/Pacific Islanders (Rebbeck, 2017). In contrast, prostate cancer mortality is the lowest among South Central, East, and South-east Asian males (2.9, 3.1, and 6.7 per 100,000, respectively).

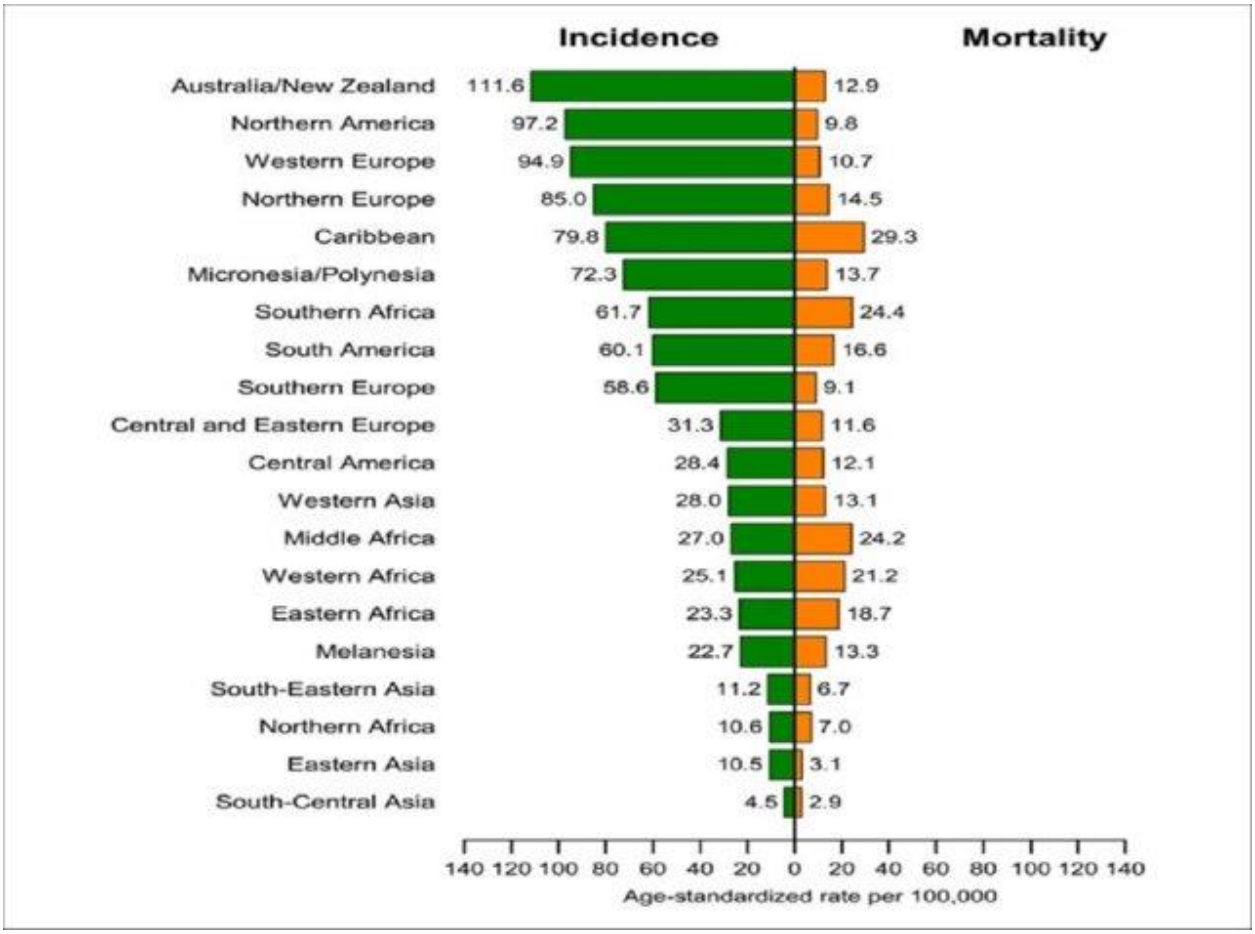

Figure 3: Prostate cancer incidence and mortality rates by geographical area

1.3 Epidemiology of Prostate Cancer in Bangladesh

Although the incidence of prostate cancer in developing countries is much lower than that in developed countries, survival rates in developing countries are low (Center et al., 2012). Presently, developing countries account for a significant proportion of prostate cancer incidence and mortality globally (Sharma, 2019). According to the Global Cancer Statistics 2020, prostate cancer ranked second in countries with higher Human Development Index (HDI) following lung cancer, and first in countries with lower HDI countries among men (Sung et al., 2021). The prostate cancer burden has increased significantly in lower HDI countries due to screening techniques. In Bangladesh, out of an estimated 1.3–1.5 million prostate cancer patients, only 0.2 million patients have been diagnosed annually due to a lack of symptoms, awareness, and screening techniques (Imtiaz et al., 2019). In 2018, the prostate cancer-associated mortality rate grew to 1.5%, with 773 deaths in Bangladesh (Rahman et al., 2022). Many factors could contribute to this increased incidence, including Bangladesh's relatively high life expectancy, the Bangladeshi population's increased life expectancy, health awareness,

and participation in health check programs (Salam, 2014). Additionally, the majority of deaths occur due to late diagnosis (Imtiaz et al., 2019).

1.4 Prostate Cancer Diagnosis

DRE: The existence of prostate cancer may be indicated by an enlarged prostate, suspicious nodule, hard nodular prostate, lobar asymmetry, indurations, and the obliteration of the median groove on digital rectal examination (DRE) (Irekpita et al., 2020).

PSA: Prostate-specific antigen (PSA) test measures the level of PSA in blood. Since high PSA levels are mostly associated with an increased prostate cancer risk, it is used as a serological marker of prostate carcinoma detection.

Imaging: When systematic prostate biopsy has failed to detect prostate cancer, imaging tools such as Magnetic Resonance Imaging (MRI) are valuable tools to detect prostate cancers in males with persistently elevated prostate-specific antigen (PSA).

Biopsy: For a prostate biopsy, small tissue samples are extracted from the prostate and examined under a microscope. Transrectal ultrasound (TRUS) guided biopsy has been the gold standard for confirming a prostate cancer diagnosis.

1.5 Prostate Cancer Staging and Grading

TNM system is the most often used staging system for prostate cancer (Prostate Cancer Stages, n.d.):

- (T-Tumor category) The extent of the primary tumor, size, location, and how far it has grown into the tissue.
- (N-Node category) Whether cancer has spread to neighboring lymph nodes
- (M-metastasis category) Whether cancer has metastasized to distant organs or tissue.

The stages of prostate cancer range from I to IV. Once the T, N, and M categories are established, the numbers following the letters describe the extent of that category; an earlier letter indicates a lower stage (Figure 4).

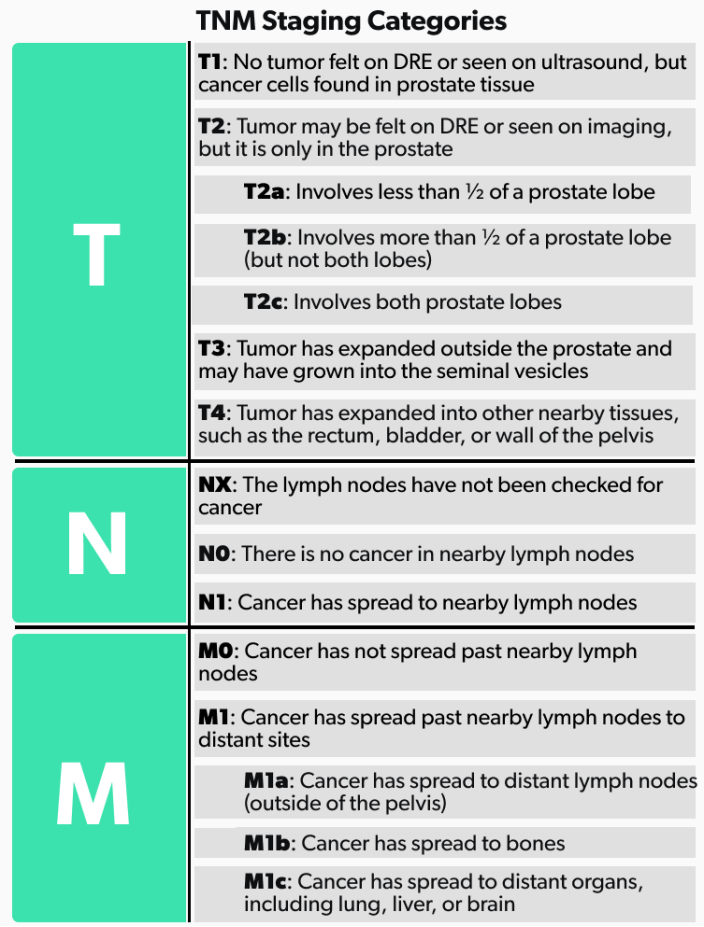

Figure 4: Stages of prostate cancer using the tumor, nodes, metastases (TNM) classification

Gleason score

The Gleason Score grading system describes how much prostate cancer cells from a biopsy resemble healthy tissue (lower score) or malignant tissue (higher score) (Gleason Score and Grade Group, 1982) (Figure 5).

Risk Group*	Grade Group	Gleason Score
Low/Very Low	Grade Group 1	Gleason Score ≤ 6
Intermediate (Favorable/Unfavorable)	Grade Group 2	Gleason Score 7 (3 + 4)
	Grade Group 3	Gleason Score 7 (4 + 3)
High/Very High	Grade Group 4	Gleason Score 8
	Grade Group 5	Gleason Score 9-10

Figure 5: Risk Groups determined by tumor grade and other measures, including PSA, and tumor stage

1.6 DNA Repair Pathways

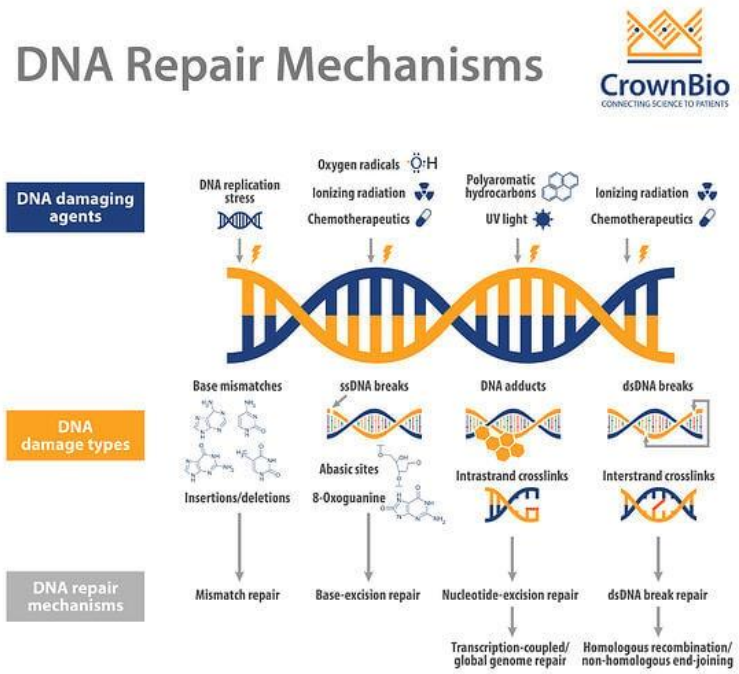

Figure 6: Types of DNA damage and DNA repair mechanisms

Human cells have four major DNA-repair processes (Figure 6): Mismatch repair (MMR), base-excision repair (BER), nucleotide-excision repair (NER), and Double-Strand Break (DSB) repair (Bourré, 2022). The MMR pathway repairs replication errors such as base-base mismatches, insertion/deletion loops, and "mispairs,". BER uses multiple enzymes to repair small base lesions, such as Single-Stranded Break (SSB), by excising and replacing a single damaged nucleotide base. The NER mechanism repairs SSB and damage from bulky DNA adducts and UV radiation. The dsDNA breaks are repaired by either homologous recombination or nonhomologous end-joining.

1.7XRCC1 and Prostate Cancer Risk

1.7.1 BER Pathway

Base excision repair (BER) repairs non-bulky single-base lesions that do not significantly alter the structure of the DNA helix. The pathway is initiated by removing the mutated base, followed by incision, repair synthesis, and ligation (Krokan & Bjørås, 2013) (Figure 7). In the stage of base removal, a damage-specific DNA glycosylase removes the damaged base leaving behind an abasic site or apurinic/apyrimidinic site (AP-site). An AP endonuclease then cleaves

the AP site, resulting in an SSB. The SSB is then resolved by DNA polymerase using either short-patch BER, in which a single nucleotide is replaced, or long-patch BER, in which 2-10 new nucleotides are synthesized. The remaining gap is sealed by DNA ligase by forming phosphodiester bonds.

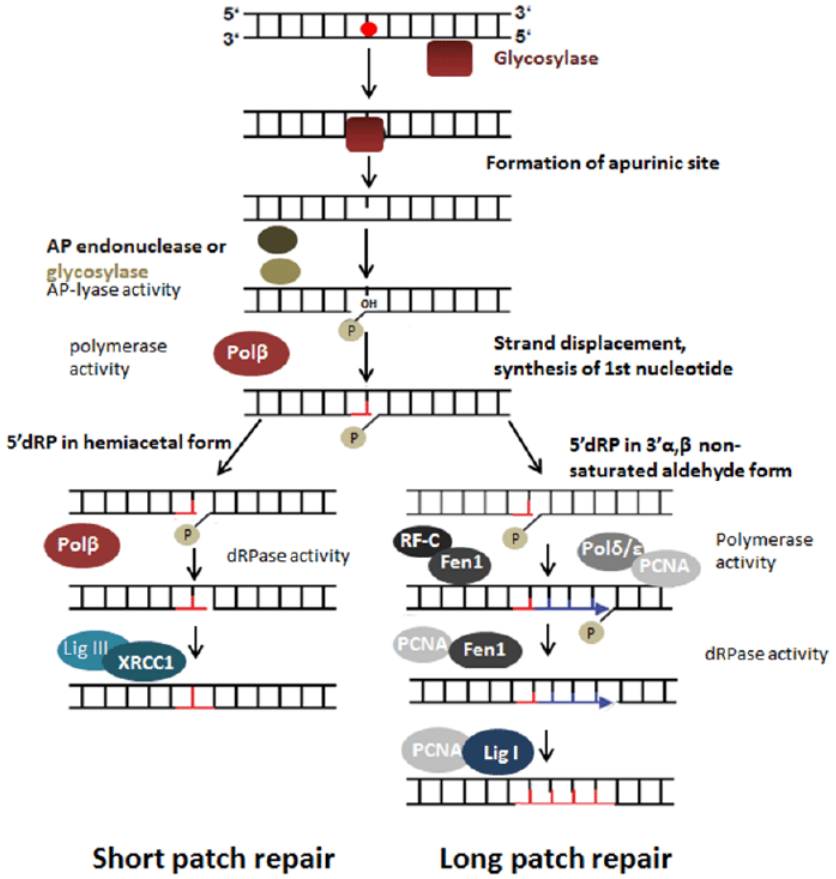

Figure 7: Basic steps of the BER pathway

1.7.2 Role of XRCC1 in BER Pathway

Human X-ray Cross Complementing 1 (XRCC1) gene maps to chromosome 19q13.32-13.3 (Mohrenweiser et al., 1989). It comprises 17 exons and spans a genomic distance of 32 kb. It encodes 633 amino acids with a molecular mass of 69477 Da.

The role of XRCC1 in BER pathway is shown in Figure 8. In Damage Detection [A], PARP 1 identifies SSBs and attaches to them, leading to the ribosylation of itself and or/other proteins. In turn, this promotes the recruitment of XRCC1 through direct interaction with ribosylated PARP and/or other ribosylated proteins. During DNA end processing [B], XRCC1 and its protein binding partners convert various types of damaged termini into typical 3'-hydroxyl and 5'-phosphate termini. In Gap filling [C], Polymerase complexed with XRCC1 replaces the

single missing nucleotide (short-patch repair) or more than one nucleotide (long-patch repair). In DNA ligation [D], LIG3 complexed with XRCC1 ligates the remaining nick.

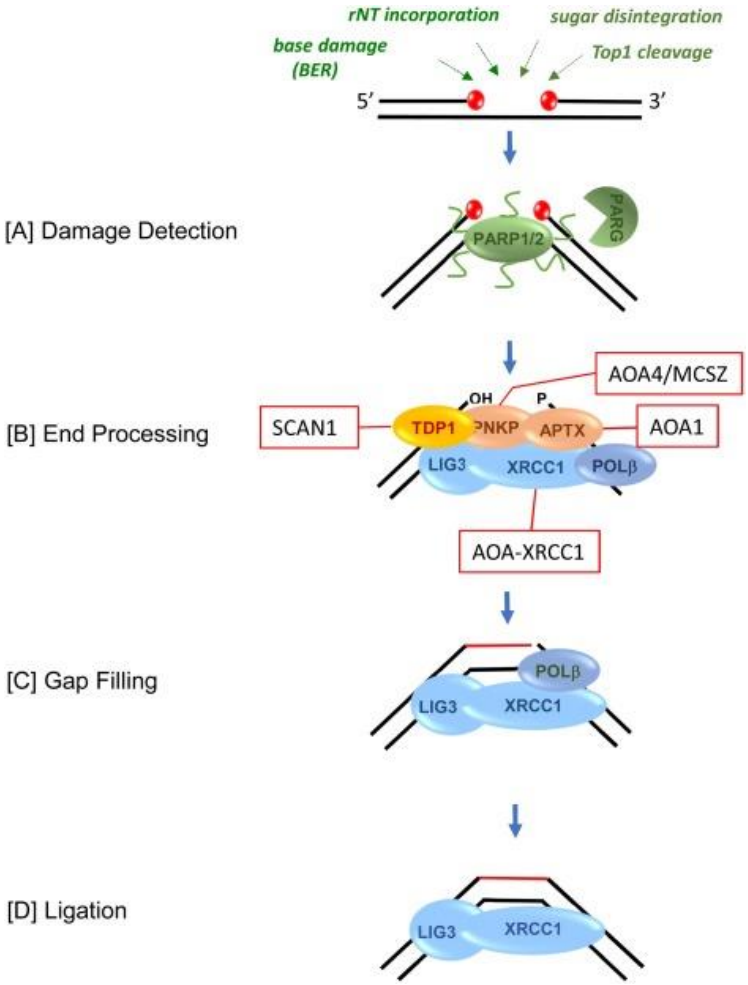

Figure 8: XRCC1 participation in BER pathway

1.7.3 Polymorphisms in XRCC1 Gene

Three particularly prevalent polymorphisms of XRCC1 gene affecting the amino acid sequence are in codon 194 (Arg/Trp), 280 (Arg/His), and 399 (Arg/Gln). Among these, XRCC1 at codon 194 (at position 26304 on exon 6, base C to T, amino acid Arginine to Trytophan, dbSNP no. rs1799782) has been shown to alter the DNA repair capacity of the protein (Wang et al., 2003).

1.7.4 XRCC1 codon 194 Polymorphism and Cancer Susceptibility

XRCC1 polymorphism in codon 194 has been linked to cancer susceptibility in case-control studies of numerous cancers. The homozygous mutant Trp allele of codon 194 XRCC1 was identified as a potential breast cancer risk factor in Kurdish ethnicity (Jalali et al., 2016). A population-based case-control study showed that the XRCC1 Trp/Trp genotype is a risk

genotype for lung cancer (Chen et al., 2002) and thyroid cancer (Wang et al., 2015) in the Chinese population.

Nonetheless, there were some discrepancies in the outcomes. Unlike the wild-type Arg allele, the mutant Trp allele was found to have a protective effect against bladder cancer (Stern et al., 2009).

1.7.5 XRCC1 codon 194 Polymorphism and Prostate Cancer

Data for prostate cancer are much more conflicting across different races. A case-control study among the Japanese population showed that individuals with the XRCC1 codon 194 Trp allele have a higher risk of developing prostate cancer (Zhu et al., 2015). The Trp/Trp genotype was also strongly associated with an elevated risk of prostate cancer in tobacco smokers, alcohol drinkers, and individuals with no family history of cancer. However, it did not show any association with clinical stage or the Gleason score of prostate cancer. Similarly, the Trp allele was also significantly observed in patients with a family history of prostate cancer; however, this allele did not correlate with clinical stages and pathological grades (Hamano et al., 2008).

In contrast, no significant correlation was discovered between Arg194Trp polymorphism and the development of toxicity following radiotherapy in prostate cancer patients (Langsenlehner et al., 2011), nor did it show predictive value for PSA recurrence after radical prostatectomy (Huang et al., 2007).

1.8 XPD and Prostate Cancer Risk

1.8.1 NER Pathway

NER repairs bulky lesions such as DNA adducts or intra-strand crosslinks. The NER pathway is divided into two sub-pathways: global genomic NER (GGR) and transcription-coupled NER (TCR), which differ only in damage recognition (Figure 9) (Menck & Munford, 2014). After lesion detection, both sub-pathways recruit transcription factor IIH (TFIIH). TFIIH is a multiprotein complex composed of XPB (3’-5’ helicase) and XPD (5'-3' helicase) subunits, as well as p62, p34, p52, p44, and p8 proteins. TFIIH helicase subunits unwind the helix near the damaged DNA site, recruiting XPA and XPG proteins. The XPA recruits the XPF/ERCC1 complex. The endonucleases XPG protein and the XPF/ERCC2 nick DNA at both sides, excising the lesion. The gap is filled by DNA polymerases which synthesize DNA from the 5’-3’ direction. The remaining nick after repair synthesis is sealed by DNA ligase.

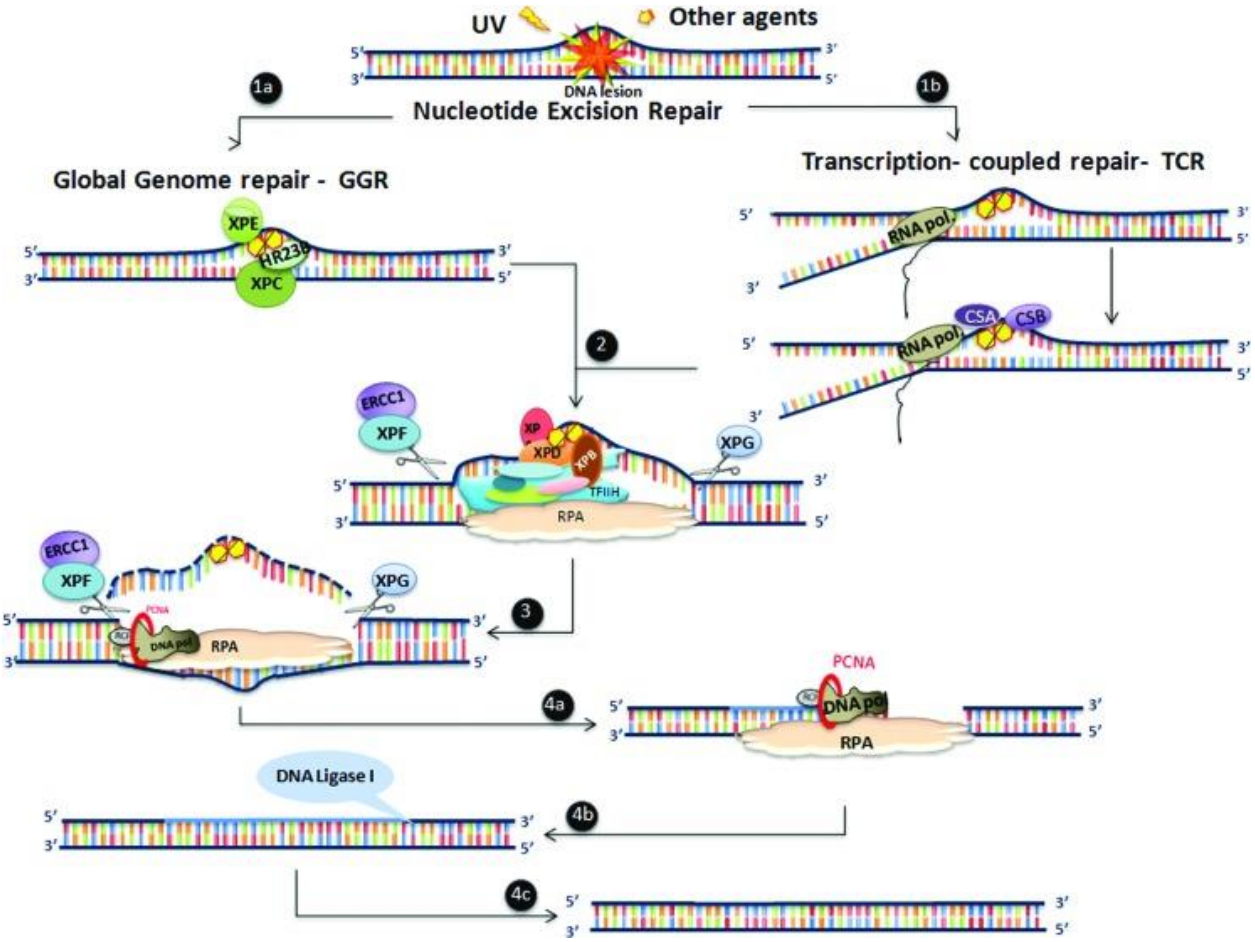

Figure 9: Schematic illustration of NER and its sub pathways: GGR and TCR

1.8.2 Role of XPD in NER Pathway

XPD, a key subunit of THIIF, is a DNA helicase involved in NER pathway (Zhu et al., 2013). The TFIIH contains XPB (3'-5' helicase) and XPD (5'-3' helicase) subunits. As helicases with opposing polarity, XPB and XPD act together to open the damaged DNA helix on opposite ends of a lesion (Sameer & Nissar, 2018). This ATP-dependent catalytic process causes conformational changes that facilitate the recruitment of additional NER proteins. While both XPB and XPD are involved in the unwinding of the DNA helix at the location of the lesion, XPD's helicase activity is more important. The XPD protein belongs to the ATP-dependent 5′-3′ superfamily 2 (SF2) helicases due to the presence of a 4Fe4S (FeS) cluster (Sameer & Nissar, 2018). The FeS cluster of the XPD helicase may play a structural role, interact directly with the damaged DNA and behave as a damaged sensor and regulatory hub for XPD's helicase

activity. XPD also serves as the verifier of the DNA lesion that was detected initially by the XPC-HR23B prior to TFIIH binding (Sameer & Nissar, 2018).

1.8.3 Polymorphisms in XPD Gene

Two common Single Nucleotide Polymorphisms (SNPs) in the XPD gene are in codon 312 (Asp/Asn) and codon 751 (Lys/Gln), located within the exon 23 of the XPD gene. Mutations in exon 23 affect the C-terminal domain of XPD helicase, which interacts with the p44 protein of the TFIIH complex (Zhu et al., 2013). Therefore, the two SNPs affect XPD helicase activity and have been linked to decreased DNA repair capacity and increased tumor risks (Zhu et al., 2013). Polymorphism at codon 751 (at position 35931 on exon 23, base A to C, amino acid lysine-to-glutamine, dbSNP no. rs13181) is the one we studied in this project.

1.8.4 XPD codon 751 Polymorphism and Cancer Susceptibility

Yong Du and colleagues showed that the XPD codon 751 polymorphism was correlated with non-small cell lung cancer in the East Chinese Han population (Du et al., 2016). The XPD homozygous mutant Gln/Gln genotype was found to be related to a twofold increase in breast cancer risk (Samson et al., 2011). Moreover, the Gln allele was also found to increase the risk of bladder cancer (Gao et al., 2010). In another study, however, the Gln/Gln genotype was only associated with a 0.51-fold increased risk of colorectal cancer-associated death compared to the Lys/Lys genotype (Gan et al., 2012).

1.8.5 XPD codon 751 Polymorphism and Prostate Cancer

Many studies have also examined the relationship between XPD Lys751Gln polymorphism and the risk of developing prostate cancer. Several investigations, however, have failed to find an elevated prostate cancer risk with the XPD Lys751Gln polymorphism in the Indian population (Sobti et al., 2012; Tabrez et al., 2008), the Taiwanese population (Bau et al., 2007), and the South Australian population (Dhillon et al., 2011).

1.9 Objectives

Although PSA and digital examination are available to screen for prostate cancer, additional efficient screening approaches are required to reduce cancer-related mortality in Bangladesh. We expect to uncover significant evidence that variations in the XRCC1 Arg194Trp, and XPD Lys751Gln are related to prostate cancer risk. It is anticipated that prostate cancer patients will demonstrate a substantial correlation with the altered genotype compared to healthy control.

Our expected findings would enable us to employ SNPs as biomarkers for detecting genetically susceptible individuals in the Bangladeshi population.

The purpose of this study is to:

i. To determine the frequency distribution of the XRCC1 codon 194 and XPD codon 751 polymorphisms in the Bangladeshi population's prostate cancer patients and healthy controls.
ii. To investigate the association of the SNPs with susceptibility and risk of prostate cancer.
iii. To study the risk of prostate cancer associated with SNPs according to smoking status and family history of cancer.
iv. To examine the association of the polymorphisms with pathological grades of prostate cancer
v. To explore the association of the SNPs with clinical and biochemical parameters of prostate cancer patients.

Chapter 2

Materials and Methods

2.1 Study Design

The study was designed as a hospital-based case-control study (Figure 10).

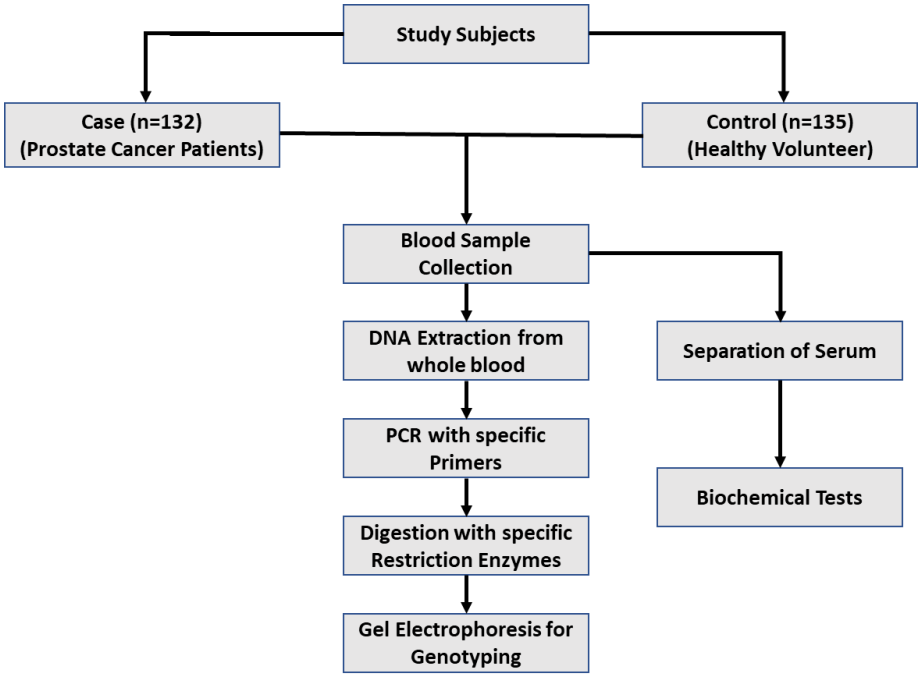

Figure 10: Schematic diagram of study design

2.2 Selection of the Patients and Controls

A total of 132 prostate cancer patients with histologically diagnosed prostate cancer from the Department of Urology of Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine, and Metabolic Disorders (BIRDEM) General Hospital, Bangabandhu Sheikh Mujib Medical University (BSMMU), Dhaka Medical College Hospital (DMCH) and other tertiary care hospitals of Dhaka formed the study group. The control group comprised 135 healthy subjects who were age-matched and unrelated to the cases from the same geographic area. Both patients and controls with a previous history of other chronic diseases such as kidney disease, cardiovascular disease, and other cancer were excluded from the study. Tribal or other ethnic groups were excluded from the study groups.

Inclusion Criteria

Case:

i. Histopathologically and biopsies confirmed cases of prostate cancer

ii. Bengali Background

Control group:

i. Age-matched healthy control from the same geographic area and ethnicity

Exclusion Criteria

i. Having a history of cancer.
ii. Having a history of any chronic diseases.
iii. Tribal or other ethnic groups

2.3 Questionnaire

A structured questionnaire was made detailing each study subject's age, smoking history, and family history of chronic diseases. The questionnaire also included a section for patients for clinical data (PSA and creatinine levels, clinical staging, histopathological grading, urine (R/E), DRE, and USG findings). Present and ex-smokers were considered smokers, and those who had never smoked were considered non-smokers. The tumor stage was classified into either low-grade (Gleason's score <7) or moderate to high-grade tumor (Gleason's score ≥ 7). The cancer stage was categorized into two groups: Localized or Organ-confined, which included T1a-c/T2a-b N0 M0, and Locally advanced or metastatic, which had T3a-b/T4/N1/M1, according to TNM classification.

2.4 Consent and Ethical Issues

Each participant was informed of the study's purpose and the investigational nature of the protocol, and informed consent was received from each patient. Participant details were obtained using a structured questionnaire. Clinical data of the patients were recorded in the presence of the attending physician. The study was conducted following the Helsinki Declaration and subsequent revisions ("World Medical Association Declaration of Helsinki, 2013," n.d.). The study was approved by the Ethical Review Committees of the Department of Biochemistry and Molecular Biology, University of Dhaka.

2.5 Sample Collection and Storage

About 3.0 mL of venous blood was drawn from each participant adhering to all aseptic precautions. The drawn blood was immediately transferred to EDTA (1.20 mg/mL) containing tubes and clot-activating plain tubes. Blood in plain tubes was centrifuged at 3,000 rpm for 10

minutes to separate serum, which was then taken in Eppendorf tubes for biochemical testing in the hospital laboratory. The EDTA tubes were kept in an icebox for transportation to the laboratory and stored at −20°C until DNA extraction.

2.6 DNA Extraction and Quantification

2.6.1 DNA Extraction from Blood

Genomic DNA was extracted from the whole blood samples using a commercial kit (FavorPrep™ Blood Genomic DNA Extraction Mini Kit) according to the manufacturer's protocol (Figure 11).

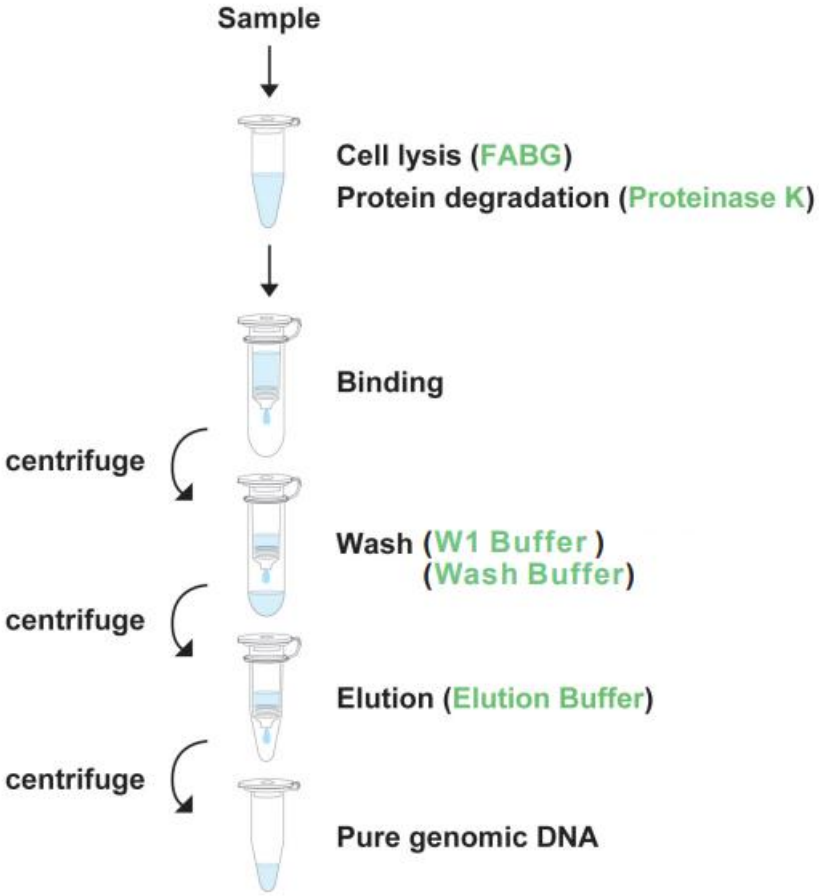

Figure 11: Steps of DNA Extraction from whole blood

Cell Lysis: Fresh human blood was collected in anticoagulant-treat collection tubes (EDTA tubes). Two hundred microliter sample of the whole blood was transferred to a microcentrifuge tube. To that, 20 µl Proteinase K and 200 µl FABG Buffer were added. It was then thoroughly mixed by pulse vortexing. The mixture was incubated at 60 ºC for 15 minutes for cell lysis. The sample was vortexed every 3 to 5 minutes during incubation to ensure uniform mixing.

Separation of the soluble DNA from cell debris: Two hundred microliter ethanol (96~100%) was added to the cell lysate. The sample was mixed thoroughly by pulse-vortexing for 30 seconds. The tubes were briefly spun to remove drops inside the lid.

Binding the DNA to a purification matrix: FABG Column was placed inside a collection tube. The sample, including any precipitate, was transferred into the FABG Column. The tubes were centrifuged for 1 minute, and the flow-through was discarded. The FABG Column containing the bound DNA is transferred to a new collection tube for washing.

Washing: The FABG column in the new collection tube was washed with 500 µl W1 Buffer and centrifuged for 1 minute. The flow-through was discarded. The second wash uses 750 µl W1 buffer. The column was then centrifuged for 1 minute, and the flow-through was discarded. For the dry spin, the columns were centrifuged for an additional 3 min to dry the column to remove any residual liquid.

Elution: The washed FABG Column was placed inside the Elution Tube. About 100~200 µl of Elution Buffer was added to the membrane of the FABG Column. The buffer was allowed to sit in the FAGB Column for 3 min. Finally, the tubes were centrifuged for 2 min to elute the DNA. The extracted DNA was stored at -20 ºC.

2.6.2 Quantification of DNA

The quantity and purity of the genomic DNA were assessed by taking absorbance values at 260 nm (A260) and at 280 nm (A280 using Thermo Scientific™ NanoDrop™. Pure DNA had an A260/A280 ratio of 1.8–2.0. The quality of DNA elutes (4 µl) was also assessed by agarose gel electrophoresis. Gel electrophoresis was performed using 2.5% agarose gel run for a long time at a low voltage (100V for 90 minutes) and visualized under a gel documentation system (AlphaImager mini, USA).

2.7 PCR-RFLP

The XRCC1 codon 194 and XPD 751 genotypes were determined using conventional Polymerase Chain Reaction- Restriction Fragment Length Polymorphism (PCR-RFLP).

2.7.1 PCR

2.7.1.1 Primer Selection

The primer sequences and PCR conditions were used by previously published work (Chacko et al., 2005; Mitra et al., 2009). The primer sequences and the resulting digested products of each amplicon are listed in Table 1.

Table 1: Primer sequences for allele determination, with amplicon size and the resultant digested products size

DNA Repair Gene	dbSNP ID	Primer Sequence	Amplicon size	Restriction Digestion Products
XRCC1 Arg194Trp	rs1799782	F: 5'-GTTCCGTGTGAAGGAGGAGGA-3' R: 5'-CGAGTCTAGGTCTCAACCCTACTCACT-3'	138 bp	Arg/Arg: 138 bp Arg/Trp: 138 bp, 75 bp and 63 bp Trp/Trp:75 bp and 63 bp
XPD Lys751Gln	rs13181	F: 5' CCCCCTCTCCCTTTCCTCTGTTC 3' R: 5' GGACCTGAGCCCCCACTAACG 3'	413 bp	Lys/Lys: 413 bp Lys/Gln: 413 bp, 322 bp and 91 bp Gln/Gln: 322 bp and 91 bp

2.7.1.2 PCR Reagents

The reagents used for PCR-RFLP are listed in Table 2.

Table 2: Reagents and the respective companies used for PCR-RFLP

	Reagents	**Company**
PCR	DreamTaq Green PCR Master Mix	Thermo Fisher, USA
	Primers	Macrogen, South Korea
	20% Dimethyl Sulfoxide	Sigma-Aldrich, Germany
	Nuclease Free Water	New England Biolabs, USA
Restriction Digestion	Restriction Enzymes (PvuII, RsaI, AluI)	New England Biolabs, USA
Gel Electrophoresis	Agarose Powder	Invitrogen, USA
	Midori Green Advance-Nucleid acid Stain	NIPPON Genetics, JAPAN
	GeneRuler 100 bp DNA Ladder, ready-to-use	Thermo Fisher, USA

2.7.1.3 Composition of PCR mix

Table 3: Composition of the PCR reaction mixture

Name of the component	**Volume (µL)**
Green PCR Master Mix	7.0
Nuclease-free water	3.0
20% Dimethyl sulfoxide	1.0
Forward Primer	0.5
Reverse Primer	0.5
Genomic DNA	3.0
Total Volume	15.0

2.7.1.4 PCR Conditions

The PCR conditions of XRCC1 194 genotype and XPD 751 genotype have been illustrated in Figure 12 and Figure 13, respectively.

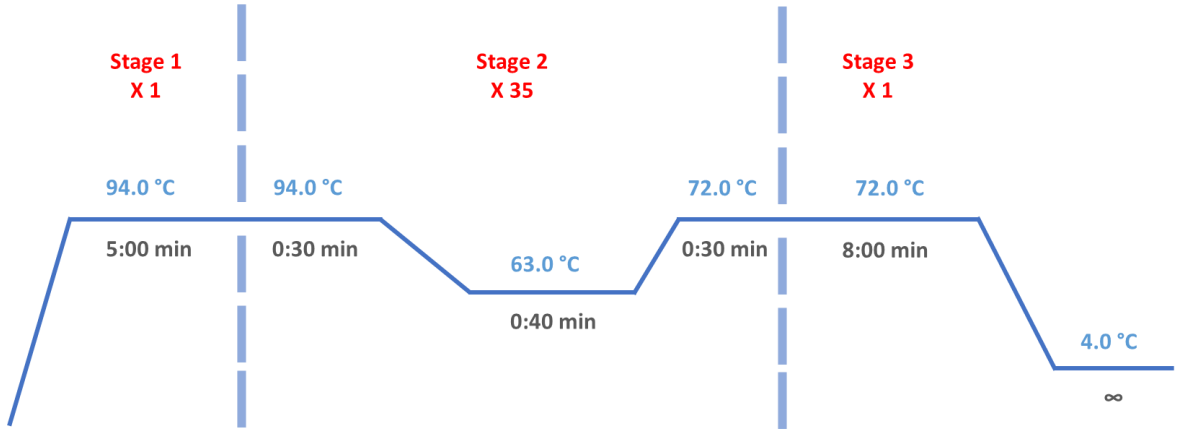

Figure 12: Thermal conditions for amplification of XRCC1 codon 194

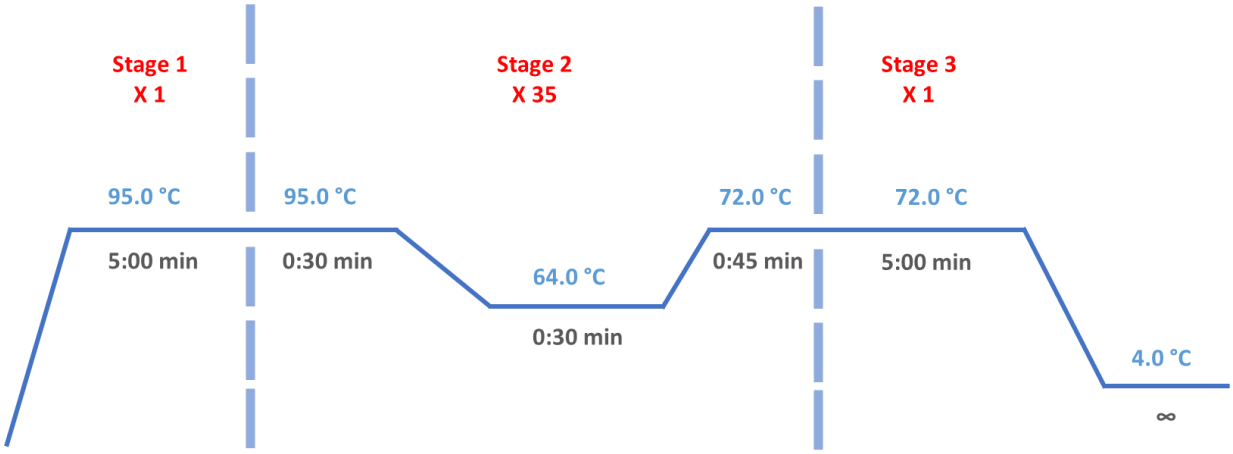

Figure 13: Thermal conditions for amplification of XPD codon 751

2.7.1.5 Evaluation of PCR

The amplified PCR products of the XRCC1 gene and XPD were checked for amplification by gel electrophoresis in 2% agarose gel stained with Midori Green stain. The amplified DNA was then visualized under UV light (Figures 14 and 15).

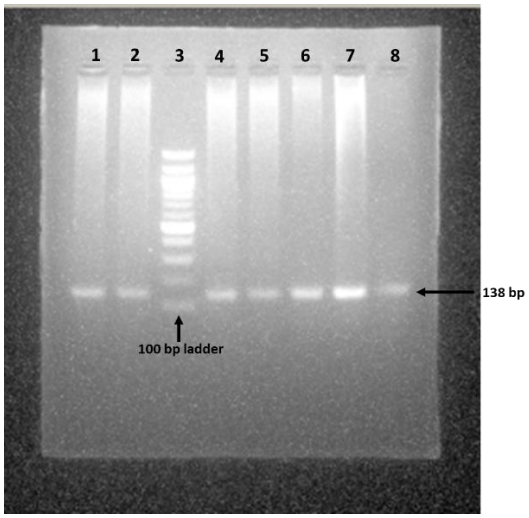

Figure 14: PCR products of XRCC1 gene in 2% Agarose gel. *PCR product of XRCC1 (138 bp) is in lanes 1,2, 4 to 8. Lane 3 contains DNA ladder*

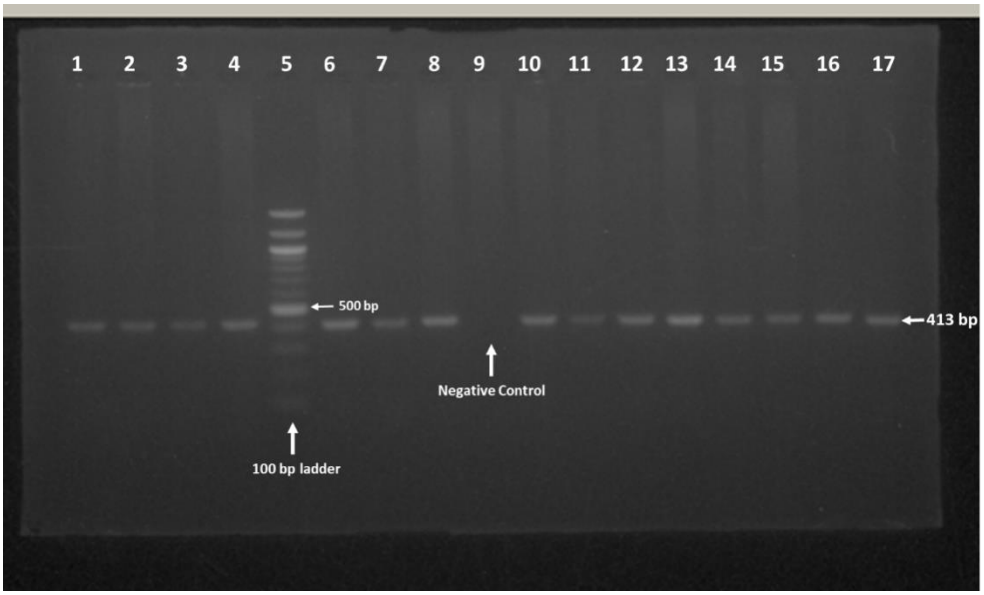

Figure 15: PCR products of XPD gene in 2% Agarose gel. *PCR products of XPD (413 bp) are in lanes 1 to 4, 6 to 8, and 10 to 17. Lane 5 contains DNA ladder. Lane 9 contains negative control.*

2.7.2 RFLP Analysis

2.7.2.1 Restriction Digestion of XRCC1 and XPD PCR Products

The restriction enzyme PvuII was used to digest the XRCC1 Arg194Trp 138 bp PCR product following the manufacturer's instructions (incubated at 37°C for 1 hour). The 138 bp PCR products of the homozygous mutant genotype (Trp/Trp) were fragmented into 75 bp and 63 bp fragments. Because 75 bp and 63 bp were only a few base pairs apart, they appeared as a single band. The wild-type allele (Arg) was not cleaved and appeared as one band (138 bp) under UV light. The heterozygous mutant genotype (Arg/Trp) produces all three bands since it has both alleles (138 bp, 75 bp, and 63 bp).

Pst1 was used to digest the PCR product (413 bp) of the XPD Lys751Gln SNP following the manufacturer's instructions (incubation at 37°C for 16 hours in a water bath). Pst1 cleaves the 413 bp into 322 bp and 91 bp when the homozygous mutation (Gln/Gln) was present. The wild-type allele (Lys) was not digested and thus shown as a single band of size 413 bp. The heterozygous genotype (Lys/Gln) was observed at positions 413 bp, 321 bp, and 91 bp.

2.7.2.2 Composition of Restriction Digestion Mix

Table 4: Composition of the reaction mixture for PvuII restriction enzyme digestion

Name of the Component	Volume (µL)
PCR grade H_2O	8.8
10X r3.1 buffer	1.0
PvuII enzyme	0.2
PCR product	5.0
Total Volume	15.0

Table 5: Composition of the reaction mixture for Pst1 restriction enzyme digestion

Name of the Component	Volume (µL)
PCR grade H_2O	8.8
10X CutSmart NEB Buffer	1.0
Pst1 enzyme	0.2
PCR product	5.0
Total Volume	15.0

2.7.2.3 RFLP Analysis of Digested Products

The enzyme digestion product was run in Midori Green stained 2% agarose gel. The gel was then visualized using gel documentation under ultraviolet (UV) light (Figure 16 and Figure 17). The product size was determined by matching against a 100-base pair DNA ladder.

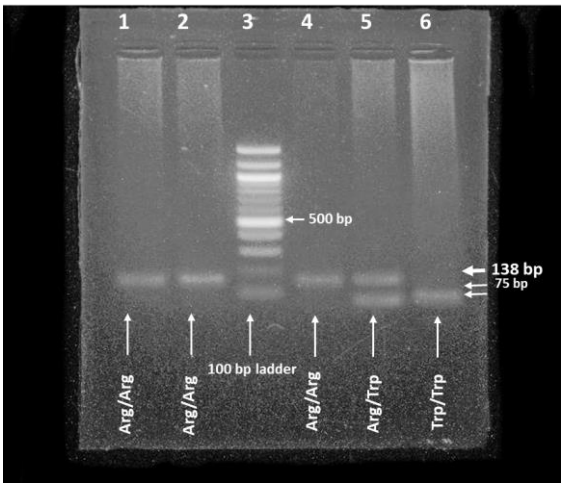

Figure 16: Restricted digested products of XRCC1 in 3% agarose Gel. *Restriction digestion fragments of XRCC1 (Lane 1,2, 4 to 6) resolved in 3% agarose gel. Lane 3 contains a DNA ladder. Lanes 1, 2, and 4, wild-type homozygote (138 bp); Lane 5, heterozygote (138, 75, 64 bp) and Lane 6, mutant homozygote (75, 63 bp).*

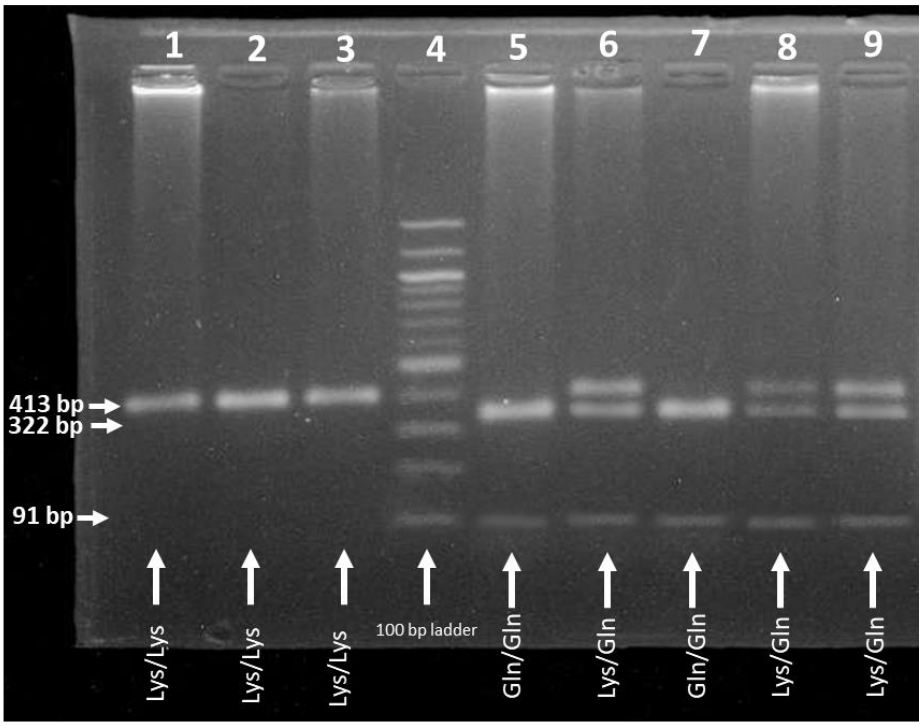

Figure 17: Restricted digested products of XPD in 3% agarose gel. *Restriction digestion fragments of XPD (Lanes 1 to 3, 5 to 9) resolved in 3% agarose gel. Lane 4 contains a molecular ruler. Lanes 1, 2, and 3, wild-type homozygote (413 bp); Lane 6, 8, and 9 heterozygotes (413, 322, and 91 bp) and Lanes 5 and 7, mutant homozygote (322 and 91 bp)*

2.8 Statistical Analysis

The Fisher's exact tests, t-test, and chi-square were performed through GraphPad Prism, version 9. The relative associations were determined by calculating the odds ratio (OR) with 95 % confidence intervals (CIs) and level of significance (p). All statistical tests were two-sided; a $p<0.05$ was taken as the level of significance.

Chapter 3

Results

3.1 Baseline Characteristics and Clinical Parameters of the Study Subjects

Table 6: Baseline characteristics and clinical parameters of the study subject

Variable	**Control (n=135) n (%)**	**Case (n=132) n (%)**	**p-value**
Gender:			
Male	135(100)[b]	132 (100)	-
Age (Year)[a]:	65.13 ± 0.74	67.34 ± 0.73	0.03 (s)
<60	29 (21.48)	21 (15.91)	
60-70	79 (58.52)	74 (56.06)	0.23 (ns)
>70	27 (20)	37 (28.03)	
Smoking status:			
Non-Smoker	83 (61.48)	52 (39.39)	
Smoker	52 (38.52)	80 (60.61)	<0.001(s)
Family History of Cancer:			
No	127 (94.07)	110 (83.33)	-
Yes	8 (3.70)	22 (16.67)	<0.01 (s)
Clinical Parameters			
Grading of tumor:			
Gleason score <7	-	78 (59.09)	-
Gleason score ≥7	-	54 (40.91)	-
Clinical Staging:			
Localized or Organ-confined	-	84 (63.64)	-
Locally advanced or metastatic	-	48 (36.36)	-
DRE:			
Soft mass with a smooth surface	-	49 (37.12)	-

Continued Table 6

Hard mass with irregular surface	-	83 (62.88)	-
Obstructive Feature with Hematuria:			
Absent	-	96 (72.73)	-
Present	-	36 (27.27)	-
Urinary tract infection:			
Absent	-	51 (38.64)	-
Present	-	81 (61.36)	-
BMI (kg/m^2):			
<18.5	-	3 (2.27)	-
18.5 – 24.9	-	107 (81.06)	-
25.0 – 29.9	-	22 (16.67)	-
> 29.9	-	0 (0)	-
Creatinine (mg/dl):			
< 0.70	-	0 (0)	-
0.7-1.3	-	91 (68.94)	-
> 1.3	-	41 (31.06)	-
PSA (ng/ml):			
≤6.5	-	2 (1.52)	-
6.6-10	-	15 (11.36)	-
>10	-	115 (87.12)	-

[a]Mean±SEM; [b]Numbers in parentheses show percentages. Digital rectal examination (DRE); Body Mass Index (BMI); Prostate-specific Antigen (PSA); $p<0.05$ was taken as the level of significance; s= significant; ns=not significant

This study included 132 prostate cancer patients and 135 controls, whose gender, age, smoking status, and family history of cancer are listed in Table 6. Patients were 49–85 years old, with a mean age of 67.34±0.73, while controls were 47–88 years old, with a mean age of 65.13±0.74. The majority (56.06%) of cancer patients belong to the 60-70 age group. Since controls were age-matched with patients at the interview, age differences between cases and controls were not statistically significant.

Compared with control subjects, patients were more likely to be smokers (60.61% in patients vs. 38.52% in control), and the difference was statistically highly significant ($P<0.001$). As expected, patients were statistically ($p<0.01$) more likely to have a family history of cancer (16.67% of the patients compared to 3.7% of controls).

Most patients (81.06%) had normal BMI (18.5-24.9/kg m^2) and normal serum creatinine levels (0.7–1.3 mg/dl) (68.94%). Most patients had low-grade (Gleason score <7) prostate cancer (59.09%) and localized tumors (63.64%). 61.36% of the patients had UTI and only 27.27% had obstructive characteristics with hematuria. However, 87.12% of the patients had PSA readings above 10 ng/ml.

3.2 Frequency distribution of XRCC1 codon 194 and XPD codon 751 genotype and Prostate Cancer risk

Table 7: Genotypic and allele frequency of XRCC1 and XPD in the study subject and risk of prostate cancer

Gene	**Genotype**	**Control (n=135) n (%)**	**Case (n=132) n (%)**	**OR (95% CI)**	**p-value**
XRCC1					
	Arg/Arg	69 (51.11)	59 (44.70)	1 (Ref)	
	Arg/Trp	65 (48.15)	66 (50.00)	1.19 (0.72-1.96)	0.54 (ns)
	Trp/Trp	1 (0.74)	7 (5.30)	8.19 (1.38-93.38)	0.029 (s)
XPD					
	Lys/Lys	68 (50.37)	64 (48.48)	1 (Ref)	
	Lys/Gln	61 (45.19)	54 (40.91)	0.94 (0.58-1.53)	0.89 (ns)
	Gln/Gln	6 (4.44)	14 (10.61)	2.48 (0.94-6.76)	0.094 (ns)
Gene	**Allele**	**Control (n=135) n (%)**	**Case (n=132) n (%)**	**OR (95% CI)**	**p-value**
XRCC1					
	Arg Allele	203 (75.19)	184 (69.70)	1 (Ref)	
	Trp Allele	67 (24.81)	80 (30.30)	1.32 (0.91-1.93)	0.18 (ns)
XPD					
	Lys Allele	197 (72.96)	182 (68.94)	1 (Ref)	-
	Gln Allele	73 (27.03)	82 (31.06)	1.22 (0.83-1.76)	0.34 (ns)

Odds ratios (OR) and 95% confidence interval (95%CI); Fisher's exact test was done to evaluate significance; $p < 0.05$ was considered as the level of significance; s= significant; ns=not significant

Genotypic and allelic distributions of XRCC1 codon 194 and XPD codon 751 polymorphisms in control and patients with their estimated risk of prostate cancer are outlined in Table 7.

In 132 prostate cancer patients, 44.70% were homozygous wild type (Arg/Arg) of XRCC1, 50.00% were heterozygous mutant variant (Arg/Trp), and 5.30% were rare homozygous mutant variant (Trp/Trp). Controls had 51.11% homozygous wild type (Arg/Arg), 48.15% heterozygous mutant variant (Arg/Trp), and 0.74% homozygous mutant variant (Trp/Trp). When compared to the homozygous wild type (Arg/Arg) genotype, the XRCC1 homozygous wild type (Trp/Trp) genotype increased PCa risk 8.2-fold (OR = 8.19; 95% CI=1.38-93.38; p 0.05). However, no significant association with prostate cancer risk was evident for heterozygous mutant (Arg/Trp) (OR=1.187; 95% CI=0.724-1.957; p=0.535). In allele frequency analysis of XRCC1, the wild-type Arg allele, and mutant Trp allele were present in 69.70% and 30.30% of patients, whereas 75.19% and 24.81% were in controls, respectively. However, neither allele significantly increased prostate cancer susceptibility (OR=1.317; 95% CI-0.905-1.932; p=0.175).

The homozygous wild type (Lys/Lys), heterozygous mutant variant (Lys/Gln), and homozygous mutant variant (Gln/Gln) of the XPD gene were present in 48.48%, 40.91%, and 10.61% respectively in patients whereas they were found in 50.37%, 45.19%, and 4.44%, respectively in controls. When Lys/Lys was used as the reference group, no significant differences were present in the frequency distribution of heterozygous mutant (Lys/Gln) (OR= 0.941; 95% CI= 0.575-1.534; p=0.898) between controls and prostate cancer patients. The homozygous mutant variant (Gln/Gln) of XPD had a non-significant 2.5-fold increased risk of prostate cancer (OR=2.479; 95% CI= 0.942-6.764; p=0.094). The frequency of the XPD codon 751 Lys allele and Gln allele for cases were 0.68 (68.94%) and 0.31 (31.06%), respectively, and 0.73 (72.96%) and 0.27 (27.03%) for controls, respectively. Neither allele was significantly associated with prostate cancer risk (OR=1.216; 95% CI-0.830-1.756; p=0.341).

3.3 Association between XRCC1 codon 194 and XPD codon 751 with Prostate Cancer using Genetic Models

Table 8: Genotype frequencies of XRCC1 codon 194 and XPD codon 751 using codominant, homozygous dominant, homozygous recessive, and over-dominant models

Gene	Genotype model	Genotype	Control (n=135) n (%)	Case (n=132) n (%)	OR (95% CI)	p-value
XRCC1	Co-dominant model	Arg/Arg	69 (51.11)	59 (44.70)	1 (Ref)	-
		Arg/Trp	65 (48.15)	66 (50.00)	1.19 (0.72-1.96)	0.54 (ns)
		Trp/Trp	1 (0.74)	7 (5.30)	8.19 (1.38-93.38)	0.03 (s)
	Dominant model	Arg/Arg	69 (51.11)	59 (44.70)	1 (Ref)	-
		Arg/Trp + Trp/Trp	66 (48.89)	73 (55.30)	1.29 (0.80-2.10)	0.33 (ns)
	Recessive model	Arg/Arg + Arg/Trp	134 (99.26)	125 (94.70)	1 (Ref)	-
		Trp/Trp	1 (0.74)	7 (5.30)	7.50 (1.23-85.12)	0.04 (s)
	Over-dominant	Arg/Arg + Trp/Trp	70 (51.85)	66 (50.00)	1 (Ref)	-
		Arg/Trp	65 (48.15)	66 (50.00)	1.07 (0.67-1.74)	0.81 (ns)
XPD	Co-dominant model	Lys/Lys	68 (50.37)	64 (48.48)	1 (Ref)	-
		Lys/Gln	61 (45.19)	54 (40.91)	0.94 (0.58-1.53)	0.89 (ns)
		Gln/Gln	6 (4.44)	14 (10.61)	2.479 (0.942-6.764)	0.09 (ns)
	Dominant model	Lys/Lys	68 (50.37)	64 (48.48)	1 (Ref)	-
		Lys/Gln + Gln/Gln	67 (49.63)	68 (51.52)	1.08 (0.67-1.75)	0.81 (ns)

	Recessive model	Lys/Lys + Lys/Gln	129 (95.56)	118 (89.39)	1 (Ref)	-
		Gln/Gln	6 (4.44)	14 (10.61)	2.55 (1.01-6.67)	0.07 (ns)
	Over-dominant	Lys/Lys + Gln/Gln	74 (54.81)	78 (59.09)	1 (Ref)	-
		Lys/Gln	61 (45.19)	54 (40.91)	0.84 (0.51-1.37)	0.54 (ns)

Odds ratios (OR) and 95% confidence interval (95%CI); Fisher's exact test was done to evaluate significance; $p < 0.05$ was considered as the level of significance; s= significant; ns=not significant

The effects of XRCC1 codon 194 and XPD codon 751 genotypes on prostate cancer risk were evaluated using codominant, dominant, recessive, and over-dominant models (Table 8).

Only the recessive model, carrying two copies of the XRCC1 194 codon Trp allele, was positively associated by 7.5 times with the risk of prostate cancer (OR=7.50; 95% CI=1.227-85.12; p=0.035) compared to carrying the other two genotypes. Over-dominant model analysis showed that the heterozygous genotype Arg/Trp did not increase prostate cancer risk.

Similarly, carrying two copies of the XPD751 codon Gln allele was non-significantly associated (2.5-folds) with the risk of PCa (OR=2.551; 95% CI=1.008-6.674; p=0.065) as observed in the recessive model analysis.

3.4 Combined Genotypic effects of XRCC1 and XPD polymorphisms on Prostate Cancer Risk

Table 9: Interaction between XRCC1 Arg194Trp and XPD Lys751Gln genotypes and prostate cancer risk

XRCC1	XPD	Controls (n=135) n (%)	Cases (n=132) n (%)	OR (95% CI)	p-value
Arg/Arg	Lys/Lys	38 (28.15)	33 (25.00)	1 (Ref)	-
Arg/Arg	Lys/Gln	29 (21.48)	21 (15.91)	0.83 (0.41-1.78)	0.71 (ns)
Arg/Arg	Gln/Gln	2 (1.48)	5 (3.79)	2.88 (0.54-15.03)	0.26 (ns)
Arg/Arg	Any Gln	31 (22.96)	26 (19.70)	0.97 (0.48-1.95)	>0.99 (ns)
Arg/Trp	Lys/Lys	29 (21.48)	26 (19.70)	1.03 (0.50-2.11)	>0.99 (ns)
Arg/Trp	Lys/Gln	32 (23.70)	3123.48)	1.12 (0.58-2.17)	0.86 (ns)
Arg/Trp	Gln/Gln	4 (2.96)	9 (6.82)	2.59 (0.69-8.08)	0.23 (ns)
Arg/Trp	Any Gln	36 (26.67)	40 (30.30)		
Trp/Trp	Lys/Lys	1 (0.74)	5 (3.79)	5.76 (0.69-69.36)	0.11 (ns)
Trp/Trp	Lys/Gln	0 (0)	2 (1.52)	-	-
Trp/Trp	Gln/Gln	0 (0)	0 (0)	-	-
Trp/Trp	Any Gln	0 (0)	2 (1.52)	-	-
Any Trp	Lys/Lys	30 (22.22)	31 (23.48)	1.19 (0.61-2.35)	0.73 (ns)
Any Trp	Lys/Gln	32 (23.70)	33 (25.00)	1.19(0.62-2.29)	0.73 (ns)
Any Trp	Gln/Gln	4 (2.96)	9 (6.82)	2.59 (0.69-8.08)	0.23 (ns)

Odds ratios (OR) and 95% confidence interval (95%CI); Fisher's exact test was done to evaluate significance; $p < 0.05$ was considered as the level of significance; s= significant; ns=not significant.

The analysis of pairwise joint associations of XRCC1 Arg194Trp and XPD Lys751Gln genotypes with prostate cancer risk are shown in Table 9.

The risk of prostate cancer was highest (5.76 folds) in individuals with XRCC1 Trp/Trp and XPD Lys/Lys genotype, but it was statistically insignificant (OR=5.578; 95% CI=0.699-69.36l; p=0.108). Because no healthy control was homozygous mutant for both genes, its cumulative effect cannot be compared.

3.5 XRCC1 and XPD Genotype on risk of Prostate cancer according to Smoking Status and Family History of Cancer

Table 10: XRCC1 and XPD genotype on risk of prostate cancer according to smoking status and family history of cancer

Baseline Characteristics	**Gene**	**Genotype**	**Control (n=135) n (%)**	**Case (n=132) n (%)**	**OR (95% CI)**	**p-value**
Smoking Status:						
Non-smoker	XRCC1	Arg/Arg	43 (31.85)	25 (18.94)	1 (Ref)	-
		Arg/Trp	39 (28.89)	26 (19.70)	1.15 (0.57-2.33)	0.73 (ns)
		Trp/Trp	1 (0.74)	1 (0.76)	1.72 (0.09-33.34)	>0.99 (ns)
	XPD	Lys/Lys	40 (29.63)	26 (19.70)	1 (Ref)	-
		Lys/Gln	39 (28.89)	21 (15.91)	0.83 (0.41-1.75)	0.71 (ns)
		Gln/Gln	4 (2.96)	5 (3.79)	1.92 (0.50-6.68)	0.48(ns)
Smoker	XRCC1	Arg/Arg	26 (19.26)	34 (25.76)	1 (Ref)	-
		Arg/Trp	26 (19.26)	40 (30.30)	1.18 (0.57-2.44)	0.72 (ns)
		Trp/Trp	0	6 (4.55)	-	-
	XPD	Lys/Lys	28 (20.74)	38 (28.79)	1 (Ref)	-
		Lys/Gln	22 (16.30)	33 (25.00)	1.11 (0.52-2.22)	0.85 (ns)
		Gln/Gln	2 (1.48)	9 (6.82)	3.32 (0.74-16.05)	0.19 (ns)

Family History of Cancer						
No	XRCC1	Arg/Arg	65 (48.15)	46 (34.85)	1 (Ref)	-
		Arg/Trp	61 (45.19)	60 (45.45)	1.39 (0.82-2.37)	0.24 (ns)
		Trp/Trp	1 (0.74)	4 (3.03)	5.65 (0.88-70.05)	0.16 (ns)
	XPD	Lys/Lys	66 (48.89)	54 (40.91)	1 (Ref)	-
		Lys/Gln	56 (41.48)	44 (33.33)	0.96 (0.57-1.61)	0.89 (ns)
		Gln/Gln	5 (3.70)	12 (9.09)	2.93 (0.95-7.84)	0.07 (ns)
Yes	XRCC1	Arg/Arg	4 (2.96)	13 (9.85)	1 (Ref)	-
		Arg/Trp	4 (2.96)	6 (4.55)	0.46 (0.10-2.13)	0.42 (ns)
		Trp/Trp	0 (0)	3 (2.27)	-	-
	XPD	Lys/Lys	2 (1.48)	10 (7.58)	1 (Ref)	-
		Lys/Gln	5 (3.70)	10 (7.58)	0.4 (0.07-2.29)	0.41 (ns)
		Gln/Gln	1 (0.74)	2 (1.52)	0.4 (0.03-8.47)	0.52 (ns)

Odds ratios (OR) and 95% confidence interval (95%CI); Fisher's exact test was done to evaluate significance; $p < 0.05$ was considered as the level of significance; s= significant; ns=not significant.

The association of XRCC1 and XPD genotypes and prostate cancer according to smoking status and family history are shown in Table 1.

A 3.3-fold greater risk of prostate cancer was observed in smokers with the XPD Gln/Gln genotype, but this was not statistically significant (OR=3.316; 95% CI=0.737-16.05; p=0.186). Surprisingly, neither XRCC1 nor XPD mutations were substantially linked to PCa risk in individuals who had a history of cancer in the family. The XRCC1 Trp/Trp genotype had a 5.7-

fold greater risk of PCa in those without a family history of cancer, whereas the XPD Gln/Gln genotype had a 2.9-fold higher risk. Both were not statistically significant (p>0.05).

3.6 Association of XRCC1 and XPD polymorphism with Clinical Parameters of Prostate Cancer Patients

Table 11: Associations of XRCC1 codon 194 and XPD 751 polymorphisms with clinical characteristics in prostate cancer patients

Tumor Grade	**Gleason score <7 (n=78) n (%)**	**Gleason score ≥7(n=54) n (%)**	**OR (95% CI)**	**p-value**
XRCC1:				
Arg/Arg	33 (42.31)	26 (48.15)	1 (Ref)	-
Arg/Trp	41 (52.56)	25 (46.30)	0.77 (0.37-1.61)	0.59 (ns)
Trp/Trp	4 (5.13)	3 (5.56)	0.95 (0.22-3.81)	>0.99 (ns)
XPD:				
Lys/Lys	40 (51.28)	24 (44.44)	1 (Ref)	-
Lys/Gln	32 (41.03)	22 (40.74)	1.15 (0.56-2.36)	0.85 (ns)
Gln/Gln	6 (7.69)	8 (14.81)	2.22 (0.74-7.70)	0.23 (ns)
Clinical Stage	**Localized or Organ-confined (n=84) n (%)**	**Locally Advanced or Metastatic (n=48) n (%)**	**OR (95% CI)**	**p-value**
XRCC1:				
Arg/Arg	37 (44.05)	22 (45.83)	1 (Ref)	-
Arg/Trp	43 (51.19)	23 (47.92)	0.89 (0.45-1.81)	0.85 (ns)
Trp/Trp	4 (4.76)	3 (6.25)	1.26 (0.29-5.04)	>0.99 (ns)
XPD:				

Continued Table 11

Lys/Lys	45 (53.57)	19 (39.58)	1 (Ref)	-
Lys/Gln	32 (38.10)	22 (45.84)	1.63 (0.77-3.55)	0.25 (ns)
Gln/Gln	7 (8.33)	7 (14.58)	2.37 (0.76-7.39)	0.21 (ns)
DRE	**Soft mass with smooth surface (n=49) n (%)**	**Hard mass with irregular surface (n=83) n (%)**	**OR (95% CI)**	**p-value**
XRCC1:				
Arg/Arg	22 (44.90)	37 (44.58)	1 (Ref)	-
Arg/Trp	26 (53.06)	40 (48.19)	0.91 (0.46-1.93)	0.86 (ns)
Trp/Trp	1 (2.04)	6 (7.23)	3.57 (0.50-42.60)	0.41 (ns)
XPD:				
Lys/Lys	24 (48.98)	40 (48.19)	1 (Ref)	-
Lys/Gln	19 (3878)	35 (42.17)	1.11 (0.53-2.36)	0.85 (ns)
Gln/Gln	6 (12.25)	8 (9.64)	0.80 (0.27-2.78)	0.77 (ns)
Obstructive Feature with Hematuria	**Absent (n=96) n (%)**	**Present surface (n=36) n (%)**	**OR (95% CI)**	**p-value**
XRCC1:				
Arg/Arg	41 (42.71)	18 (50.00)	1 (Ref)	-
Arg/Trp	53 (55.21)	13 (36.11)	0.56 (0.24-1.23)	0.21 (ns)
Trp/Trp	2 (2.08)	5 (13.89)	5.69 (1.01-29.84)	0.04 (s)
XPD:				
Lys/Lys	45 (46.88)	19 (52.78)	1 (Ref)	-
Lys/Gln	41 (42.71)	13 (36.11)	0.75 (0.32-1.65)	0.54 (ns)
Gln/Gln	10 (10.42)	4 (11.11)	0.95 (0.30-3.56)	>0.99 (ns)

Urinary tract infection	**Absent (n=51) n (%)**	**Present surface (n=81) n (%)**	**OR (95% CI)**	**p-value**
XRCC1:				
Arg/Arg	24 (47.06)	35 (43.21)	1 (Ref)	-
Arg/Trp	25 (49.02)	41 (50.62)	1.13 (0.53-2.38)	0.85 (ns)
Trp/Trp	2 (3.92)	5 (6.17)	1.71 (0.31-9.11)	0.69 (ns)
XPD:				
Lys/Lys	24 (47.06)	40 (49.38)	1 (Ref)	-
Lys/Gln	23 (45.10)	31 (38.27)	0.81 (0.39-1.65)	0.71 (ns)
Gln/Gln	4 (7.84)	10 (12.35)	1.50 (0.42 to 4.70)	0.76 (ns)

Odds ratios (OR) and 95% confidence interval (95%CI); Digital rectal examination (DRE); Fisher's exact test was done to evaluate significance; $p < 0.05$ was considered as the level of significance; s= significant; ns=not significant.

The genotype distribution of XRCC1 codon 194 and XPD codon 751 polymorphisms in prostate cancer patients by tumor grade and clinical Stage is shown in Table 11.

Neither XRCC1 Arg194Trp nor XPD Lys751Gln polymorphism affected tumor grade, clinical stage, DRE results, or UTIs ($p>0.05$) (Table 5). Although this was not statistically significant, patients carrying the XPD Gln/Gln genotype was twice as likely to develop moderate to high-grade tumors (Gleason score 7) and metastatic tumor. Hematuria risk was significantly elevated 5.6-fold ($p=0.04$) and prostate hard mass with uneven surface risk was increased insignificantly 3.57-fold ($p=0.41$) in patients with the XRCC1 Trp/Trp genotype.

Table 12: Association of the XRCC1 codon 194 and XPD codon 751 polymorphisms with biochemical parameters in prostate cancer patients

Gene	**XRCC1**						**XPD**					
	Arg/Arg		**Arg/Trp**		**Trp/Trp**		**Lys/Lys**		**Lys/Gln**		**Gln/Gln**	
Parameter	**Mean ±SEM**	**p-value**	**Mean±SEM**	**p-value**	**Mean±SEM**	**p-value**	**Mean±SEM**	**p-value**	**Mean ±SEM**	**p-value**	**Mean±SEM**	**p-value**
Creatinine (mg/dl)	1.19 ± 0.04	(Ref)	1.22 ± 0.036	0.53 (ns)	1.43 ± 0.18	0.04 (s)	1.17 ± 0.03	(Ref)	1.23 ± 0.05	0.25 (ns)	1.34 ± 0.11	0.04 (s)
PSA (ng/ml)	45.44 ± 4.50	(Ref)	40.74 ± 4.47	0.46 (ns)	73.14 ± 9.73	0.04 (s)	43.75 ± 4.15	(Ref)	44.09 ± 4.96	0.96 (ns)	50.07 ± 11.75	0.55 (ns)

T-test was performed. $p < 0.05$ was considered as the level of significance; Prostate-specific antigen (PSA); s= significant; ns=not significant.

The association of XRCC1 codon 194 and XPD codon 751 polymorphisms with biochemical parameters of prostate cancer patients is shown in Table 12.

Patients with the XRCC1 Trp/Trp genotype had higher mean creatinine levels (1.43 mg/dl vs. 1.19 mg/dl) and higher mean PSA values compared to those with Arg/Arg genotype (73.1 ng/ml vs. 45.44 ng/ml). Accordingly, higher mean levels of both creatinine and PSA were substantially linked to the Trp/Trp variation (P=0.04). High creatinine levels in prostate cancer patients were likewise related to the homozygous mutant Gln/Gln genotype of the XPD gene (p=0.04).

Chapter 4

Discussion

4.1 Association of XRCC1 codon 194 Polymorphism with Prostate Cancer Risk

The Arg194Trp polymorphism in XRCC1 is located in the linker region that separates the NH_2-terminal domain and the BRCT1 (BRCA1 C-terminus) domain. Therefore, this polymorphism has a direct effect on the enzymatic activity of the XRCC1 protein (Galvez et al., 2021). It is hypothesized that XRCC1 polymorphisms do not make the protein completely non-functional since studies in mice have demonstrated that the lack of XRCC1 activity is embryo-lethal (Tebbs et al., 1999).

According to our research, men with the XRCC1 codon 194 Trp/Trp genotype had an 8.2 times greater chance of developing prostate cancer than those with the Arg/Arg genotype (OR =8.186; 95% CI=1.378-93.38; p=0.0296) (Table 7). In line with our findings, in Chinese population, the genotype Trp/Trp was shown to be linked to a higher risk of prostate cancer (OR=2.04; 95% CI=1.24-3.41; p= 0.003). (Zhu et al., 2015). However, in a Japanese population, the genotype Arg/Trp was significantly linked to prostate cancer patients compared to controls (OR=2.19; 95% CI=1.28- 3.73, p=0.0058) (Hamano et al., 2008). In the same study, they also found that one Trp allele (Arg/Trp and Trp/Trp genotypes) raised the risk of Prostate cancer by 2-folds (OR=2.03; 95% CI=1.23– 3.36, p=0.008). Their discovery conflicts with our findings from the recessive model analysis, which showed that the presence of both copies of the Trp allele increased the risk of prostate cancer compared to the presence of the Arg/Arg and Arg/Trp genotypes (OR=7.50; 95% CI=1.227-85.12; p=0.035). Their result contradicts our findings from the dominant model analysis, which showed that the genotypes Arg/Trp and Trp/Trp did not significantly raise the risk of prostate cancer. However, the recessive model analysis suggested that the risk of prostate cancer was increased when both copies of the Trp allele were present (OR=7.50; 95% CI=1.227-85.12; p=0.035) in comparison to the presence of Arg/Arg and Arg/Trp genotype (Table 8). As their study only included patients with a familial prostate cancer, their conclusions might not be applicable to us. Additionally, there is contradictory evidence that suggests codon 194 does not significantly increase the risk of prostate cancer (Langsenlehner et al., 2011; Huang et al., 2007). Differences in ethnic, regional, and environmental factors may be responsible for the lack of correlation in their study.

The 194 Arg/Trp or Trp/Trp genotype likely causes a decrease in DNA repair ability and accelerated tumor growth, but it's unclear to what extent this polymorphism affects DNA repair. In contrast, Rahman & Zein et al. found that the increase in mean sister chromatid exchange (SCE), a marker of gene damage, in response to the genotoxic chemical was greater in the wild-type Arg/Arg compared to Arg/Trp variant (Abdel-Rahman & El-Zein, 2000). These results suggest that the wild-type variation, but not the mutant variants, may be related to genomic instability. The Trp/Trp genotype could be studied for its effect on SCE due to the lack of participants carrying the genotype. Despite this, the link between SCP and the Arg/Arg genotype was only identified in a sample group in their study and was not statistically significant. In addition, Wang and colleagues found that the wild-type Arg allele, as opposed to the mutant Trp allele, caused significantly more chromosomal breaks and thus may be more likely to raise the risk of cancer (Wang et al., 2003). More functional studies to evaluate the DNA repair capacity of XRCC1 codon 194 polymorphism need to be conducted to reach a consensus, particularly because all studies use the wild-type variant as the reference. The possibility that there were other cancer susceptibility genes in linkage disequilibrium with XRCC1 might account for the discrepancy in the results. In addition, the effect of the polymorphism on the capacity to repair DNA may be different depending on the nature and severity of the DNA-damaging agents. There is also the possibility that some of these results were the result of random chance.

4.2 Association of XPD codon 751 Polymorphism with Prostate Cancer Risk

XPD is a possible cancer-predisposing gene in linkage disequilibrium with XRCC1, which maps close near XRCC1 on chromosome 19. The SNP at 751 resides in the COOH-terminal domain of XPD, which is vital for TFIIH. Therefore, changes in the XPD gene may have an impact on how it interacts with other subunits, leading to decreased XPD DNA helicase activity in TFIIH and NER abnormalities (Coin et al., 1998; Coin et al., 1999). However, it has been hypothesized that TFIIH transcriptional activity is very tolerant of changes in the XPD protein's amino acid composition. The 751 residue is located close to the polypeptide's C-terminal end, far from where disease-associated mutations have been detected (Clarkson & Wood, 2005).

In our study, we found no statistically significant connection between the XPD codon 751 variants and the risk of prostate cancer (Table 7). XPD codon 751 Gln/Gln was associated with a higher risk of prostate cancer compared to the wild-type variation, although the correlation

was not statistically significant (OR=2.479; 95% CI= 0.942-6.764; p=0.094). Our results are consistent with some earlier research on the relationship between the XPD codon 751 polymorphism and prostate cancer carried out in other nations (Sobti et al., 2012; Tabrez et al., 2008). We also examined the combined effect of the two polymorphisms that may influence DNA repair outcomes together, but the results did not reach statistical significance (Table 9). The absence of a statistically significant correlation may be attributable to the selection of different DNA repair pathway genes.

4.3 XRCC1 codon 194 and XPD codon 751 Polymorphisms on Prostate Cancer Risks based on Smoking Status and Family History of Cancer

Tobacco smoke has a high level of carcinogens and reactive oxygen species (ROS), such as polycyclic aromatic hydrocarbons (PAHs), and N-nitroso compounds, which can cause DNA lesions such as DNA bulky adducts, SSBs, and DSBs (Barnes et al., 2018; Nair et al., 1996). PAH-DNA adduct formation in the prostate due to cigarette smoke exposure has been shown to differ among races, suggesting individual susceptibility to cancer risk due to genetic variations in the DNA repair genes (Nock et al., 2007).

We only found the mutant homozygous Gln/Gln genotype of XPD showed an increased risk of prostate cancer in smokers, however, the association was not statistically significant (OR=3.316; 95% CI=0.737-16.05; p>0.186) (Table 10). While early research on XPD gave consistent results as ours (Sobti et al., 2012), a subsequent study discovered an association between the XRCC1 Arg/Trp and Trp/Trp genotypes and the risk of prostate cancer in smokers (Zhu et al., 2015), pointing to a significant gene-environment interaction in prostate cancer risk. The same study also showed that the XRCC1 Arg/Trp and Trp/Trp genotypes increased prostate cancer risk in individuals without a family history of cancer. In our investigation, homozygous mutant genotypes of both genes were associated with prostate cancer in subjects with no history of cancer, but not statistically significantly. The different distributions of genetic and environmental factors in the study cohort, which may affect the consequences of any specific genetic variant, could be the cause of inconsistent results among epidemiological studies.

4.4 XRCC1 codon 194 and XPD codon 751 Polymorphisms and Clinical and Biochemical Parameters of Prostate Cancer Patients

Finally, we investigated the relationships between the polymorphisms of prostate cancer patients and their clinical and biochemical parameters. We discovered that our studied SNPs had no impact on the DRE results, UTIs, tumor grade, or tumor aggressiveness ($p>0.05$) (Table 11). Among the clinical characteristics, only the genotype XRCC1 Trp/Trp substantially raised the incidence of hematuria by 5.6 times ($p=0.04$). The XRCC1 Trp/Trp genotype was also significantly associated with higher mean serum creatinine levels and PSA compared to patients having the wild type (Arg/Arg) genotype. Patients with the XPD Gln/Gln genotype were likewise affected in terms of higher serum creatine levels. Serum creatinine level a U-shaped association with prostate cancer risk; low and high creatinine levels have a significantly increased prognostic risk of prostate cancer (Gu et al., 2022). The majority of the prostate cancer patients in our study (68.94%) had blood creatinine levels that fell within the normal limit of 0.7-1.3 mg/dL. However, the prognosis risk was not a focus of our analysis as we did not measure survival rates in our study. XRCC1 and XPD polymorphisms have been linked to a higher risk of cancer, however, it is still unknown how SNPs modify clinical variables such as clinical stage, tumor grade, creatinine, and PSA levels. Hematuria and elevated levels of creatinine are symptoms of impaired renal function. The higher incidence of hematuria and higher serum creatinine levels associated with the XRCC1 homozygous wild-type Trp/Trp genotype suggests that individuals with this genotype are especially at risk for compromised kidney function. Even when serum creatinine levels are normal, Vacher and colleagues (Launay-Vacher et al., 2009) discovered that renal dysfunction is particularly frequent in men with prostate cancer. Although most of our cases had serum creatinine levels in the normal range, patients with the Trp/Trp genotype and prostate cancer should have had their renal function assessed.

4.5 Strengths and Limitations

Our control group comprises both hospital-based and non-hospital-based subjects, which minimizes the chance of a selection bias and thus is one of the strengths of our study. We excluded tribal or other ethnic groups from participating in this study, ensuring all subjects had a uniform genetic background. However, we were unable to sequence-cross-check our results due to financial constraints. To ensure the reproducibility of our results, genotype assays were

repeated on 10% of the randomly chosen samples chosen at random, and the repetitions were 100% concordant.

Conclusion

The identification of gene polymorphisms in prostate cancer-associated pathways might enhance early detection, enable targeted chemoprevention, and improve understanding of the complex biological processes involved in prostate carcinogenesis (Salvi et al., 2016). SNPs found in genes responsible for DNA repair show potential as noninvasive biomarkers with diagnostic and prognostic use. In the current molecular population-based case-control study we investigated whether the XRCC1 codon 194 and XPD 751 polymorphisms affect the incidence of prostate cancer in Bangladeshi men.

Our findings indicate a potential link between genetic variations and increased prostate cancer risk. We discovered that the codon 194 Trp/Trp variant of XRCC1 is associated with prostate cancer risk in Bangladeshi men. However, the XPD Lys751Gln gene polymorphism was not significantly associated with an elevated risk of developing prostate cancer. The XRCC1 Trp/Trp genotype was found to be associated with increased hematuria risk, higher mean PSA, and mean serum creatinine. Only higher mean serum creatinine levels were linked with the XPD 751 Gln/Gln genotype in prostate cancer patients. To examine the link between these polymorphisms and serum creatinine and PSA levels, additional research with larger sample sizes is required.

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