Republic of Iraq

Ministry of Higher Education and Scientific Research

University of Babylon

College of Medicine

Department of Chemistry and Biochemistry

Polymorphism of 3-beta-hydroxysteriod dehydrogenase type I (HSD3B1) and androgen receptor (AR) genes and their serum level in patients with prostate cancer (PCa) in middle Euphrates

Project for PhD Degree

in Clinical Biochemistry

Presented by:

Dr. Zainab Abdulhussein AbdYasser

Under supervision of

Prof. Dr.

Abdulsamie Hassan Alta'ee

PhD. Biochemistry

College of Medicine / University of Babylon

Prof. Dr.

Mohammed Ridha Joodi

FICMS (Uro)

College of Medicine / University of Babylon

صدق الله العلي العظيم

المجلة (11)

Dedication

To my family, to my mother and all people who might get benefit from this research
I dedicate this work

Zainab

Acknowledgements

First, and above all, I praise God, the almighty, for providing me this opportunity and granting me the capability to proceed to this stage.

Then, I would like to express my sincere gratitude to my advisor Prof. Dr. **Abdulsamie Hassan Alta'ee** for the continuous support, patience, motivation, and immense knowledge. His guidance helped me in all the time of my research and writing of this thesis.

Special thanks then go to my second advisor Prof. Dr. **Mohammed Ridha Joodi**.

In addition, my sincere thanks go to the staff of Department of Urology and archived in Department of Clinical and Molecular Pathology of the Imam Hussein (peace be upon him) Teaching Hospital in the Holy Karbala Governorate and Merjan Medical City in Babylon Governorate/ Iraq and Biochemistry Department at the College of Medicine. Without their precious support it would not be possible to conduct this research.

Moreover, I thank my fellows for their stimulating discussions, for working together before deadlines, and for all the fun we have had for the last two years.

Last, but not least; I would like to thank all the subjects who participated in this study for their help.

Zainab

Supervisors' Certification

We certify that this PhD. thesis entitled: -

" **Polymorphism of 3-beta-hydroxysteriod dehydrogenase type I (HSD3B1) and androgen receptor (AR) genes and their serum level in patients with prostate cancer (PCa) in middle Euphrates "**
was prepared under our supervision in laboratories at the Department of Biochemistry – College of Medicine / University of Babylon as a partial fulfillment of the requirements of **PhD Degree** in **Clinical Biochemistry**.

Prof. Dr.

Abdulsamie Hassan Alta'ee

PhD. Biochemistry

College of Medicine / University of Babylon

Prof. Dr.

Mohammed Ridha Joodi

FICMS (Uro)

College of Medicine / University of

In the view of the available recommendation, I forward this PhD. thesis for debate by the examining committee.

Prof. Dr. Abdulsamie Hassan Alta'ee

Head of Biochemistry Department

College of Medicine - University of Babylon

Examining Committee Certification

We, the examining committee, certify that we have read this PhD. thesis entitled: - " **Polymorphism of 3-beta-hydroxysteriod dehydrogenase type I (HSD3B1) and androgen receptor (AR) genes and their serum level in patients with prostate cancer (PCa) in middle Euphrates**

" We have examined the student (**Dr. Zainab Abdulhussein AbdYasser**), at the Department of Biochemistry-College of Medicine / University of babylon) as a partial requirement for the (PhD Degree in Clinical Biochemistry).

Index

Paragraph	Title	Page
	Abstract	I
	Abbreviations	V
	List of Tables	VIII
	List of Figures	IX
Chapter One: Introduction and Literature Review		
1.	Introduction and Literature Review	1
1.1.	Prostate	1
1.1.1	Definition and structure of prostate	1
1.1.2	Development of prostate	3
1.1.3	function of prostate	4
1.1.4	role of androgen in development of prostate	6
1.1.5	Conditions of prostate	7
1.1.5.1	Benign Prostatic Hyperplasia	7
1.1.5.2	Prostatitis	8
1.1.5.3	Prostate cancer	8
1.1.6	Risk Factors of Prostate Cancer	11
1.1.6.1.	Age of patient	12
1.1.6.2	Ethnicity of patient	13
1.1.6.3.	Genetic Changes of patient	13

1.1.6.4.	Inflammation	14
1.1.6.5	Smoking	15
1.1.6.6.	Obesity	15
1.1.6.7.	Hormones	16
1.1.6.7.1.	Insulin-Like Growth Factors	16
1.1.6.7.2.	Testosterone	17
1.2	AR and prostate cancer	18
1.2.1	Androgen receptor	21
1.2.2.	AR structure and function in prostate cells	21
1.2.3.	AR function in the adult prostate	26
1.2.4.	Generalized function of AR in prostate diseases	28
1.3.	3β-hydroxysteroid dehydrogenase-1	29
Chapter Two: Subjects, Material and Methods		
2.	Materials, Methods	32
2.1	Samples	32
2.1.1.	Patient group	32
2.1.2.	Control	32
2.1.3.	Inclusion Criteria	32
2.1.4.	Ethical Issues	32
2.1.5.	Specimen Collection	33
2.2.	Materials	33
2.2.1.	Instruments	33
2.2.2.	Chemical Materials	35
2.3.	Methods	36

2.3.1.	Molecular Analysis	36
2.3.1.1	Genomic DNA Extraction Protocol	36
2.3.1.2	Polymerase Chain Reaction	37
2.3.1.2.1	Polymorphism Detection for AR-E211	38
2.3.1.2.2	Agarose Gel Electrophoresis	38
2.3.1.2.3	Polymorphism Detection for HSD3B1 (rs1047303)	40
2.3.1.2.4	Amplification Reaction Mixture	40
2.3.2	Biochemical assay	41
2.3.2.1.	Determination of Human Androgen Receptor	41
2.3.2.1.1	Reagent Preparation	42
2.3.2.1.2	Assay Procedure	43
2.3.2.1.3	Calculation of Results	44
2.3.2.2.	Determination of Human 3 Beta-Hydroxysteroid Dehydrogenase Type 1 ELISA Kit	44
2.3.2.2.1	Reagent Preparation	44
2.3.2.2.2	Assay Procedure	46
2.3.2.2.3	Calculation of Results	47
2.4.	Statistical Analysis	47
Chapter Three: The Results		
3.1.	Demographic Comparison between Prostate Cancer and Control groups	48
3.1.1.	Age	48
3.1.2.	Gleason	49
3.1.3.	Family history	51

3.1.4.	Smoking	52
3.2.	Androgen Receptor (AR)	54
3.2.1.	Androgen Receptor Level	54
3.2.2.	Androgen Receptor ROC Curve	55
3.2.3.	Genetic Result for AR-E211 G>A Polymorphism	57
3.3	β-Hydroxysteroid Dehydrogenase Isoenzyme-1 (HSD3B1)	59
3.3.1.	β-Hydroxysteroid Dehydrogenase Isoenzyme-1 Levels	59
3.3.2.	HSD3B1 ROC Curve	60
3.3.3.	Genetic result for HSD3B1 (rs1047303) Genotyping	62
Chapter Four: Discussion		
4.1.	Demogrphic Comparison between Prostate Cancer and Control groups	66
4.1.1.	Age	66
4.1.2.	Gleason	66
4.1.3	Family history	68
4.1.4	Smoking	69
4.2.	Androgen Receptor (AR)	68

4.3.	β-Hydroxysteroid Dehydrogenase Isoenzyme-1 (HSD3B1)	71
Chapter Five: Conclusions and Recommendations		
5.1.	Conclusions	76
5.2.	Recommendations	77
Chapter Six: References		
6.1	References	78

Abstract

Background

Prostate cancer is the second most common non-cutaneous cancer in men and a leading cause of death. Prostate cancer is in fact very heterogeneous and potentially multifocal.

Androgen receptor (AR) has a role in the normal growth and development of the prostate gland, in prostate carcinogenesis and androgen-dependent (AD) or androgen-independent (AI) progression of the disease.

Androgens play critical roles in prostate carcinogenesis as well as prostate cancer progression. Dihydrotestosterone synthesis in prostate cancer from adrenal DHEA/DHEA-sulfate requires enzymatic conversion in tumor tissues. 3β-hydroxysteroid dehydrogenase-1 is an absolutely necessary enzyme for such dihydrotestosterone synthesis and is encoded by the gene HSD3B1

Objectives

The pathogenesis of prostate cancer (CaP) involves alterations in a gene structure of the androgen receptor (AR). The single nucleotide polymorphism AR-E211 G>A localized in exon 1 of the AR gene (G1733A) was detected using direct polymerase chain reaction and restriction digestion (PCR-RFLP) method on blood. The genetic polymorphism in HSD3B1 encoding 3β-hydroxysteroid dehydrogenase-1 has been shown to be associated with oncological outcome and develop of CaP.

Subjects and method

Blood samples were obtained from 90 healthy group and 90 patients at the 54–88 ages diagnosed with histopathological confirmed prostate cancer (CaP). The CaP

patients were recruited from Department of Urology and archived in Department of Clinical and Molecular Pathology of the Imam Hussein (peace be upon him) Teaching Hospital in the Holy Karbala Governorate and Merjan Medical City in Babylon Governorate/ Iraq.

PCR-RFLP directly from blood. Agarose gel electrophoresis showing 416 bp PCR products detected after PCR where blood was used as a template. A 416 bp DNA fragment indicates the presence of the allele A. The allele G is determined a presence of the 329 bp and 87 bp DNA fragments.

Genome DNA was obtained from patient whole blood samples, and genotyping on HSD3B1 (rs1047303, 1245C) was performed by sent the samples to Macrogen Inc. Geumchen, Seoul, South Korea company.

Results

The statistical analysis showed significant differences between the healthy and patient group for the age groups (60-64), (65-69), and (70-74) years, while no significant difference appeared between the age group (55-59) and the healthy and sick groups, as well as for the age group (> 75) while the statistical analysis showed a high significant among all age groups studied between patients and healthy subjects.

The results of the statistical analysis indicated that there is no significant relationship appeared between cancer and healthy people living in rural and urban areas.

There was a highly significant relationship, and there were also high significant differences between the group of patients as well as in terms of the presence or absence of history between the healthy group and the injured group.

Abstract

The results of the statistical analysis showed that there were significant differences between smokers for the two groups of patients and healthy people. The statistical results also high statistical signs were recorded between smokers and non-smokers in patients and control group.

There was no significant difference in AR serum level between patients and control group, the mean value in both groups were compared using t test (p value =0.42) which is not significant at p value<0.05 (mean AR serum level in patient =9.709, mean AR serum level in control= 9.05).

The genotypes association of AR. The model codominant was non-significant change with (P = 0.7) when compared with healthy control.

The model dominant was shown non-significant change with (P = 0.7) when compared with healthy control according to the percentage of A/G –G/G is genotypes response and related with disease RA. The recessive model was shown non-significant change with (P = 0.7) when compared with healthy control.

The present study measures the serum level of HSD3B1 in PCa patient and noted if change and has role in disease or change with gene mutation. The mean value of HSD3B1 serum level in the patients was 5.91 ng/ml. The mean serum value of HSD3B1 in control is 6.20 ng/ml. although the mean serum level of HSD3B in patients group seems to be less than that of the control group but he difference was not statically significant (P value 0.82).

There was significant difference in expression of SNP between patients group and control group. where it was expressed more often in patients than in control. P value 0.015 which is significant at p value <0.05.

Conclusion:

Prostate cancer is more commune in elderly men than middle and young men. Also this cancer is distributing in rural areas more than in urban areas as shown in result and discussion. The genetic factors have main role in development of CaP in HDS3B1 and many other genes. The smoker is one of the important and influencing factors on the incidence of prostate cancer. The AR play important role in CaP progression, there was **no** significant difference in AR serum level between patients and control group. Frequency of alleles of the AR-E211 G>A polymorphism in blood samples. The genotypes association of AR the model codominant was non-significant change when compared with healthy control. The all AR polymorphism not significant with CaP. The study measures the serum level of this enzyme in PCa patient and noted if change and has role in disease or change with gene mutation. Although the mean serum level of HSD3B in patients group seems to be less than that of the control group but the difference was not statically significant. HSD3B1 (rs1047303) genotyping was performed by sequencing technology. There was significant difference in expression of SNP between patients group and control group where A allele changes to C allele.

Abbreviations

Abbreviations	Meaning
AR	Androgen receptor
AD	androgen-dependent
AI	androgen-independent
ADT	androgendeprivation therapy
AF	activation function
BPH	Benign prostatic hyperplasia
CRPC	castration-resistant prostate cancer
CaP	Cancer of prostate
CNP/CPPS	chronic nonbacterial prostatitis/ chronic pelvic pain syndrome
D4A	Δ4-abiraterone
DBD	DNA-binding domain
DHT	dihydrotestostero
DHEA	Dehydroepiandrosterone
DRE	Digital rectal examination
EHPCCG	Endogenous Hormones and Prostate Cancer Collaborative Group
EDTA	ethylenediaminetetraacetic acid
ETS	E-twenty-six
FOXA1	fork head box A1
gDNA	genomic DNA

Abbreviations

Abbreviations	Meaning
GnRH	gonadotropin-releasing hormone
GWAS	Genome-wide association studies
HSD3B1	3β-hydroxysteroid dehydrogenase-1
HSP	heat shock protein
HSPC	hormone-sensitive prostate cancer
IGF	Insulin-like growth factors
IGFBP	Insulin-like growth factors binding proteins
LBD	C-terminal ligand-binding domain
LUTS	lower urinary tract symptoms
mpMRI	Multiparametric magnetic resonance imaging
NFIB	nuclear factor IB
NIH	National Institutes of Health
NTD	N-terminal domain
PCa	Prostate cancer
PCR	Polymerize chain reaction
PPV	positive predictive value
PSA	Prostate specific antigene
RFLP	Restriction Fragment Length Polymorphism
RNS	reactive nitrogen species
ROS	reactive oxygen species
SHBG	sex hormone binding globulin
SNPs	single nucleotide polymorphisms
SRD5A1	5a-reductase 1
SRD5A2	5a-reductase 2

Abbreviations

Abbreviations	Meaning
SRD5A3	5a-reductase 3
T	Testosterone
TBE	Tris Borate EDTA
TOP2B	topoisomerase II beta
UGE	urogenital epithelium
UGM	urogenital mesenchyme

List of Tables

Number	Title	Page
Chapter 2		
2-1	laboratory equipment and supplies	34
2-2	chemical and biological materials used in the research	35
2-3	AR-E211 G>A forward primer and reverse primer	37
2-4	Primers Detection for HSD3B1 (rs1047303)	40
Chapter 3		
3-1	The effect of age on the incidence of the disease	48
3-2	The correlation	50
3-3	Distribution of Patient and Control Groups According to Family History	51
3-4	Distribution of Patient and Control Groups According to Smoking	53
3-5	comparison of AR serum between patient and control groups.	54
3-6	values of ROC curve to AR serum level	56
3-7	Allelic Association of AR Gene Polymorphism in Prostate Cancer Males Cases and Controls Males	59
3-8	comparison between patient and control groups for HSD3B1.	60
3-9	values of ROC curve to HSD3B1 serum level	61
3-10	Allele Frequency of HDS3B1 gene	65
3-11	Chi-square statistic analysis	65

List of Figures

Number	Title	Page
Chapter 1		
1-1	Side View of the Prostate	1
1-2	The Function of risk factor in the Growth of Cancer	12
1-3	The Androgen Signaling Pathway Promotes Terminal Differentiation of Normal Prostate Cells	14
1-4	The unique molecular features of the androgen receptor and its coregulatory recruitment	25
1-5	Pathway of steroid biosynthesis and the conversion of T to DHT	26
Chapter 2		
2-1	standard curve for ELISA assay to AR serum level	44
2-2	standard curve for ELISA assay to HSD3B1 serum level	47
Chapter 3		

List of Figures

3-1	Predictive Value Section for Patient Using the Empirical ROC Curve for Androgen Receptor Level	56
3-2	Electroph. For pcr prod. to the AR gene on 70 volt for 1 hr 3% agaros.	57
3-3	PCR-RFLP of AR-E211 G>A Polymorphism after Addition of restriction enzyme.	58
3-4	Predictive Value Section for Patient Using the Empirical ROC Curve for HSD3B1	62
3-5	Electroph. For pcr prod. To the HSD3B1 gene on 70 volt for 1 hr. 2% agaros.	63
3-6	site of A → C snp for HDS3B1 gene	64

1. Introduction and Literature Review

1.1. Prostate:

1.1.1. Definition and structure:

The prostate is both an accessory gland of the male reproductive system and a muscle-driven mechanical switch between urination and ejaculation. The prostate gland is of the male reproductive system. In adults, it is about the size of a walnut (1) and it is an average weight of about 11 grams, usually ranging between 7 and 16 grams (2). The prostate is located in the pelvis. It sits below the urinary bladder and surrounds the urethra. The part of the urethra passing through it is called the prostatic urethra, which joins with the two ejaculatory ducts (1). The prostate is covered in a surface called the prostatic capsule or prostatic fascia figure (1-1) (3).

The internal structure of the prostate has been described using both lobes and zones (4)(1). Because of the variation in descriptions and definitions of lobes, the zone classification is used more predominantly (1).

The prostate has been described as consisting of three or four zones (1)(3). Zones are more typically able to be seen on histology, or in medical imaging, such as ultrasound or MRI (1)(4).

The prostate gland divides anatomically into five lobes: anterior and posterior lobes, two lateral lobes, and one median lobe. This description is in many anatomical textbooks. In clinics, it is described as having two lateral lobes right and left and a median lobe. The prostate gland is composed of histologically different zones; based on these, the gland divides into three anatomical zones [5]

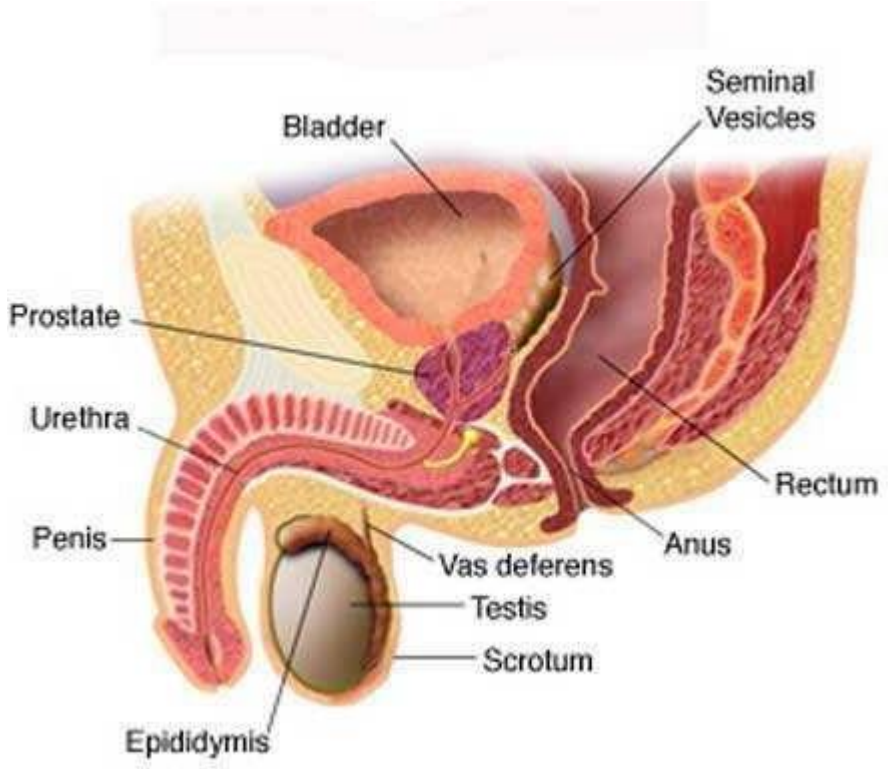


Figure (1-1) Side View of the Prostate (3)

About 20,000 protein coding genes are expressed in human cells and almost 75% of these genes are expressed in the normal prostate (6)(7). About 150 of these genes are more specifically expressed in the prostate, with about 20 genes being highly prostate specific (8). The corresponding specific proteins are expressed in the glandular and secretory cells of the prostatic gland and have functions that are important for the characteristics of semen, including prostate-specific proteins, such as the prostate specific antigen (PSA), and the Prostatic acid phosphatase (9).

Normally, the prostate reaches its mature size at puberty, between ages 10 and 14. Around age 50, the size of the prostate and the amount of its secretions

commonly decrease. Enlargement of the prostate in size after midlife, often making urination difficult, may occur as a result of inflammation or malignancy (9).

It contains a system of branching ducts comprising pseudo-stratified epithelium surrounded by a fibromuscular stroma. The prostate is a male sex accessory gland that functions by producing and secreting fluids that contribute to the ejaculate, and thereby significantly enhances male fertility. Intriguingly, the prostate is highly susceptible to oncogenic transformation at a frequency significantly greater than that of other male secondary sexual tissues, such as the seminal vesicles. Indeed, approximately one in seven men will be diagnosed with prostate cancer during their lifetime (Siegel et al., 2016) (10).

1.1.2.Development of prostate:

The prostate gland starts to develop laterally as epithelial buds from the urogenital sinus wall. These buds branch into solid cords which canalize to form the ducts and acini. The surrounding urogenital sinus mesenchyme forms the inter fascicular fibroblasts and the smooth muscle of the prostate (11). With androgenic stimulation of the androgen receptor expressed in prostatic Mullerian mesenchyma, the prostate start to forms (12). In development, androgen secretion by Leydig cells is the chief regulator of prostate growth (13).

In the developing embryo, at the hind end lies an in pouching called the cloaca. This, over the fourth to the seventh week, divides into a urogenital sinus and the beginnings of the anal canal, with a wall forming between these two inpouchings called the urorectal septum (14). The urogenital sinus divides into three important parts, with the middle part forming the urethra; the upper part is largest and becomes the urinary bladder, and the lower part then changes depending on the biological sex of the embryo (14).

The prostatic part of the urethra develops from the middle, pelvic, part of the urogenital sinus, which is of endodermal origin (15). Around the end of the third month of embryonic life, outgrowths arise from the prostatic part of the urethra and grow into the surrounding mesenchyme (15). The cells lining this part of the urethra differentiate into the glandular epithelium of the prostate (15). The associated mesenchyme differentiates into the dense connective tissue and the smooth muscle of the prostate (16).

Condensation of mesenchyme, urethra, and Wolffian ducts gives rise to the adult prostate gland, a composite organ made up of several tightly fused glandular and non-glandular components. To function properly, the prostate needs male hormones (androgens), which are responsible for male sex characteristics. The main male hormone is testosterone, which is produced mainly by the testicles. It is dihydrotestosterone (DHT), a metabolite of testosterone, that predominantly regulates the prostate. The prostate gland enlarges over time, until the fourth decade of life (17).

1.1.3.Function of prostate:

The prostate secretes fluid which becomes part of semen or seminal fluid. Semen is the fluid emitted (ejaculated) by males during the sexual response The prostate secretes fluid that nourishes and protects sperm. During ejaculation, the prostate squeezes this fluid into the urethra, and it's expelled with sperm as semen (18).] When sperm is emitted, it is transmitted from the vas deferens into the male urethra via the ejaculatory ducts, which lie within the prostate gland. Ejaculation is the expulsion of semen from the urethra (18). Semen is moved into the urethra following contractions of the smooth muscle of the vas deferens and seminal vesicles, following stimulation, primarily of the glans penis. Stimulation sends nerve signals via the internal pudendal nerves to the upper lumbar spine; the

nerve signals causing contraction act via the hypogastric nerves. After traveling into the urethra, the seminal fluid is ejaculated by contraction of the bulbocavernosus muscle (18). The secretions of the prostate include proteolytic enzymes, prostatic acid phosphatase, fibrinolysin, zinc, and PSA (prostate specific antigen), which can be raised in patients who have an enlarged prostate gland (BPH), prostate cancer, urinary tract infection, or following recent surgery or instrumentation (eg: putting in a catheter) to the lower urinary tract (19). Together with the secretions from the seminal vesicles, these form the major fluid part of semen (19).

It is possible for some men to achieve orgasm solely through stimulation of the prostate gland, such as via prostate massage (20)(21). This has led to the area of the rectal wall adjacent to the prostate to be popularly (yet inaccurately) referred to as the "male G-spot" (22). The prostate's changes of shape to do it function, which facilitate the mechanical switch between urination and ejaculation, are mainly driven by the two longitudinal muscle systems running along the prostatic urethra. These are the urethral dilator (musculus dilatator urethrae) on the urethra's front side, which contracts during urination and thereby shortens and tilts the prostate in its vertical dimension thus widening the prostatic section of the urethral tube (23)(24), and the muscle switching the urethra into the ejaculatory state (musculus ejaculatorius) on its backside (25).

In case of an operation, e.g. because of benign prostatic hyperplasia (BPH), damaging or sparing of these two muscle systems varies considerably depending on the choice of operation type and details of the procedure of the chosen technique. The effects on postoperational urination and ejaculation vary correspondingly (26).

1.1.4. role of androgen in development of prostate:

The prostate is completely dependent on testicular androgens for both its development and the maintenance of its structural and functional integrity. In humans this is reflected in the natural history of the organ. The prostate is small in boys during childhood, weighing around 2 g. At puberty it undergoes a phase of exponential growth, increasing in size to about 20 g. This corresponds to the rise in serum testosterone to adult levels. The human prostate reaches its normal adult size by 18e20 years of age and subsequently halts growth despite sustained circulating levels of androgen. This transition from prostatic growth to a steady-state phase is maintained by a balance of cell proliferation/death and is controlled by AR signaling in both the stroma and epithelium. After puberty, mean prostatic weight stabilizes and remains fairly constant until the end of the fifth decade of life. At this point, mean prostatic weight in the population begins to rise slowly, predominantly reflecting the incidence of BPH as men age (27). The AR is a transcription factor that mediates the effects of androgens in concert with a number of, often tissue specific, co-factors (28,29). The AR is expressed early in prostatic development and androgens are essential for development of the prostate gland and other reproductiveorgans in males in utero (30). The AR is expressed in the mesenchyme of the fetal urogenital sinus and androgen action on these stromal receptors is necessary to induce the initiation of prostatic buds in the epithelium of the sinus. AR expressionin the epithelium lags that of the mesenchyme but can be detected in mice as epithelial prostatic buds start to form around embryonic Day 18 (31). Throughout development, epithelial AR signaling increases during epithelial bud protrusion and extension (32,33). Deletion and mutations of the AR leading to a nonfunctional receptor result in the lack of a prostate, showing the AR is critical for prostatic cell growth and development (34). Work by Cunha and others demonstrated that stromal

AR is required for epithelial cell proliferation and differentiation during normal development, while epithelial AR expression is essential for the differentiated functions of the gland (35,36). The primary source of androgens, namely testosterone, is from testicular secretion. Once androgens reach prostatic tissue, three 5a-reductase isozymes (produced by the SRD5A1, SRD5A2, and SRD5A3 genes) convert testosterone to dihydrotestosterone (DHT), which binds the receptor with two-to five-fold higher affinity in prostate cells and elevates AR signaling 10-fold compared to testosterone (37,38). In the prostate the dominant 5a-reductase is isozyme 2. Individuals without functional 5a-reductase 2 (SRD5A2) have small or undetectable prostates in addition to ambiguous genitalia throughout childhood, but at the time of puberty increased testosterone production induces masculinization of the external genitalia. These individuals maintain small prostates throughout adulthood but treatment with DHT can increase prostate size. Together, these data indicate that DHT is required for complete development of the adult prostate (39). Androgen availability, AR expression, and 5a-reductase activity all serve critical roles in prostate development.

1.1.5 Conditions of prostate:

1.1.5.1 Benign Prostatic Hyperplasia:

Benign Prostatic Hyperplasia (BPH) is an age-related and progressive neoplastic condition of the prostate gland (40). BPH may only be defined histologically. BPH in the clinical setting is characterized by lower urinary tract symptoms (LUTS). There is no causal relationship between benign and malignant prostatic hypertrophy (41).

1.1.5.2 Prostatitis:

Prostatitis is a common urinary tract condition that many doctors find problematic to treat effectively. It is estimated that half of all men suffer from symptoms of prostatitis at some stage in their lives (42). It is the 3rd most commonly found urinary tract disease in men after Benign Prostate Hyperplasia (BPH) and prostate cancer (43,44). Prostatitis may account for 25% of all office visits made to the urological clinics complaining about the genital and urinary systems all over the world and up to 15–25% of visits to the clinics of urology in Korea (45). Contrasting to prostate cancer and Benign Prostate Hyperplasia (BPH), which are predominantly diseases of elder men, prostatitis affects men of all ages, especially high impact on the middle age group. In more recent studies, it has shown that the occurrence of prostatitis ranges from 3 to 16% in Europe, North America and Asia (46), of which half have repeated episodes (47) highlighting that prostatitis is an important universal health problem.

Classification of prostatitis by the National Institutes of Health (NIH) has classified prostatitis into new categories. According to this classification, acute and chronic bacterial prostatitis remains the same. There is a formation of a new group called chronic nonbacterial prostatitis/ chronic pelvic pain syndrome (CNP/CPPS). This new group is a combination of chronic nonbacterial prostatitis and prostadynia prostatitis. The fourth and final category is the asymptomatic prostatitis (48).

1.1.5.3 Prostate cancer:

Prostate cancer is the second most common non-cutaneous cancer in men and a leading cause of death, with an estimated 174,650 new cases and 31,620 deaths in the United States in 2019 (49). Age and family history are key risk factors for

prostate cancer, and black men have a higher risk of prostate cancer incidence and death compared to men from white or Asian backgrounds (50).

The majority of cases of prostate cancer are diagnosed in men from western countries in the Americas and Europe, and this has largely been driven by the introduction of prostate-specific antigen (PSA) for prostate cancer detection in the 1990s. The widespread use of PSA has proven controversial as the evidence for benefit as a screening test in asymptomatic men is still subject to debate, and PSA is prone to false positives and false negatives in men with symptoms suggestive of a possible diagnosis of prostate cancer (51). Work is ongoing to find better ways to risk stratify men for further investigation, and more accurate methods to differentiate between clinically significant and clinically insignificant prostate cancer to inform treatment decisions.

Most prostate cancer diagnoses are made in symptomatic men. Prostate cancer should be suspected in men over 50 years old presenting with lower urinary tract symptoms (LUTS), visible haematuria or erectile dysfunction (52). LUTS are also a common presenting symptom of benign conditions affecting the prostate, such as benign prostatic hyperplasia (BPH) and prostatitis, creating a diagnostic challenge. There is no strong evidence of association between the severity of LUTS and the likelihood of prostate cancer or the stage at diagnosis (54). Digital rectal examination (DRE) is recommended in many countries alongside PSA to aid decision-making about referral for diagnostic testing. A recent systematic review suggests that DRE has a high specificity and positive predictive value (PPV) for prostate cancer in symptomatic patients. Many prostate cancers are detected on the basis of elevated plasmatic levels of prostate-specific antigen (PSA > 4 ng/mL), a glycoprotein normally expressed by prostate tissue. However, because men without cancer have

also been found with elevated PSA, a tissue biopsy is the standard of care to confirm cancer's presence (54).

In light of the limitations of PSA, a number of other tests have been investigated to aid the diagnosis of clinically significant prostate cancer. PSA is a kallikrein serine protease, and other related biomarkers have been assessed for a potential role in prostate cancer detection (55). Genome-wide association studies (GWAS) have identified more than 100 single nucleotide polymorphisms (SNPs) related to risk of prostate cancer (56). Multiparametric magnetic resonance imaging (mpMRI) has gained much interest in recent years, both as a diagnostic test for prostate cancer and for monitoring men with localised prostate cancer on active surveillance for signs of disease progression (57).

Prostate cancer may be asymptomatic at the early stage and often has an indolent course, and may require minimal or even no treatment. However, the most frequent complaint is difficulty with urination, increased frequency, and nocturia, all symptoms that may also arise from prostatic hypertrophy. More advanced stage of the disease may present with urinary retention and back pain, as axis skeleton is the most common site of bony metastatic disease. Diet and physical activity play an important role in prostate cancer development and progression. Dietary factors are mainly associated with the observed worldwide and ethnic differences in the incidence rates of prostate cancer (55,57).

Although the AR is involved in diverse activities, its primary functions are related to male physiology, such as sex differentiation and sex-specific pathology (58). Defects in the AR gene can prevent the normal development of both internal and external male structures in 46, XY individuals and result in androgen insensitivity syndrome, which is the partial or complete inability of cells to respond to androgens (59,60). Defects in the AR gene can be caused by four types of

mutations: (i) Single point mutations resulting in substitutions or premature stop codons, (ii) nucleotide insertions and deletions resulting in frameshifts, (iii) complete or partial deletion of the gene, or (iv) intronic mutations that affect AR RNA splicing (61). The 1029 distinct mutations have been identified in the human AR gene and are distributed predominantly over the AR DBD- and LBD-coding regions. These mutations are well documented in the Androgen Receptor Gene Mutations Database World Wide Web Server at the Lady Davis Institute for Medical Research (62). Several investigations have associated the polymorphic polyglutamine repeats in the NTD with Kennedy's Disease, also known as spinobulbar muscular atrophy, a progressive neurodegenerative condition (63,64,65). There are an increasing number of studies relating the action of the AR to breast (66), larynx (67), liver (68), and testicular cancers (69).

1.1.6. Risk Factors of Prostate Cancer:

Causal factors to cause cancer usually fall into two categories, environmental and genetic. The 90-95% are environmental factors or lead to them, while the 5-10% of cancer has been genetic factors (70) as shown in figure 1-2(71).

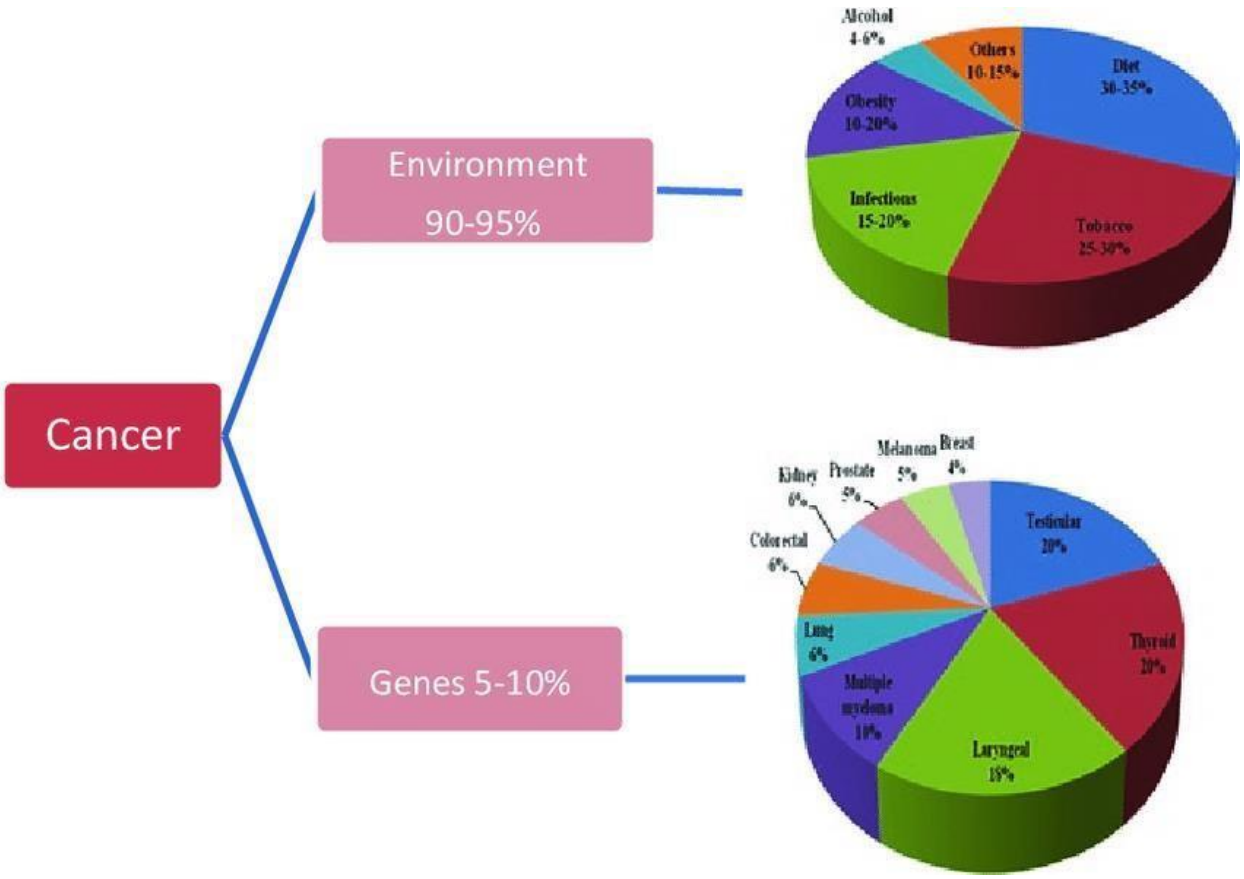


Figure 1-2. The Function of risk factor in the Growth of Cancer (71).

Age, ethnicity and family history of PCa are widely recognized risk factors. There were also unknown factors affecting PCa risk, including education, workplace, fat, meat, smoking habits, alcohol consumption, marriage problems, vasectomy, sexual behavior, diabetes mellitus, and the like. In addition, the probable risk factors were described, such as the sex steroid hormones and the insulin-like growth factor (72).

1.1.6.1. Age

The incidence of prostate cancer rises with age; thus, the prevalence of PCa in people over 65 years of age can still rise. Prostate cancer is the most prevalent disease among older men (73). About 64% of new United States PCa cases in men older than 65 years and 23% in men older than 75 years of age have been diagnosed (74).

While the prevalence of disease in the elderly has been expected to increase, most studies investigating optimal treatment regimens have focused on men under 75 years of age (75).

1.1.6.2. Ethnicity:

Ethnicity is one of the PCa risk factors (76). Data of PCa incidence in England indicate that Black men (Black Caribbean, Black African, and Other Black) are significantly more likely, and Asian men (Pakistani, Bangladeshi, Indian, and Other Asian) significantly less likely, to be diagnosed with the disease compared to white men (77). The Prostate Cancer in Ethnic Subgroups study (PROCESS) (78), and others (79,80), calculated Black men are 2 to 3 times more likely to be diagnosed with PCa compared to white men of the same age in the UK. In addition, the PROCESS study showed black men may be diagnosed 5 years younger than white men (81,82), despite equal access to diagnostic services between ethnic groups (82).

1.1.6.3. Genetic Changes:

Prostate cancer is in fact very heterogeneous and potentially multifocal. Age and ethnicity are among the most important risk factors (83). In addition to these factors, a well-established risk factor is genetic predisposition. Throughout most of the years, several essential inherited elements in PCa susceptibility have been identified in genetic-epidemiological studies (84). This type of cancer is tissue-dependent on androgen; PCa etiopathological investigation tend to be targets most promising for genes along the steroid hormone pathways (85). In addition, there are studies that have also detected a connection between PCa risks and polymorphisms through androgen's metabolism in several genes, including the

androgen receptor (86). and 5–reductase, that catalyze dihydrostosterone (DHT) as its active form (87), as shown in figure 1–3(88).

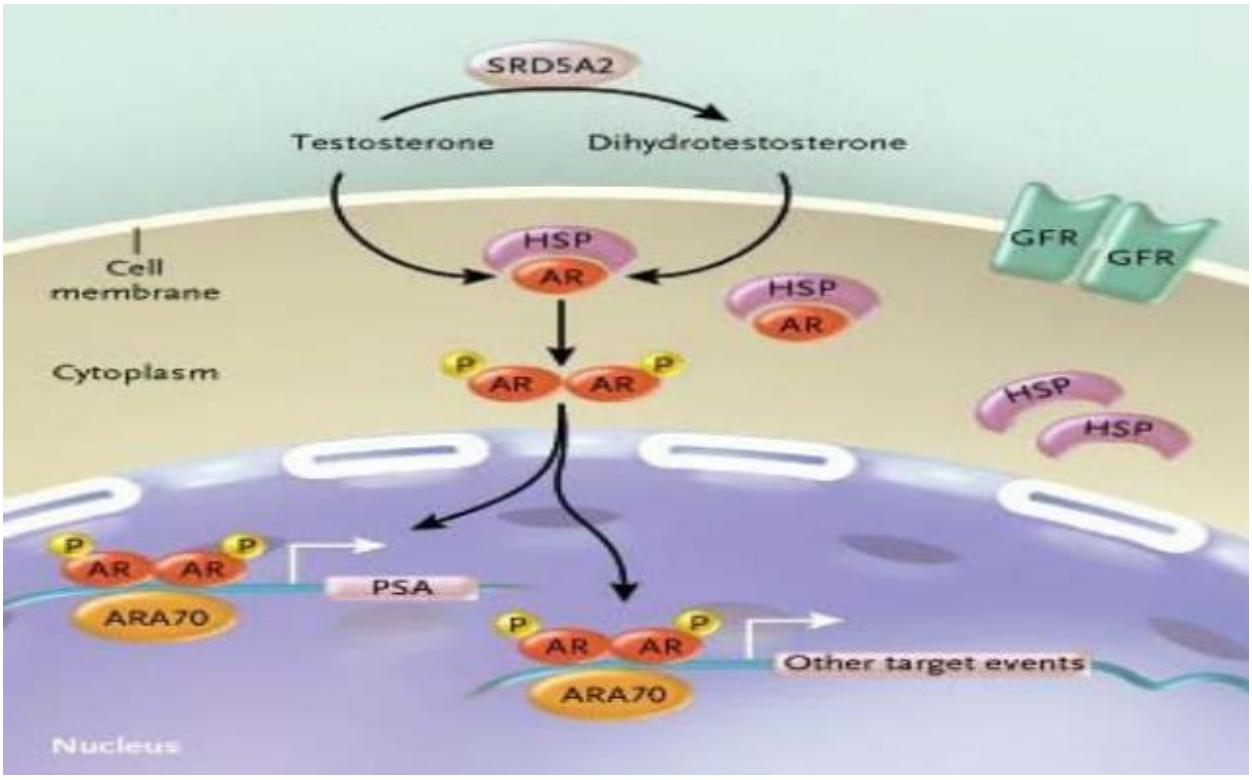


Figure 1-3. The Androgen Signaling Pathway Promotes Terminal Differentiation of Normal Prostate Cells (88).

1.1.6.4. Inflammation:

Inflammation is a basic physiological process that can occur for disease, after ischemic, toxic or autoimmune damage in any tissue.

Inflammation causes damage to cells and genomes, and promotes cellular transformation liked to a sustained inflammatory microenvironments providing a constant supply to a wide range of reactive nitrogen and oxygen species, chemokines, reactive aldehydes, cytokines, and growth factors that may influence

essential biological processes responsible for maintaining normal cell homeostasis (89).

Inflammation is an adaptive and innate immune response after the infection or injury. Innate human immune system begins an inflammation response with the production of a wide range of cytokine, reactive oxygen species (ROS), as well as reactive nitrogen species (RNS) (90). Continuous cytokine, ROS, and RNS supplies can, over time, cause genomic instability in a microenvironment with continuous inflammation; and tumor development after that (91).

1.1.6.5 Smoking:

Smoking is significant for many cancers and an association between PCa and cigarette smoking has not been shown by most studies (92). Cigarette smoking can stimulate carcinogenesis in the prostate and may result in increased exposure to carcinogenic compounds such as nitrosamines, heterocyclic aromatic amines and polycyclic aromatic hydrocarbons (93).

The effects of environment play a role in PCa development as the probability of PCa has risen five times with environmental change as migration studies by Japanese immigrants to the United States have shown. Cigarette smoking is a significant potential risk factor (94).

1.1.6.6. Obesity:

In addition, there has been accumulation of evidence that obesity is a significant risk for many malignancies, including aggressive PCa (95). Adipose tissue dysfunction activity was widely recognized as a major cause for cancer, which is often seen in obesity (96). The prostatic gland has a capsular structure and adipose

tissue surrounds it. Sometimes, prostate tumor cells enter the fat pad by transposition or penetration of the capsule, leading to immediate proximity to the fat tissue. Once cancer cells extend beyond the capsule, the per prostatic adipose tissue–secreted factors, extracellular matrix components or direct cell–cell contact may influence the phenotypic behavior of malignant cells (97).

1.1.6.7. Hormones:

1.1.6.7.1. Insulin-Like Growth Factors:

Insulin-like growth factors (IGF) and their associated binding proteins (IGFBP) are involved in the regulation of cell proliferation, differentiation, and apoptosis, and there has been a considerable interest in their role in the development of prostate cancer. Previous individual prospective studies and our 2008 pooled analysis of individual participant data from 3,700 men with prostate cancer in the Endogenous Hormones and Prostate Cancer Collaborative Group (EHPCCG) have indicated that men with high IGF-I concentrations have an elevated risk for the disease (98). However, insufficient data were available to provide accurate risk estimates for increased prostate cancer (both from the case number and the number of tests studied), either individually or combined, with the concentrations for other IGF biomarkers of the axis (IGF-II, IGFBP1 and IGFBP -2). Further, previous research failed to check out whether circulating IGF concentration affects prostate initiation or development, or whether both, of the advanced or of high-grade diseases (99,100).

The potential role of reverse causalities in understanding the relation between IGF-I and prostate cancer risk also needs further study, with the preclinical tumors potentially influence the IGF levels at the blood drawing level in both cross-sectional and prospective research, especially with short lag between blood collection and a

relatively short period of time between diagnosis and blood collection. The EHPCCG (now extended as the Endogenous Hormones, Nutritional Biomarkers and Prostate Cancer Collaborative Group, EHNBPCCG) was developed to conduct cooperative reanalyses of data collected on the association between prediagnostic circulating sex hormone and IGFs concentrations and risk for prostate cancer (101).

1.1.6.7.2. Testosterone:

In men, most (approximately 90 percent) testosterone is synthesized by Leydig cells in the testes and about 10 percent more are generated in the adrenal glands. Testosterone is irreversibly transformed by 5 alpha- reductase enzyme into primary effector androgen, 5 α -dihydrotestosterone (DHT). DHT binds to the cytoplasmic receptor and the DHT androgen receptor complex moves into the cell nucleus where it activates the androgen- gene transcription (102). Normal gene expression is required to balance proliferative signals with apoptotic signals to support healthy prostatic tissue and to produce PSA and other prostate proteins (103).

The levels of testosterone go up and down, and for each point there are typical values. As a result, people change in childhood, adolescence, teens and adulthood. As an older adult, these changes may be linked to their testosterone levels. All these variations in testosterone levels during life influence the behavior and health of men. The rise in testosterone levels is linked to a concern for sex and high sperm count in puberty and youth (104).

A link between testosterone and PCa is that, PCa is a major concern in men with symptoms of lower urinary tract, especially if they suffer from their family history. Testosterone levels in men have basic facts that raise the risks of prostate cancer, but this theory is valid when Huggins and Hodges published their study evaluating castration, estrogens, and androgen injections on prostate cancer in 1941.

They concluded that development and growth of prostate cancer are directly related to androgenic activity (105).

However, several studies find the correct opposite years later in the evaluation of testosterone replacement therapy (106). behind this discrepancy, Huggins and Hodges have still the original androgen theory, and prostate cancer can be treated usefully. However, there are many side effects which hormone therapy may have. Testosterone contributes in vitro to prostate cancer cell proliferation and tumor development. Cancer cells undergo apoptosis (cell death) because they have no testosterone (107).

In fact, the experiments are only in vitro, in optimal laboratory conditions which a living human probably doesn't follow. In reality serum testosterone levels are not representative of intra prostatic levels (those in the blood). In other words, there may be a significant rise in free blood testosterone in patients that undergo testosterone therapy. Nevertheless, the testosterone levels do not change considerably in the prostate tissue (108).

Additionally, the problem seems to have led to the production of androgens in cancer cells and their surrounding tissue, which added to the problem independent of their levels of serum testosterone. Through that view, we can look at a Miyoshi *et al.* analysis. The prostate and testosterone levels in 196 prostate cancer patients were examined by researchers. Higher levels of testosterone were associated with higher Gleason scoring for advanced or aggressive prostate cancer. However, they also found that testosterone levels remain the same in both, when evaluating the benign and malignant portions of each biopsy (109).

1.2 AR and prostate cancer:

AR activity is intimately linked to prostate cancer, which is far the most commonly diagnosed cancer among American men and the second leading cause of

cancer death (110,111). In 2010, direct medical costs for prostate cancer were projected to reach $12 billion and are expected to further increase by 2020 (112). Of the 1029 mutations found in gene that encodes the AR, 159 mutations predispose males to prostate cancer (62). Previous work has suggested that the length of the repeats in the NTD influences prostate cancer risk in men (113,114). A meta-analysis of 19 studies including Caucasian, African-American and Asian subjects predicted an increased risk of prostate cancer in men with shorter (≤21) CAG repeats. However, a Swedish study suggests that men with shorter AR CAG lengths (eg, ≤22 repeats) are at a greater risk of developing prostate gland cancer. Other studies found no association between the AR CAG repeat length and prostate cancer risks (115). Although evidence that mutations in the AR predispose men to prostate cancer is undisputed, AR NTD CAG repeat length association with prostate cancer risk thus remains controversial.

Prostate cancer cells, like normal prostate cells, require androgens to grow and survive. Growth of prostate cancer depends on the ratio of the rate of cell proliferation to the rate of cell death (116). In prostate cancer, the rate of proliferation is higher than that of death, resulting in continuous net growth. Androgens and the AR are the main regulators of this ratio. More than 70 years ago, Charles HUGGINS demonstrated that androgen deprivation by orchiectomy (removal of the testes) caused regression of prostate cancer (117,118). Increased serum levels of PSA suggest that AR activity is elevated in prostate cancer patients. According to the American Cancer Society, a PSA level above 4 ng/mL has been recognized to be abnormal, and these patients are advised to undergo a biopsy (111,119).

The initiation of prostate cancer can be attributed to the activation of distinct growth-promoting pathways. One prominent example is the androgen-dependent

upregulation of members of the E-twenty-six (ETS) family of transcription factors by gene fusions between the AR-regulated TMPRSS2 gene promoter and the coding region of the ETS family members erythroblast transformation-specific (ERG) and ETS variant 1 (ETV1), that have been estimated to occur in ~50% of prostate tumors (120,121,122). These fusions confer androgen responsiveness to ETS transcription factors, that lead to cell-cycle progression. The induction of this fusion is itself dependent on the DHT/AR-stimulated recruitment of three DNA-directed enzymes, activation-induced cytidine deaminase (AID), LINE-1 repeat-encoded ORF2 endonuclease, and topoisomerase II beta (TOP2B), that trigger chromosomal translocation (123,124). Other signaling pathways shown to be involved in prostate cancer initiation and progression include the PI3K and RAS/RAF pathways; dysregulation of these pathways in both early and late stage prostate cancer was implicated through genomic profiling (125). In this study, analysis of the AR signaling pathway revealed a greater alteration compared with the other pathways, indicating that the AR is still the "master regulator" of prostate cancer.AR pathway perturbation is the mechanistic rationale for the use of androgen suppression methods to treat prostate cancer. Initial treatment includes androgen suppression via castration through surgical (orchiectomy) or chemical (gonadotropin-releasing hormone (GnRH) analogues such as leuprolide and goserelin) means (126). GnRH agonists desensitize the GnRH receptor by interrupting its physiological intermittent stimulation, whereas the GnRH antagonist degarelix blocks GnRH stimulation directly (127). Patients are then placed on androgen deprivation therapy, which is usually combined with leuprolide for total androgen blockade (128,129).

1.2.1 Androgen receptor:

Androgen receptor (AR) has a role in the normal growth and development of the prostate gland, in prostate carcinogenesis and androgen-dependent (AD) or androgen-independent (AI) progression of the disease. Functional AR is expressed during various stages of prostate carcinogenesis from the very early stage of prostate intraepithelial neoplasia to organ-confined or locally invasive primary tumors, in metastatic tumor and before or after androgendeprivation therapy (ADT) (130_134). When activated by the endogenous androgenic ligands, testosterone (T) and dihydrotestosterone (DHT), AR becomes phosphorylated and the ligand-receptor complex translocates into the nucleus, and in association with coregulatory factors, binds to specific genomic DNA (gDNA) sequences in the regulatory regions of ARtarget genes (135).

1.2.2. AR structure and function in prostate cells:

The AR is a member of the steroid receptor and nuclear receptor family of transcription factors which contains an N-terminal domain (NTD), DNA-binding domain (DBD), hinge region, and a C-terminal ligand-binding domain (LBD) as show in figure 1-4. The NTD contain an activation function (AF)-1 region and can promote transcriptional activity of the AR in the presence or absence of the LBD. The DBD contains two zinc-finger domains that recognize androgen-response elements for DNA-binding and transcriptional activation but also to dimerization (136). The hinge region allows flexibility between domains of the protein but also contains a nuclear localization signal that is exposed upon the ligand binding to AR (137). The last on, LBD contains the androgen binding site and an AF-2 region which is necessary for binding of AR coactivators (138,139). Throughout the last decade, the discovery of AR variant, which is produced from alternative splicing of

the full-length AR mRNA transcript has complicated our perception of androgenic signaling. Numerous splice variants have been discovered to-date, most of it maintain the NTD and DBD, but contain truncated, missing, or variable C-termini (140,141). AR variants have been discovered in normal tissues, and one example has been shown to have a regulatory role of full-length AR in PCa cells (141,142). Without the LBD, AR variants can dimerize without ligand (either homodimerize with one another or heterodimerize with full-length of AR) and promote activation of AR target gene (143). When unbound to ligand, the inactive AR resides in the cytoplasm in complex with other proteins. In this inactive state, AR is bound to the chaperone proteins heat shock protein (HSP) 70 and HSP40 along with co-chaperones (144) to form a mature apo receptor complex maintaining the AR in a conformation suitable to binding of DHT (145,146). In the absence of ligand, the AR degrades quickly to end the chaperone cycle and the chaperones become free and available to form new complexes in an ATP-dependent process (133). Binding of ligand, such as DHT, induces a conformational change in the ligand-binding domain of AR causing release of the chaperone complex and stabilization, phosphorylation, and dimerization of AR (145). HSP27 binding to the AR homodimer prompts translocation to the nucleus and facilitates transcriptional activity of AR figure (1-4) (146). In the nucleus, AR acts as a transcription factor by recognizing sequence-specific regions of DNA, AREs, where it can bind and promote transcription of specific gene (147).

Other transcription factors have been demonstrated to associate with AR, such as it direct interaction with fork head box A1 (FOXA1) or FOXO1, with further complex formation with nuclear factor IB (NFIB) (148,149). The targets of AR-driven transcription are dependent upon the profile of transcription factors

expressed in a cell. This profile varies between organs and tissues resulting in different target genes being activated on a cells type-specific basis (150).

Prostatic epithelial cell express the androgen receptor (151). From the beginning of embryonic differentiation life to pubertal maturation and beyond, androgens are a prerequisite for the normal development and physiological control of the prostate gland (152). Androgens also help maintain the normal metabolic and secretory functions of the prostate gland. They are also implicated in the development of abnormal condition for prostate such as BPH and prostate cancer. Androgens do not act in isolation and other hormones and growth factors are being investigated (153). Androgen also interact with prostate stromal cell which release soluble paracrine factors that are important for the growth and development of the prostate epithelium (154)

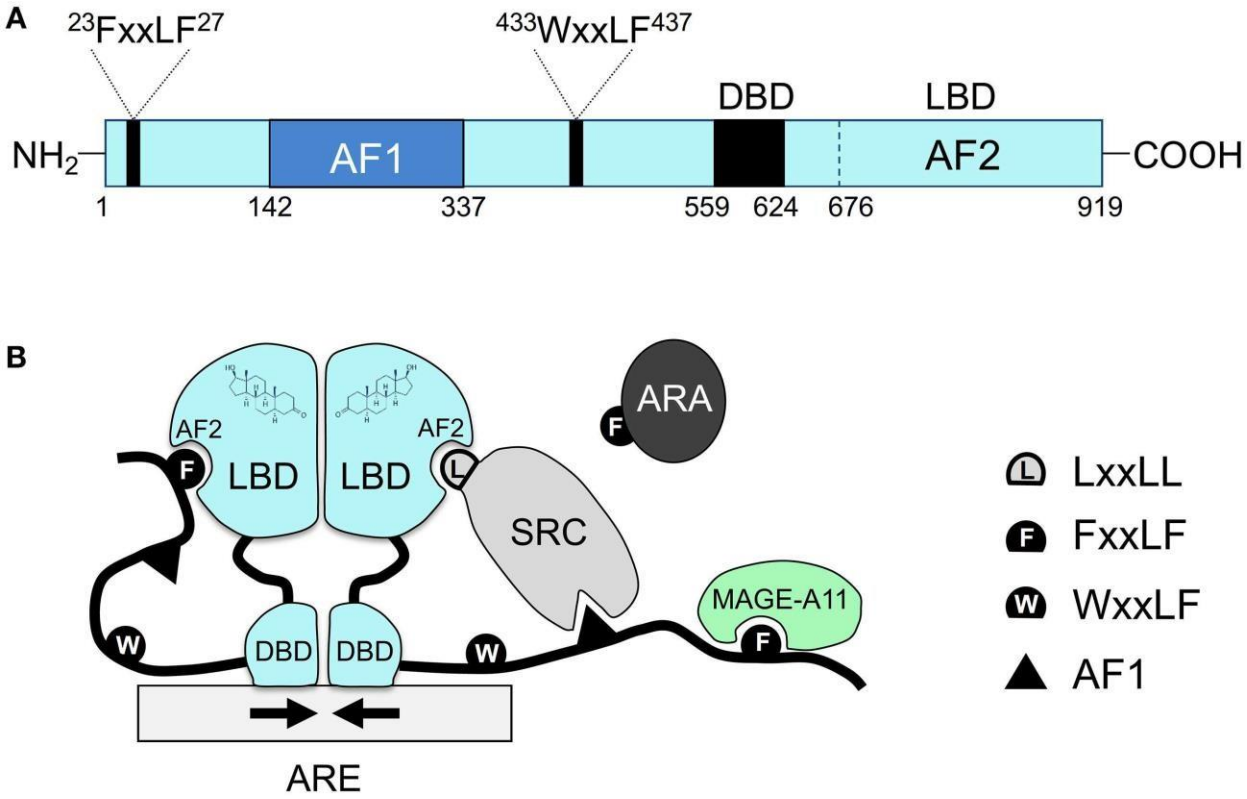


Figure (1-4) | The unique molecular features of the androgen receptor and its coregulator recruitment.

These paracrine pathways may be critical in regulation of the balance between proliferation and apoptosis of prostate epithelial cells in the adult (153) (22).

The appropriate balance between testosterone and its 5 alpha reduced metabolites is key for the normal prostate physiology. The metabolism of testosterone to dihydrotestosterone (DHT) and its aromatization to estradiol are recognized as the key events in prostatic steroid response. Testosterone, to be maximally active in the prostate gland, must be converted to DHT by the enzyme 5-alpha reductase as shown in figure 1-5(155) as show in figure 1-5. DHT has a much greater affinity for the androgen receptor than does testosterone which allows it to accumulate in the prostate gland even when circulating levels of testosterone are low (156,157). DHT is about twice as potent as testosterone in studies of rats at equivalent androgen concentrations (158). Therefore, DHT concentrations may remain similar to those in young men in the prostate of elderly men, despite the fact that serum testosterone levels may decline with age (159). In the prostate gland, the total level of testosterone is about 0.4 ng/g and the total of DHT is 4.5 ng/g (119). The total concentration of testosterone in the blood 18.2nnmol/L (160), is approximately 10 times higher than that of DHT. Circulating DHT, by virtue of its low serum plasma concentration and tight binding to plasma proteins, is of diminished importance as a circulating androgen affecting prostate growth (161). Indeed, intra-prostatic androgens appear remarkably independent of serum levels (162) and circulating androgen levels are not necessarily reflected within the prostate (163).

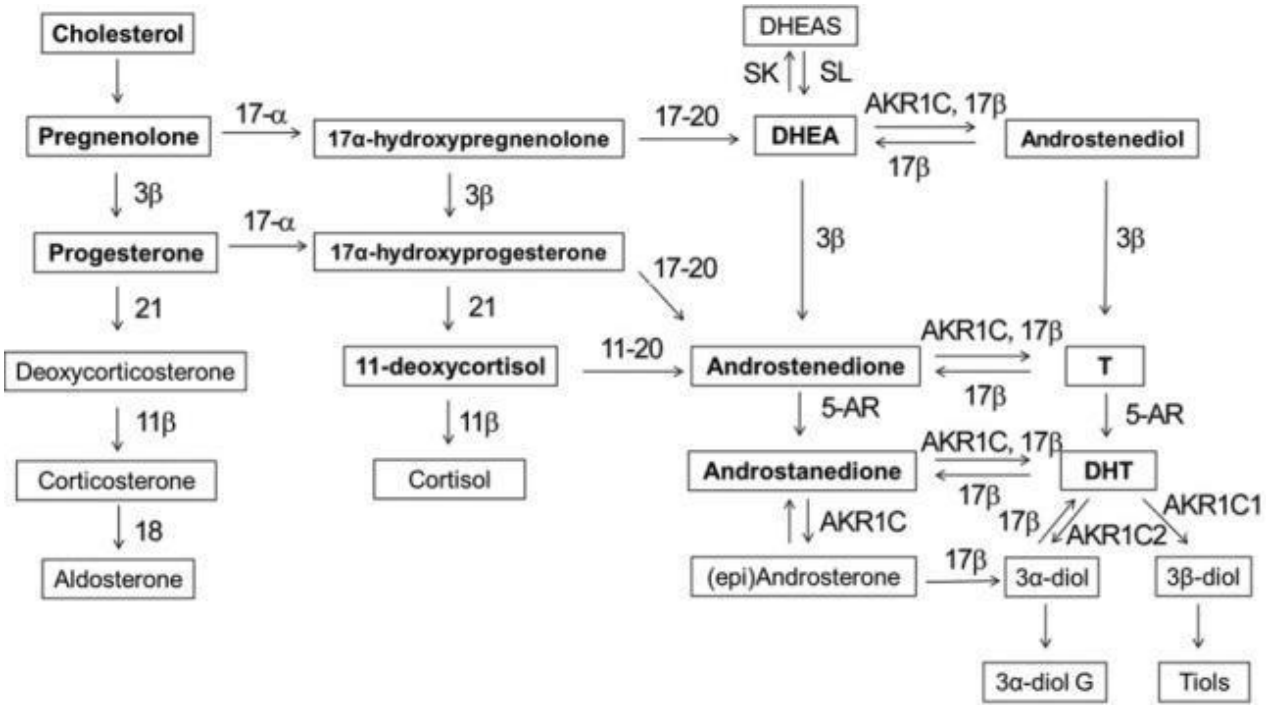


Figure 1-5 Pathway of steroid biosynthesis and the conversion of T to DHT by 5-AR. C21 precursors (pregnenolone and progesterone) are converted to C19 adrenal androgens (DHEA and androstenedione) by sequential hydroxylase and lyase activities.

1.2.3 AR function in the adult prostate

In the adult prostate, the AR continues to be critical for maintenance of the organ. Unlike in the developing prostate, AR in the adult organ is primarily expressed in the prostatic luminal epithelial cell rather than stromal cell. At any age, depletion of testosterone through surgical or chemical castration causes the prostate to involute and decrease in size due to loss of secretory luminal epithelial cell. The prostate has incredible self-renewal potential, since many cycles of involution followed by restoration with androgen supplementation demonstrated that the organ can continuously regenerate for its original size (164). This ability reflects cyclic changes in androgen levels and prostate size that occur in many seasonally-breeding mammals (165,166). In vivo modeling suggests that involution of the prostate,

consistent with development, is a function of loss of androgen action on stromal rather than on epithelial cells (167,168). Initial cellular changes following castration in rodent models are first seen in the endothelial cells sub-adjacent to the epithelium, with epithelial apoptosis apparently occurring as a secondary consequence (169). Circulating androgen is primarily bound by proteins in the serum, but only androgens which are "free" of association with proteins are presumed to be capable of diffusing into the cells of target tissue, allowing for a cellular response to circulating hormones (170). However, androgens, primarily testosterone or DHT, bound to sex hormone binding globulin (SHBG) secure increased half-life and allow serum androgen levels to rise while simultaneously preventing hypertrophy of reproductive organs (171). Circulating testosterone levels have been demonstrated to decrease with age while SHBG levels increase with age, meaning that free testosterone becomes less available (172,173). On the other hand, prostatic diseases become more prevalent with age and both BPH and PCa utilize androgens for their development and progression, indicating that prostatic cells in these diseased states are still able to function in the environment of lower circulating testosterone in aged men. Nonetheless, both androgens and the AR are required for proper development and function of the adult prostate and there is an ongoing research effort to understand the mechanisms involved in androgen availability, transport, and AR activity in the prostate.

1.2.4. Generalized function of AR in prostate diseases:

AR plays important role in multiple cellular processes such as differentiation, secretory function, metabolism, morphology, proliferation, and survival. AR activity is heavily involved in maintaining prostate function; in one analysis, researchers found that almost one-half of androgen-regulated genes are involved in synthesis and modification of secretory proteins (174). Ligand occupancy of AR in luminal epithelial cells was determined to promote cellular quiescence and induce the completion of differentiation into secretory cells detectable by expression of PSA and other prostate luminal-secretory differentiation markers (175). Toivanen & Shen, 2017 (176) demonstrated that when wild-type urogenital mesenchyme (UGM) was recombined with AR-null urogenital epithelium (UGE), the resulting prostatic tissues lacked fully differentiated luminal cells expressing secretory proteins. Similarly, selective deletion of luminal AR using targeted Cre recombinase expression resulted in poorly differentiated and hyper-proliferative epithelial structures (177). Proteins involved in apoptosis were also determined to be regulated by AR activity, namely p53 regulator MDM2, caspase-2, and c-FLIP (178-180). Mechanistically, luminal cell AR signaling induces G0 arrest by inhibiting c-MYC and RB function, while promoting p21 and p27 expression (181). AR is recognized as a growth suppressor for a variety of other normal human epithelial cell types, such as thyroid and adrenocortical epithelial cells, where ligand-dependent endogenous AR signaling suppresses growth (182,183). Further, Chua et al. (184) demonstrated that expression of AR suppressed the growth of murine prostate epithelial cells using an in vivo prostate regeneration system. These observations demonstrate an important growth suppressive and pro-differentiation role for AR in the normal prostate, particularly as it reaches adult steady-state size and function (185). In contrast, studies of AR in PCa have clearly demonstrated that AR signaling acquires an oncogenic gain-offunction that drives cancer proliferation and tumor progression.

The vast majority of prostate tumors express AR, and androgen-deprivation therapy is the predominant therapeutic strategy to reduce tumor burden and block cancer progression. Furthermore, AR remains a critical oncogenic driver in castration-resistant PCa (186). Castration-resistant PCa cells do not undergo apoptosis when androgens are depleted or androgen antagonists are used (187); however, mechanistic studies in cell lines demonstrated that such cells stop proliferating and activate cell death if the AR protein level is reduced below a critical level both in vitro and in vivo (188-190). Thus, both clinical and mechanistic studies have documented an oncogenic dependency of cancer cells to AR signaling and have justified continued development of novel anti-androgens targeting AR. In BPH, AR function within luminal cells mimics that of a tumor suppressor, and there is little evidence of AR converting to a driver of cell proliferation such as in PCa. Thus, luminal epithelial cells in BPH remain differentiated and proliferatively quiescent. This supports a model whereby AR signaling within the prostate epithelium retains its normal adult function in BPH but instead inflammation and fetal reprogramming disrupt normal stromal/epithelial paracrine signaling. Such changes to the prostate microenvironment enable prostate epithelial cells to exit a steady-state of proliferation/death and enter into an abnormal growth phase leading to cellular overexpansion (164).

1.3. 3β-hydroxysteroid dehydrogenase-1:

Androgens play critical roles in prostate carcinogenesis as well as prostate cancer progression. Since 1941, androgen-deprivation therapy (ADT), which reduces testosterone production and inhibits androgen action in prostate cancer cells, has been the criterion standard therapy for metastatic prostate cancer. Although initially most prostate cancers respond well to ADT, most patients eventually

progress to castration-resistant prostate cancer (CRPC), which is mainly thought to be because androgen receptor reactivation is induced by several mechanisms (191). One of those mechanisms have been identified to be intratumoral androgen synthesis mostly from adrenal precursor steroids and at least in part due to de novo synthesis from cholesterol (192,193,194) which is supported by increased expression of several genes encoding steroidogenic enzymes including HSD3B, HSD17B, and SRD5A in CRPC (195). Among them, HSD3B1 encodes 3β-hydroxysteroid dehydrogenase-1, which is mainly expressed in peripheral tissues including the prostate, breast, skin, and placenta (another isoform, 3β-hydroxysteroid dehydrogenase-2was mainly expressed in adrenal gland and gonad in human) and is a rate-limiting enzyme required for all pathways of dihydrotestosterone synthesis (196).

Dihydrotestosterone synthesis in prostate cancer from adrenal DHEA/DHEA-sulfate requires enzymatic conversion in tumor tissues. 3β-hydroxysteroid dehydrogenase-1 is an absolutely necessary enzyme for such dihydrotestosterone synthesis and is encoded by the gene HSD3B1 which comes in 2 functional inherited forms described in 2013. The adrenal-permissive HSD3B1(1245C) allele allows for rapid dihydrotestosterone synthesis. The adrenal-restrictive HSD3B1(1245A) allele limits androgen synthesis (197). Studies from multiple cohorts show that adrenal-permissive allele inheritance confers worse outcomes and shorter survival after castration in low-volume prostate cancer and poor outcomes after abiraterone or enzalutamide treatment for castration-resistant prostate cancer (198).

The upfront abiraterone in combination with primary ADT has been shown to improve survival for metastatic hormone-sensitive prostate cancer (HSPC) (199). It remains unclear who is suitable for upfront abiraterone therapy tometastatic HSPC. Intriguingly, it has been reported that abiraterone is converted by 3β-hydroxysteroid

dehydrogenase to Δ4-abiraterone (D4A), which blocks multiple steroidogenic enzymes and antagonizes the androgen receptor, providing an additional explanation for clinical activity by abiraterone (200). Therefore, tumors in men carrying variant genotype in HSD3B1 showing higher enzymatic activity of 3β-hydroxysteroid dehydrogenase-1may be vulnerable to abiraterone owing to higher concentration of D4A. The studies have demonstrated that genetic polymorphism in HSD3B1 is associated with oncological outcome among residents in the United States treated with ADT, where men carrying variant alleles showed worse prognosis (201-203).

Aim:

The main aim of study is found if there are relationship between two gene involve in development of androgen receptor and HSD1B3 enzyme which has role in the production of androgen hormones function and synthesis in male and development of CaP.

2. Materials, Methods:

2.1 Samples:

2.1.1. Patient group:

In this case control study the blood samples were obtained from 90 patients at the 54–88 ages diagnosed with histopathological confirmed prostate cancer (CaP). The CaP patients were recruited from Department of Urology and archived in Department of Clinical and Molecular Pathology of the Imam Hussein (peace be upon him) Teaching Hospital in the HolyKarbala Governorate and Merjan Medical City in Babylon Governorate/ Iraq. The CaP patients were divided into two groups of patients with Gleason grade<7 who had histopathologically designed well moderately differentiated CaP (Grade 1 and 2), and in patients with Gleason grade≤7 who had poorly differentiated or undifferentiated CaP (Grade 3 and 4).

2.1.2. Control

Ninety candidates of this group have neither symptoms nor signs of essential PCa by doing PSA serum level with per rectal examination of prostate and they are healthy otherwise.

2.1.3. Inclusion and exclusion Criteria

Data was collected by ways of a personal interview for every individual to obtain information about their smoking status and about their age, ethnicity and family history. Included all patient with CaP newly diagnosis or receive therapy and excluded patient who had another type of cancer or any chronic autoimmune disease.

2.1.4. Ethical Issues

The ethical issues in the present study were depended on the following:

a) Approval by Biochemistry Department and Scientific Committee of College of Medicine at University of Babylon.

b) Approval of the Research and Development Department of the Babylon Health Directorate in Babylon Province.

c) The goals and methods of this study were explained to all participants in the present study through a verbal agreement from all the patients involved in the research before sampling.

2.1.5. Specimen Collection

Almost 10 ml venous blood was drawn from each candidate when visit the oncology center for treatment. Two ml of this sample were collected in EDTA tube for DNA extraction and PCR. The remaining was transferred into a clean plain tube, and left at room temperature for nearly thirty minutes for clotting, then centrifuged. Serum was divided into two parts one for AR.

2.2. Materials:

2.2.1. Instruments

Table 2-1 shows the used laboratory equipment and supplies.

Table 2-1 The laboratory equipment and supplies used in this study, the manufacturing company and the origin.

Number	The name of the device or accessory	Manufacturer and Origin
1	Cosmoplast EDTA tube	UAE
2	)ReliaPrep™Binding Columns (50/pack)	Geneaid (Korea)
3	Laminar flow cabinet	Biobse (China)
4	Collection Tubes (40/pack)	Geneaid (Korea)
5	Gel electrophoresis apparatus	Drawell (China)
6	Photo documentation system	Cleaver scientific (USA)
7	Thermal cycler DNA incubator	Biobase (China)
8	Cooling Centrifuge	Biobase (China)
9	Centrifuge	Biobase (China)
10	UV light transillminator	)UV Transilluminator China)
11	Water bath	Biobse (China)
12	Vortex	Biobse (China)
13	Automatic Micropipettes	Bio Basic (Canada)
14	Deep Freezer	Japan
15	Microwave oven	Samsung(Korea)
16	Micropipettes tips	Bio Basic Canada
17	Electrophoresis constant power supply	Cleaver scientific (USA)
18	Electrical sensitive balance	Biobse (China)
19	Gel tube	UAE
20	ELISA	UK

2.2.2. Chemical Materials:

Table (2-2) chemical and biological materials used in the research

NUNBER	NAME OF MATERIAL	MANUFACTURER AND ORIGIN
1	Agarose	Marliju (Korea)
2	Bstul Restriction enzyme	Bioneer (Korea)
3	Ethidium Bromide	BIO BASIC (Canada)
4	Primers	(Korea) Bioneer
5	DNA loading Dye	(Korea) Bioneer
6	gsync™ Blood DNA Extraction Kit	Geneaid (Korea)
7	Deionized water	(Canada) Aquarama
8	Acetylated BSA	Promega (USA)
9	PCR PreMix	(Korea) Bioneer
10	10 X AccCut TM Red Buffer	Bioneer(Korea)
11	1X Dilution Buffer	Bioneer(Korea)
12	10X TBE Buffer Solution	BIO BASIC (Canada)
13	10X NE Buffer	BioLabs (New ENGLAND
14	10X RE Buffer	Promega (USA)
15	Cell Clean Solution	Germany
16	Cell Pack Solution	Germany
17	Stromato lyser-WH Solution	Germany
18	Vidas,25(OH)2D3	BioMrieux ® sa. France
19	DNA ladder Marker(100-2000 bp)	(Korea) Bioneer

20	Standard Solution (64ng/ml)	Bioassay technology laboratory china
21	Standard Diluent	Bioassay technology laboratory china
22	Streptavidin-HRP	Bioassay technology laboratory china
23	Stop Solution	Bioassay technology laboratory china
24	Substrate Solution A Substrate Solution B	Bioassay technology laboratory china
25	Wash Buffer Concentrate (25x)	Bioassay technology laboratory china
26	Biotinylated Human HSD3B1 Antibody	Bioassay technology laboratory china

2.3. Methods:

2.3.1. Molecular Analysis:

2.3.1.1 Genomic DNA Extraction Protocol:

a) Twenty µl of Proteinase K (obtained from 25mg Proteinase K dissolved in 1.25ml nuclease-free water) added to a clean 1.5 ml tube.

b) Two hundred µl of whole blood applied to the tube containing proteinase K

c) Two hundred µl of Binding buffer (GC) added to the sample and mix immediately by vortex mixer.

d) Incubated at 60℃ for 10 min.

e) One hundred µl of Isopropanol added and mixed well by pipetting

f) The lysate carefully transferred into the upper reservoir of the Binding column tube (fit in a 2 ml tube)

g) The tube centrifuged at 8,000 rpm for 1 min

h) The Binding column tube transferred to a new 2ml tube for filtration

i) Five hundred µl of Washing buffer 1 (W1) added and centrifuge at 8,000 rpm for 1 min

j) The solution poured from the 2 ml tube into a disposal bottle

k) Five hundred µl of Washing buffer 2 (W2) was added and centrifuged at 8,000 rpm for 1 min.

l) Centrifuged once more at 12,000 rpm for 1 min to completely remove ethanol

m) The Binding column tube transferred to a new 1.5 ml tube for elution add 100 µl of Elution buffered onto Binding column tube, and wait for 5 min at RT (15~25℃) until EL is completely absorbed into the glass fiber of Binding column tube

n) Centrifuged at 8,000 rpm for 1 min to elute.

o) Then a measurement of the obtained DNA concentration and purity done by NanoDrop technique.

2.3.1.2 Polymerase Chain Reaction:

The AR-E211 G>A polymorphism was detected by amplification of a 416 bp fragment using forward primer 416 and reverse primer as shown in table 2-3:

	5′- CAC AGG CTA CCT GGT CCT GG−3′	Forward primer
	5′- CTG CCT TAC ACA ACT CCT TGG C –3′	reverse primer

2.3.1.2.1 Polymorphism Detection for AR-E211:

The G1733A single nucleotide polymorphism of the AR-E211 gene (UCSC code: rs6152), designated the G and A allele is a synonymous change. The presence of the G allele at nucleotide 1733 abolished a StuI restriction enzyme recognition site, which is recognized on the A allele.

Amplification Reaction Mixture for AR-E211 rs6152 two primers were used in single PCR reaction. The PCR mixture is prepared by mixing 1µl from each primer (p1, p2) of 10 pmol/µl primers with 5 µl of extracted DNA were mixed in total volume of 20 µl. Then the mixture was added to lyophilize PCR premix formula.

The reaction mixture has been incubated and the following program is used to amplify the mixture.

a) 95°C for 7 minutes (initial denaturation)

b) 95°C for 45 seconds (denaturation, 35 cycles)

c) 58°C for 45 seconds (annealing, 35 cycles)

d) 72°C for 45 second (extension, 35 cycles)

e) 72°C for 7 minutes (Final extension) and 4°C (hold phase)

Bands for the required product sizes were obtained shown in figure (2-3).

2.3.1.2.2 Agarose Gel Electrophoresis

a) Agarose gel was prepared by dissolving 2 g of agarose powder in 100 ml of 10% Tris Borate EDTA (TBE) buffer solution and boiling by electric heater.

b) The solution was cooled to 50˚C.

c) Two microliters of ethidium bromide solution were added.

d) The comb was fixed at one end of the tray for making wells used for loading the PCR products samples.

e) The agarose was poured gently into the tray, and allowed to solidify at room temperature for 30 min.

f) The comb was removed gently from the tray.

g) The tray was fixed in electrophoresis chamber. The chamber was filled with TBE buffer 10%.

h) Ten microliters of each PCR products sample were loaded into the wells in agarose gel.

i) The voltage of the electrophoresis apparatus was fixed at 100 volts to ensure an electrical field adjusted with (5-8) v.cm-1 for 16cm distance between cathode and anode.

j) At the end of the run about (1:30 min) ultraviolet trans-illuminator was used for bands detection.

k) The gel was photographed using digital camera.

The PCR-RFLP (Restriction Fragment Length Polymorphism) assay was performed from blood samples for sample collection. In the first step, genomic DNA was amplified directly from 2 µl whole blood using, the AR-E211 G>A polymorphism was detected by amplification of a 416 bp fragment using forward primer 416/F 5′- CAC AGG CTA CCT GGT CCT GG −3′ and reverse primer 416/R 5′- CTG CCT TAC ACA ACT CCT TGG C – 3′ [203] on high-speed Piko® Thermal Cycler (Finnzymes, Espoo, Finland) by initial

denaturation at 98 °C for 7 min, followed by 30 cycles of denaturation at 98 °C for 45 s, annealing at 58 °C for 45 s, elongation at 72 °C for 45s, and the final extension at 72 °C for an additional 7 min after the last cycle. After PCR, the reactions were centrifuged at 2,500 rpm for 3 min and the supernatants were collected for restriction digestion. The digestions were prepared from 5 µl of supernatants by addition of 10 units of StuI (New England Biolabs, Ipswich, MA) and incubated at 37 °C over night. Digested products were electrophoresed through a 3 % agarose gel and visualized by ethidium bromide staining. The band size 416 represent A allele while band size 329 represent G allale as shown in picture:

2.3.1.2.3 Polymorphism Detection for HSD3B1 (rs1047303):

The primers that have been used to detect this polymorphism are shown in table (2-4).

Forward primer	5′-GTCAAATAGCGTATTCACCTTCTCTTAT-3′
reverse primer	5′-GAGGGTGGAGCTTGATGACATCT-3′

2.3.1.2.4 Amplification Reaction Mixture

The PCR mixture is prepared by mixing 1µl from each primer (p1, p2) of 10 pmol/µl primers with 5 µl of extracted DNA were mixed in total volume of 20 µl. Then the mixture was added to lyophilize PCR premix formula. The reaction mixture incubated and the following program was used to amplify the mixture:

a) 95°C for 5 minutes (initial denaturation)

b) 95°C for 30 seconds (denaturation, 30 cycles)

c) 60°C for 30 seconds (annealing, 30 cycles)

d) 72°C for 30 second (extension, 30 cycles)

e) 72°C for5 minutes (Final extension) and 4°C (hold phase)

Then we used Agarose Gel Electrophoresis to detect the polymorphism for HSD3B1 (rs1047303). The bands for the required product sizes were obtained 215 bp and the gel was photographed using digital camera as shown in picture.

After performing the PCR assay, 4 samples were selected for each gene (3 samples from patients versus 1 control sample), those samples with their primers were sent to Macrogen Corporation in Korea (Macrogen Inc. Geumchen, Seoul, South Korea) specialized in analyzing the sequencing products of those samples. The PCR products of the HSD3B1 (rs1047303) polymorphism gene were dependent on the lengths (bp 215), the result of the sequences was compared with those of the DNA sequences of the previously mentioned and globally registered genes, from which the reference was extracted. https://www.ncbi.nlm.nih.gov PCR sequencing products were analyzed, purified, lined up and analyzed along with NCBI samples using BioEdit 6 GenBank (DNASTAR reference database) software, Madison. Sequence Alignment Editor Software Version 7.1 www.ncbi.nlm.nih.gov (https://www.ncbi.nlm.nih.gov/) National Center for Biotechnology Information.

2.3.2 Biochemical assay:

2.3.2.1. Determination of Human Androgen Receptor:

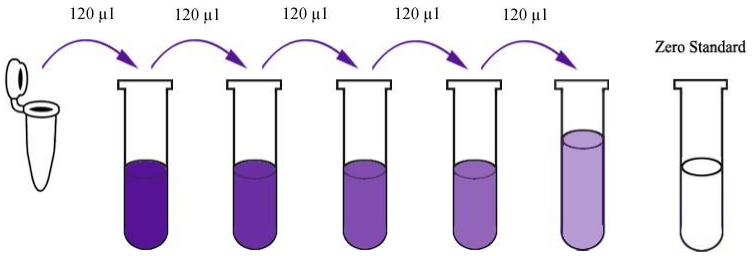
Determination of Human Androgen Receptor was done using ELISA Kit for BT LAB Bioassay Technology Laboratory／China

2.3.2.1.1 Reagent Preparation:

All reagents should be brought to room temperature before use.

Standard Reconstitute the 120µl of the standard (80ng/ml) with 120µl of standard diluent to generate a 40ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (40ng/ml) 1:2 with standard diluent to produce20ng/ml, 10ng/ml, 5ng/ml and2.5ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

40ng/ml	Standard No.5	120µl Original Standard + 120µl Standard Diluent
20ng/ml	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
10ng/ml	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
5ng/ml	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
2.5ng/ml	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
80ng/ml	40ng/ml	20ng/ml	10ng/ml	5ng/ml	2.5ng/ml

2.3.2.1.2 Assay Procedure:

1. All reagents, standard solutions and samples were prepared as instructed. All reagents were brought to room temperature before use. The assay is performed at room temperature.
2. The number of strips required for the assay were determined. The strips in the frames for use were insert. The unused strips should be stored at 2-8°C.
3. A volume of 50µl standard to standard well was added.

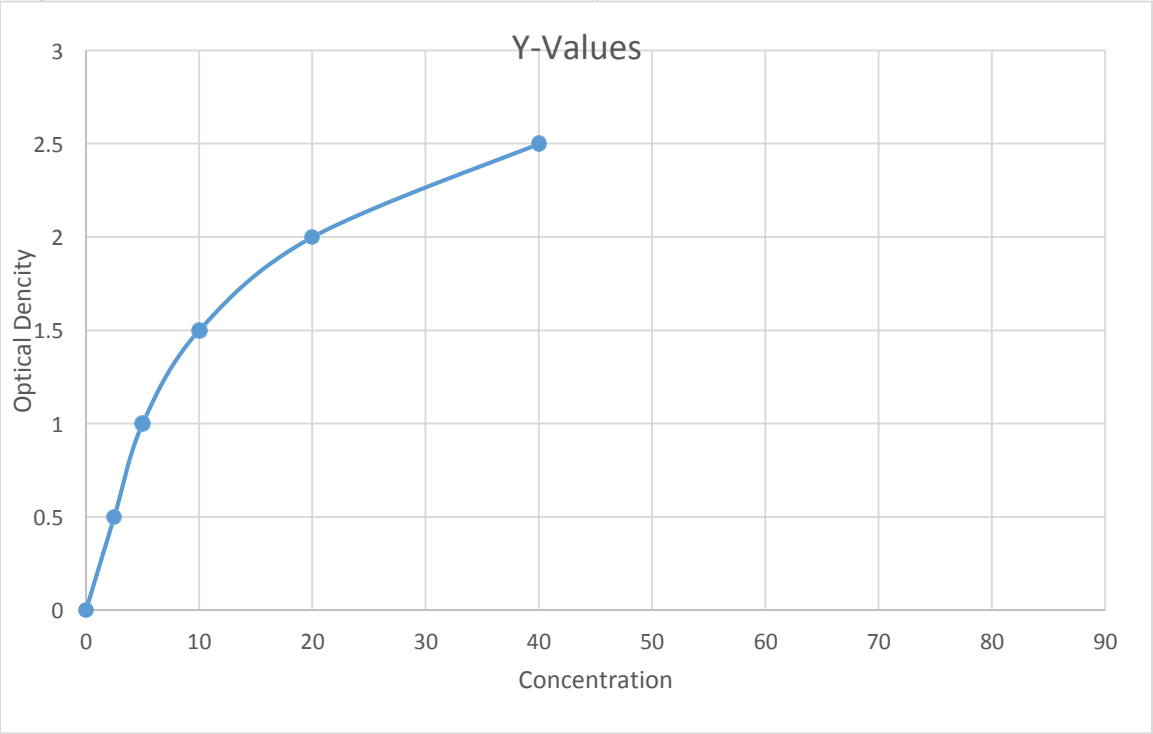
   Note: Don't add antibody to standard well because the standard solution contains biotinylated antibody.
4. A volume of 40µl sample to sample wells and then added 10µl anti-AR antibody to sample wells was added, then 50µl streptavidin-HRP was added to sample wells and standard wells (Not blank control well). The plate was mixed well and covered with a sealer, and incubated for 60 minutes at 37°C.
5. Removed the sealer and washed the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.
6. We were added 50µl substrate solution A to each well and then added 50µl substrate solution B to each well. Incubated plate covered with a new sealer for 10 minutes at 37°C in the dark.
7. Added 50µl Stop Solution to each well, the blue color was change into yellow immediately.

8. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

2.3.2.1.3 Calculation of Results:

The standard curve is draw by PT⁄PT LOG LUN by used the stander concentration**.**

Figure 2-1 standard curve for elisa assay to AR serum level



2.3.2.2. Determination of Human 3 Beta-Hydroxysteroid Dehydrogenase Type 1 ELISA Kit:

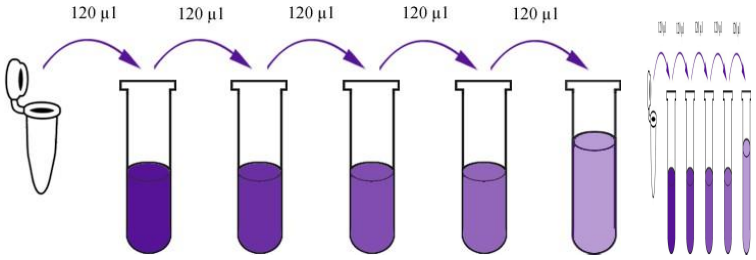
Determination of Human 3 Beta-Hydroxysteroid Dehydrogenase Type 1 was done using ELISA Kit for BT LAB Bioassay Technology Laboratory⁄China.

2.3.2.2.1 Reagent Preparation

All reagents should be brought to room temperature before use.

Standard Reconstitute the 120µl of the standard (64ng/ml) with 120µl of standard diluent to generate a 32ng/ml standard stock solution. Allow the standard to sit for 15 mins with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution (32ng/ml) 1:2 with standard diluent to produce16ng/ml, 8ng/ml, 4ng/ml and2ng/ml solutions. Standard diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C and used within one month. Dilution of standard solutions suggested are as follows:

32ng/	Standard No.5	120µl Original Standard + 120µl Standard Diluent
16ng/	Standard No.4	120µl Standard No.5 + 120µl Standard Diluent
8ng/	Standard No.3	120µl Standard No.4 + 120µl Standard Diluent
4ng/	Standard No.2	120µl Standard No.3 + 120µl Standard Diluent
2ng/	Standard No.1	120µl Standard No.2 + 120µl Standard Diluent



Standard Concentration	Standard No.5	Standard No.4	Standard No.3	Standard No.2	Standard No.1
64ng/ml	32ng/ml	16ng/ml	8ng/ml	4ng/ml	2ng/ml

● **Wash Buffer** Dilute 20ml of Wash Buffer Concentrate 25x into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

2.3.2.2.2 Assay Procedure:

1. All reagents, standard solutions and samples were prepared as instructed. All reagents were brought to room temperature before use. The assay is performed at room temperature.

2.The number of strips required for the assay were determined. The strips in the frames for use were insert. The unused strips should be stored at 2-8°C.

3. Added 50µl standard to standard well. **Note**: Don't add antibody to standard well because the standard solution contains biotinylated antibody.

4. A volume of 40µl sample to sample wells and then added 10µl anti-HSD3B1 antibody to sample wells, then added 50µl streptavidin-HRP to sample wells and standard wells (Not blank control well). Mix well. Cover the plate with a sealer. Incubate 60 minutes at 37°C.

5.We were Removed the sealer and washed the plate 5 times with wash buffer. Soak wells with at least 0.35 ml wash buffer for 30 seconds to 1 minute for each wash. For automated washing, aspirate or decant each well and wash 5 times with wash buffer. Blot the plate onto paper towels or other absorbent material.

6. Added 50µl substrate solution A to each well and then add 50µl substrate solution B to each well. Incubate plate covered with a new sealer for 10 minutes at 37°C in the dark.

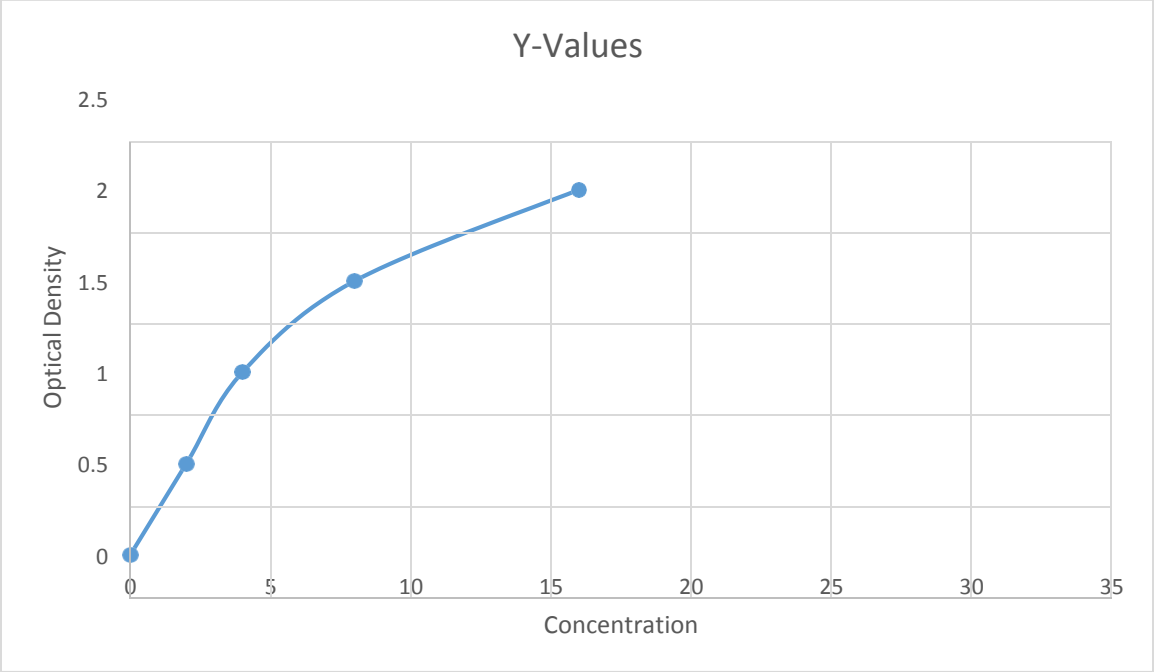
7. Added 50µl Stop Solution to each well, the blue color will change into yellow immediately.

8. Determined the optical density (OD value) of each well immediately using a microplate reader set to 450 nm within 10 minutes after adding the stop solution.

2.3.2.2.3 Calculation of Results:

The standard curve is draw by PT⁄PT LOG LUN by used the stander concentration.

Figure 2-2 standard curve for elisa assay to HSD3B1 serum level



2.4. Statistical Analysis

Statistical analysis was done by SPSS statistical software (SPSS 22 for Windows, standard version). The results were presented as mean ± standard deviation (SD). Continuous variables were tested by T-test and for genetic analysis we performed it by using chi square to compare the results. The correlation analysis was done using the Pearson test, a P value of <0.05 was considered statistically significant.

3. Result:

3.1. Demographic Comparison between Prostate Cancer and Control groups

Through the information collected from 90 patients with prostate cancer and 90 healthy as a control group, it became clear the effect of some important factors on the incidence of prostate cancer, including residence, smoking, family history of the disease and age.

3.1.1. Age:

Table 3-1 shows there are no different in age group between patient group were mean equal 69.79 and control group effect mean equal 7.463 with p value 0.281.

Table 3-1 The relation of age between two groups

	N.	Mean	SD±	P value
patient	**90**	**69.79**	**10.907**	**0.281**
control	**90**	**70.31**	**7.463**	

The statistical analysis showed no significant differences association between the age and biochemical marker for AR and HSD1B3 serum level in patient group, also not association with smoking as risk factor and gleason of disease as shown in table of correlation 3-2.

3.1.2. Gleason

The Gleason grading system refers to how abnormal your prostate cancer cells look and how likely the cancer is to advance and spread. A lower Gleason grade means that the cancer is slower growing and not aggressive.

- Grade group 1: Gleason score 6 or lower (low-grade cancer)
- Grade group 2: Gleason score 3 + 4 = 7 (medium-grade cancer)
- Grade group 3: Gleason score 4 + 3 = 7 (medium-grade cancer)
- Grade group 4: Gleason score 8 (high-grade cancer)
- Grade group 5: Gleason score 9 to 10 (high-grade cancer)

Higher numbers indicate a faster growing cancer that is more likely to spread.

When compared the severity of cancer with AR level and HSD3B1 level there are no significant relationship p value equal 0.206 and 0.684 respectively. Also no relation with age p=0.374 and smoking p=0.424. Gleason score was grouped into low grade (Gleason score < 8) and high grade (Gleason score 8-10). The most prostate cancer cases were Gleason score 9 (44,64%), histopathological grading poorly differentiated/ undifferentiated (76,78%), grade group 5 (48,21%).

Table 3-2 The correlation

		Age	Receptor	enzyme	smoking	gleason
Age	pearson correlation	1	-0.075	-0.10	0.151	0.096
	P		0.488	0.925	0.164	0.374
	N	90	90	90	90	90
Receptor	pearson correlat	-0.075	1	0.322	0.091	-0.136
	P	0.488		0.002	0.397	0.206
	N	90	90	90	90	90
Enzyme	pearson correlat	-0.01	0.322	1	0.072	0.044
	P	0.925	0.002		0.506	0.684
	N	90	90	90	90	90
Smoking	pearson correlat	0.151	0.091	0.072	1	0.086
	P	0.164	0.397	0.506		0.424
	N	90	90	90	90	90
Gleason	pearson correlat	0.096	-0.136	0.044	0.086	1
	P	0.374	0.206	0.684	0.424	
	N	90	90	90	90	90

3.1.3 Family history:

The family history of the disease is one of the most important risk factors affecting the incidence of prostate cancer. As the results of Table 3-3 and in the field of the impact of the family history of the disease on the incidence of prostate cancer show that 16 (18%) of patients with prostate cancer have a family history compared to patients without a family history by 74 (82%). However, in the healthy group, none of them had a family history of the disease, and the results of the statistical analysis indicated that there were high significant differences between the two groups of patients and healthy people who had prostate cancer.

Table 3-3 Distribution of Patient and Control Groups According to Family History

Family History	Number and Ratio		P value
	Control	Patient	
Not found	90 (100%)	74 (82%)	0.00002
Found	0 (0%)	16 (18%)	

There was a highly significant relationship, and there were also high significant differences between the group of patients as well as in terms of the presence or absence of history between the healthy group and the injured group, there were high moral differences as shown in figure 3-3.

3.1.4 Smoking:

Smoking is one of the important and influencing factors on the incidence of prostate cancer. The results of distributing samples according to smoking and non-smoking in Table 3-4 showed that the highest incidence of the disease was 65 (72%) in smoking patients compared to 25 (28%) in non-smokers. While the healthy group had 74 (82%) non-smokers, higher than 16 (18%) healthy smokers.

While in table of correlation there are no significant relationship between severity of disease and smoking. Also on relation between serum level of receptor and enzyme with the smoking, so only has relation with cancer..

Table 3-4 Distribution of Patient and Control Groups According to Smoking

Smoking	Number and Ratio		P value
	Control	Patient	
Not smoking	74 (82%)	25 (28%)	0.00
Smoking	16 (18%)	65 (72%)	

The results of the statistical analysis showed that there were significant differences between smokers for the two groups of patients and healthy people. The statistical results also high statistical signs were recorded between smokers and non-smokers in patients and control group.

3.2. Androgen Receptor (AR)

3.2.1. Androgen Receptor Level

AR remains one of the most import nuclear transcription factor from the steroid hormone receptor superfamily of genes. Normal prostate growth and development, prostate carcinogenesis and AI progression of PCa are mainly dependent on AR expression and function. As a brief and oversimplified statement, the prostate gland at any state of normal or neoplastic growth is addicted to the AR. Alterations in AR structure, expression and signaling could have a determining role in PCa progression toward an incurable AI state. These alterations which occur could be secondary to somatic or germline mutations, presence or absence of non-androgenic ligands, cytoplasmic signaling crosstalk with other kinases or cross-modulation by other nuclear transcription factors. This review provides a concentration of AR in serum with development of CaP. There was **no** significant difference in AR serum level between patients and control group as show in table 3-5, the mean value in both groups were compared using t test (p value =0.42) which is not significant at p value<0.05 (mean AR serum level in patient =9.709, mean AR serum level in control= 9.05).

AR level	patient	control
Mean (ng/ml)	9.709	9.05
±SD	3.65	3.08
P value	0.42	

Table 3-5 comparison of AR serum between patient and control groups

3.2.2. Androgen Receptor ROC Curve

A receiver operating characteristic curve, or ROC curve, is a graphical figure that shows how a binary classifier system's diagnostic capacity changes as the discrimination threshold is changed. Starting in 1941, the approach was created for operators of military radar receivers, hence the name. Plotting the true positive rate (TPR) against the false positive rate (FPR) at various threshold levels yields the ROC curve. Sensitivity, recall, and chance of detection are all terms used to describe the true-positive rate. The likelihood of false alarm, commonly known as the false-positive rate, can be expressed as (1- specificity).

Figure 3-1 Predictive Value Section for Patient Using the Empirical ROC Curve for Androgen Receptor Level

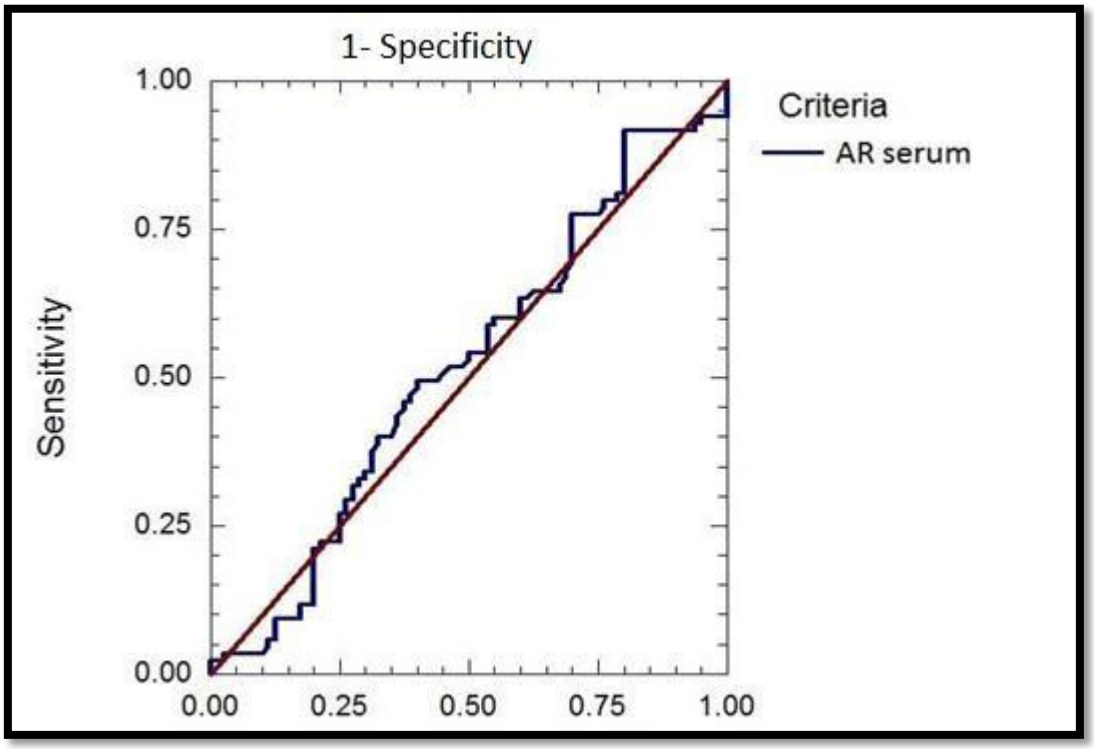


Table 3-6 values of ROC curve to AR serum level

Parameter	Value
specificity	**75%**
Sensitivity	**25%**
AUC	**0.5168**
P.Value	**0.42**
95% CI	**(0.42209-0.60042)**
Cut-off point	**≥ 10 (ng/ml)**
positive predictive value	**0.51220**
negative predictive value	**0.48387**

3.2.3. Genetic Result for AR-E211 G>A Polymorphism

It is still studied by the assumption that the minor allele A is associated with higher risk of prostate cancer [204]. Our finding indicates that the minor allele A could be associated with transformation-induced changes of the modified androgen receptor gene or induced another changes caused by tumor transformation. Frequency of alleles of the AR-E211 G>A polymorphism in blood samples. Since the AR gene is located on the X chromosome, males have only one allele on their one X chromosome and females have two alleles on their two X chromosomes [205]. The PCR-RFLP assay was performed from blood samples without examine DNA purification. In the first step, genomic DNA was amplified directly from 2 µl whole blood, the AR-E211 G>A polymorphism was detected by amplification of a 416 bp fragment using forward primer 416/F 5'- CAC AGG CTA CCT GGT CCT GG –3' and reverse primer 416/R 5'- CTG CCT TAC ACA ACT CCT TGG C – 3' (Fig. 3-2).

Figure 3-2 showing Electrophoresis. For pcr production to the AR gene on 70 volts for 1 hr 3% agaros.

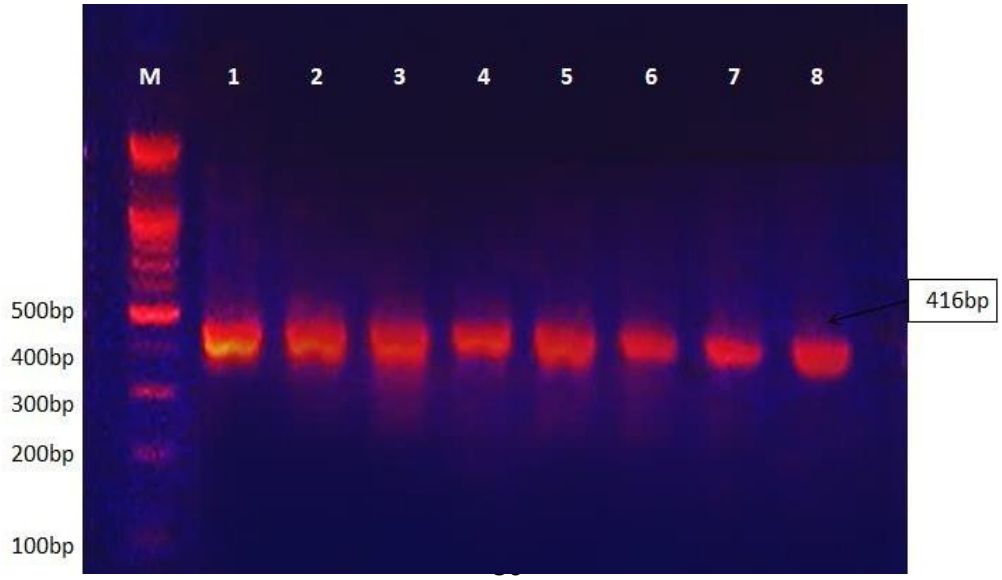
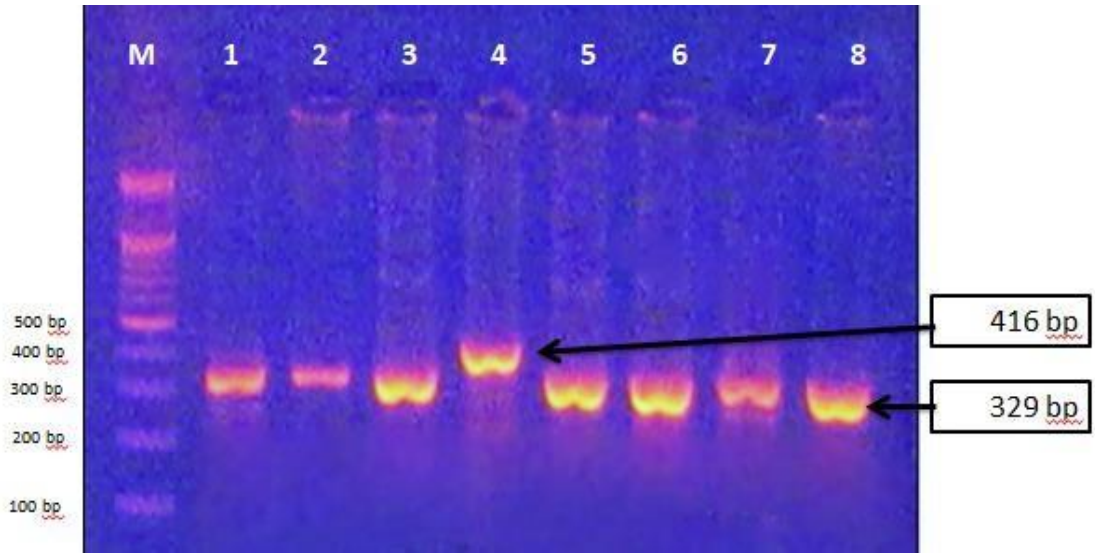


Figure 3-3 PCR-RFLP of AR-E211 G>A Polymorphism after Addition of restriction enzyme.



Comparison of allele frequencies between the groups of CaP patients and between the groups of control were analyzed by χ2 – tests.

The genotypes association of AR is shown in Table 3-8. The model codominant was non-significant change with (P = 0.7) when compared with healthy control.

The model dominant was shown non-significant change with (P = 0.7) when compared with healthy control according to the percentage of A/G –G/G is genotypes response and related with disease RA.

The recessive model was shown non-significant change with (P = 0.7) when compared with healthy control.

Table 3-7 Allelic Association of AR Gene Polymorphism in Prostate Cancer Males Cases and Controls Males

Model	Genotype	Case	Control	OR (95% CI)	P-value
Codominant	A/A	8 (8.8%)	7 (7.7%)	1.15(0.401-3.33	
	A/G	0 (0%)	0 (0%)		0.7
	G/G	82 (91.1%)	83 (92.2%)		
Dominant	A/A	8 (8.8%)	7(7.7%)		0.7
	A/G-G/G	82 (91.1%)	83 (92.2%)	1.15(0.401-3.33)	
Recessive	A/A-A/G	8 (8.8%)	7(7.7%)		0.7
	G/G	82 (91.1%)	83 (92.2%)	1.15(0.401-3.33)	
Overdominant	A/A-G/G	90 (100%)	90 (100%)		
	A/G	0 (0%)	0 (0%)		

3.3 β-Hydroxysteroid Dehydrogenase Isoenzyme-1 (HSD3B1)

3.3.1. β-Hydroxysteroid Dehydrogenase Isoenzyme-1 Levels

The enzyme 3b-hydroxysteroid dehydrogenase isoenzyme-1 (3b-HSD1, encoded by HSD3B1), which catalyzes the rate-limiting step of potent androgen synthesis from adrenal precursor steroids in peripheral tissues. The present study measures the serum level of this enzyme in PCa patient and noted if change and has role in disease or change with gene mutation. The mean value of HSD3B1 serum level in the patients was 5.91 ng/ml. The mean serum value of HSD3B1 in control is 6.20 ng/ml. although the mean serum level of HSD3B in patients group seems to be

less than that of the control group but he difference was not statically significant (P value 0.82) as show in Table 3-8.

Table 3-8 comparison between patient and control groups for HSD3B1.

HSD3B1	patient	control
mean	5.91	6.20
±SD	2.56	2.32
P	0.82	

3.3.2. HSD3B1 ROC Curve

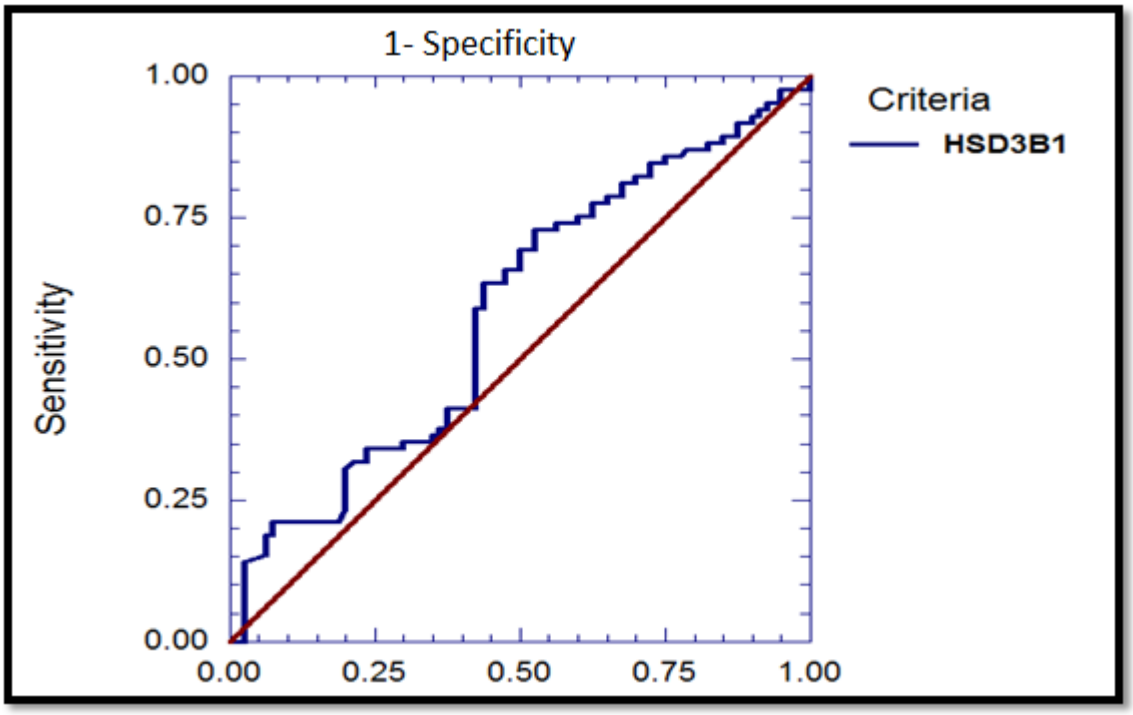
A receiver operating characteristic curve, or ROC curve, is a graphical figure that shows how a binary classifier system's diagnostic capacity changes as the discrimination threshold is changed. Starting in 1941, the approach was created

for operators of military radar receivers, hence the name. Plotting the true positive rate (TPR) against the false positive rate (FPR) at various threshold levels yields the ROC curve. Sensitivity, recall, and chance of detection are all terms used to describe the true-positive rate. The likelihood of false alarm, commonly known as the false-positive rate, can be expressed as (1- specificity).

Table 3-9 values of ROC curve to HSD3B1 serum level

Parameter	Value
specificity	**94%**
Sensitivity	**7.5%**
AUC	**0.58360**
P.Value	**0.82**
95% CI	**(0.48903-0.66462)**
Cut-off point	**≥ 10 (ng/ml)**
positive predictive value	**0.51948**
negative predictive value	**0.54545**

Figure 3-4 Predictive Value Section for Patient Using the Empirical ROC Curve for HSD3B1

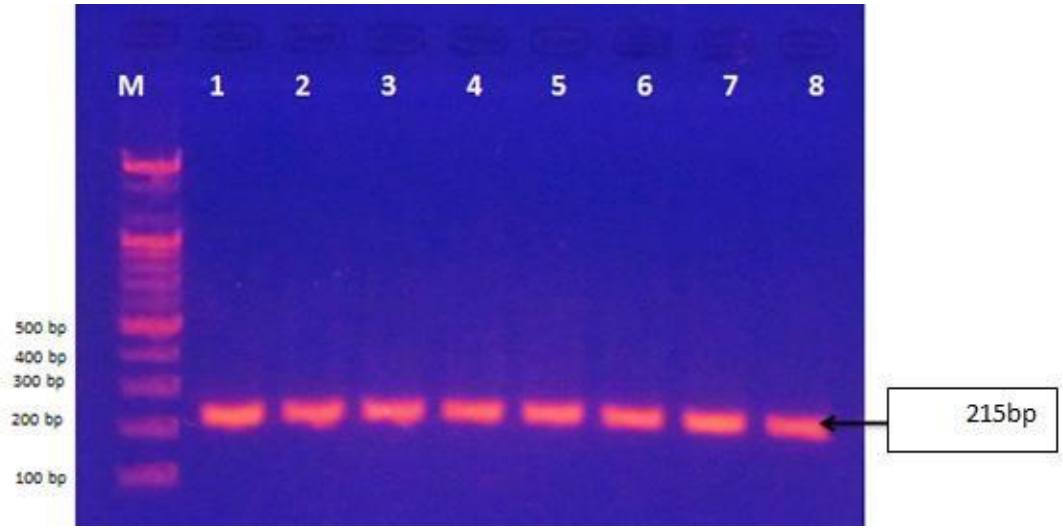


3.3.3. Genetic result for HSD3B1 (rs1047303) Genotyping

Genomic DNA was extracted from patient whole blood samples. HSD3B1 (rs1047303) genotyping was performed by sequencing technology by sent the samples to Macrogen Inc. Geumchen, Seoul, South Korea company. Briefly, pathologic complete response amplification was performed using special PCR Master Mix kit. The primers, annealing temperature, and cycle numbers were as follows: 5'-GTCAAATAGCGTATTCACCTTCTCTTAT-3' and 5'-GAGGGTGGAGCTTGATGACATCT-3', annealing temperature: 65°C, 35 cycles, respectively. The pathologic complete response products were purified and sequenced using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) on a Genetic Analyzer 3130XL (Applied Biosystems). Sequence data were visualized using Sequence Scanner Software version 1.0 (Applied Bio edit) as

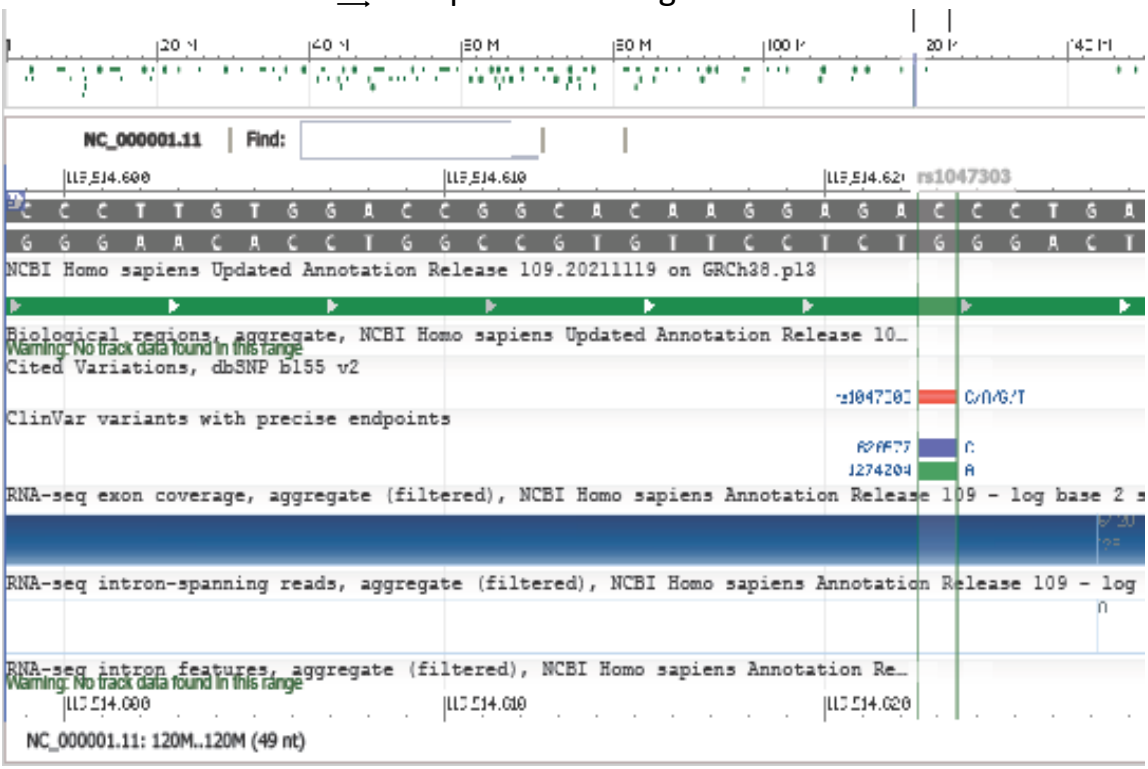
shown in fig. 3-5.

Figure 3-5 Electrophoresis for pcr production to the HSD3B1 gene on 70 volt for 1 hr. 2% agaros.



The result of gene sequencing shows the change A allele with C allele at site of SNP as show in figure 3-6

Figure 3-6 show site of A → C snp for HDS3B1 gene.



There was significant difference in expression of SNP between patients group and control group. where it was expressed more often in patients than in control. P value 0.015 which is significant at p value <0.05 as show in below table 3-10

Table 3-10 Allele Frequency of HDS3B1 gene

	C dominant N % SD	A dominant N % SD	Marginal Row Totals
Patients	87 (96.66) (10.3)	3 (3.33) [1.7]	90
Control	72 (80) [9.45]	18 (20) [4.12)	90
Marginal Column Totals	159	21	180

Table 3-11 The chi-square statistic is 12.129. The p-value is .0004. Significant at p< .05. The chi-square statistic with Yates correction is 10.566.

Chi-square	12.129
p-value	0.000496
Yates' chi-square	10.566
Yates' p-value	0.001151

4. Discussion:

4.1. Demographic Comparison between Prostate Cancer and Control groups

4.1.1. Age:

The results of the current study are in agreement with the findings of (Deuker et al. 2021) (206) which indicated that the age factor is one of the most important risk factors affecting prostate cancer. This has a close relationship with the disease, and that infection before the age of 40 is very rare. (Cunningham *et al.*, 2021) (207) reported that about 75% of patients diagnosed with prostate cancer ranged between 60 and 80 years old, with the possibility of prostate cancer occurring in young people and even in children and adolescents in a small way (Cunningham *et al.*, 2021) explaining the increased incidence. Prostate cancer with advancing age leads to aging of cells and loss of their important metabolic capabilities such as removal of toxins and carcinogens and the occurrence of genetic imbalance in them in addition to an increase in the period of exposure to carcinogens. The incidence of some diseases such as chronic inflammation in the prostate gland or benign enlargement increase with age, which may develop into a cancerous infection for these gland (Freeland *et al*., 2021) (208).

4.1.2 Gleason

The Gleason grading system is the main method used to stage prostate cancer (209). This system mainly considers the histological pattern of cells in H&E-stained sections of prostate tissue. The strength of the Gleason grading system is that it is useful for follow-up of a large variety of patients. The Gleason system has five grades (1 to 5) based on the morphology of the most common cell type, and another

five grades (1 to 5) based on the morphology of the second-most common cell type. Thus the total histological score ranges from 2 to 10. The common practice is to consider a Gleason score of 2-4 as well-differentiated carcinoma, 5-7 as moderately differentiated carcinoma, and 8-10 as poorly differentiated carcinoma. However, a Gleason score of 7 usually has characteristics of high-grade carcinoma. The AR has a critical role in regulation of the proliferation of androgen-refractory prostate cancer cells, even in the absence of androgens (210). Some studies reported same results as Park et al. (211) and Husain et al. (212) which found statistically insignificant correlation with Gleason score.

Our findings indicate that Gleason score had no relationship with AR level and HSD1B3 level. Also no relation between other physical singe like age and smoking with Gleason score as shown in table 3-2.

4.1.3 Family history:

The risk of prostate cancer increases with the presence of a family history of prostate cancer, as the results of the current study agreed with the results of studies (Taha *et al*., 2020) (213) and (Ni, Raghallaigh and Eeles, 2021) (214) which indicated an increase in the incidence of this type of cancer with a family history of this disease. And (Leão, and James, 2018) (215) pointed out in his study the possibility of prostate cancer due to the inheritance of mutation-carrying genes that cause prostate cancer. (Increased chance of have disease with relatives)

4.1.4 Smoking:

The smoking is one of the main causes of prostate cancer because cigarettes contain carcinogenic substances such as aromatic cyclic amines and free radicals, which can damage the DNA, where the risk of prostate cancer causing higher risk among smokers (Deuker *et al*. 2021) (216), also indicated (Factors, 2020) (217) the difference in the effect of the smoking factor on prostate cancer incidence according to the amount of smoking, increase and decrease, and this is in agreement with the results of the current study, while (Jiménez - Mendoza *et al*., 2018) (218) indicated that moderate smoking has no association with prostate cancer rates other than excessive smoking, which is a very important risk factor.

a. Androgen Receptor (AR):

Androgen acts on its target cells by binding to AR, causing transformation into a DNA-binding form that can interact with hormone responsive genes (219). The AR gene is located on the X chromosome and is composed of 8(A–H) coding exons.

Several studies on mutations in the AR gene in human prostate cancer have already been reported. Yang *et al*. (220) found that 25% of advanced lesions had a mutation at codon 877, which lies within the hormone-binding domain. This was the same as that detected in LNCaP. Further, Estébanez-Perpiñá *et al*. (221), detected a high rate of AR gene mutation in primary stage C and stage D prostate cancer prior to initiation of hormone therapy, 50% of these mutations being in exon A. The concept of androgen dependence of prostate cancer is based on the fact that a dramatic reduction in tumor mass is achieved after surgical castration or estrogen therapy. The development and progression of cancer in the prostate therefore appear to be androgen dependent. However, after androgen deprivation treatment, this androgen dependence is lost in most cases and progression to a more malignant status occurs. Qualitative and/or quantitative abnormalities in the AR gene may be involved in this phenomenon, hence information relating to AR expression levels and genetic alterations in prostate cancer is important.

Many studies have suggested that polymorphisms of the AR gene could influence the risk of prostate cancer development and progression in patients [222–223]. However, the CAG and GGC polymorphic repeats e nucleotide in the AR gene have been studied extensively as markers of prostate cancer susceptibility, with inconclusive results [224–226].

Given the AR gene is highly conserved, with only a single dimorphic marker reported in Caucasian populations, we investigated the role of this polymorphism on androgen related prostate cancer and risk factors for prostate cancer (age, country of birth, family history. As for the number of CAG repeats in the AR gene. Rutkowski, (227) reported a contraction of CAG repeats in a prostate cancer case which showed a paradoxical agonistic response to hormone therapy with an anti-androgen flutamide.

Guilherme *et al*. has been reported that an expansion of CAG repeats causes a linear decrease in the transactivation function of the AR protein, even though they are not included in the DNA-binding domain (228).

The E211 G>A is in partial linkage disequilibrium with both CAG and GGC repeats (229), its used as the genetic marker has been limited. It is well established that nucleotide repeat sequences are highly polymorphic that can be reflecting a high rate of mutation (230), whereas dimorphic polymorphisms are more stable that can be reflecting lower rates of mutation (231), and thus ideal markers in association studies. We therefore utilized the E211 marker to ascertain association with prostate cancer and known risk factors.

Result of the genetic analysis of the AR-E211 G>A polymorphisms of patients that confirmed with histological study has prostate cancer was association of the A genotypic variant we found no overall association between the presence of the A allele and CaP patients with Gleason grades. So many far studies have been carried out searching for a relationship between the AR-E211 G>A polymorphism detected in blood and various degrees changes in tumor prostatic tissue [232]. Although, we analyzed only a small sample of patients, in several cases we have repeatedly

identified in non-tumor and tumor tissues, both alleles of the AR-E211 G>A polymorphism. It is still studied by the default that the minor allele A is associated with higher risk of prostate cancer [233]. Our finding indicates that the minor allele A could be associated with transformation-induced changes of the modified androgen receptor gene or induced another changes caused by tumor transformation.

In our study, the A allele is not associated with overall prostate cancer risk, but decreases the risk of metastatic disease. Shorter CAG repeats have been reported to be more common in prostate tumors and older age at diagnosis.

The author of currant of the study opinion there are no change in serum level of AR (p value =0.42) which is not significant at p value<0.05. (Lolli *et al*., 2019) discuss the serum level of AR in CaP and show no significant change in its level association with extend of cancer (234)

b. β-Hydroxysteroid Dehydrogenase Isoenzyme-1 (HSD3B1):

There are several enzymes that convert adrenal androgens to testosterone and/or DHT have increased expression in castration-resistant disease (235). One such enzyme is 3b-hydroxysteroid dehydrogenase isoenzyme-1 (3b-HSD1, encoded by HSD3B1), which catalyzes the rate-limiting step of potent androgen synthesis from adrenal precursor steroids in peripheral tissues (e.g., prostate, breast, skin, placenta) (236, 237). Here, the study discusses the impact of an A→C missense-encoding single nucleotide polymorphism (SNP) in HSD3B1 nucleotide position 1245 (clustered refSNP ID 1047303) to augment potent androgen synthesis from adrenal precursors.

The study discusses the impact of an A→C missense-encoding single

nucleotide polymorphism (SNP) in HSD3B1 nucleotide position 1245 (clustered refSNP ID 1047303) to augment potent androgen synthesis from adrenal precursors. We and others researchers have referred to the HSD3B1(1245C) allele as the variant allele because it is minor allele. However, HSD3B1(1245C) is also the allele in the HSD3B1 reference sequence (238, 239)

In agreement with HSD3B1 function is convert DHEA to D4 -androstenedione (AD), piling up of 3bHSD1(367T) from HSD3B1(1245C) resulted to increased production of AD and ultimately DHT from the adrenal precursor DHEA, resulting in an adrenal-permissive phenotype that warrant more rapid development of CRPC. In contrast, the HSD3B1 (367N) protein from HSD3B1(1245A) was readily degraded

and thus incapable of strongly producing potent androgens from adrenal precursors, leading to an adrenal-restrictive phenotype (240).

Shiota *et al*. (241) again study the relationship between the adrenal-permissive genotype and worse postADT clinical outcomes in 104 Japanese men treated with AD for metastatic disease. Men possessing one or two copies of the adrenal-permissive allele had a higher HR of progression compared with homozygous wild-type men. Similarly, in a cohort of 44 Spanish men with PCa treated with ADT, Gil *et al*. (242) discover the adrenal-permissive genotype to be associated with inferior PFS compared with the adrenal-restrictive one.

Further, a post hoc analysis of 197 patients in a randomized phase 3 clinical trial (243) shows that inheritance of one or two copies of the adrenal-permissive allele is associated with more rapid development of CRPC and shorter OS in men with low-volume metastatic PCa treated with ADT 6 docetaxel (244).

The adrenal-permissive genotype occurs in CRPC as either a germline variant or a somatic mutation. The frequency of the germline 1245C allele varies widely with ancestry (34% European, 20% American, 16% South Asian, 9% African, and 8% East Asian) (245). Remarkably, the HSD3B1(1245C) allele was found to be selected for following androgen deprivation; 3/11 (27%) tumors from patients with CRPC with germline heterozygosity exhibited loss of heterozygosity with loss of HSD3B1(1245A) after ADT, and 3/25 (12%) of germline homozygous HSD3B1(1245A) CRPC tumors acquired the somatic mutation (246).

Previous studies investigating the effect of HSD3B1 on oncological outcome have demonstrated higher risk of progression and all-cause mortality in men carrying the variant allele (247,248). Although ethnic distribution in the study by

Agarwal *et al*. (250) is not presented, the study by Hearn et al (249) included mainly white men. In contrast to these US reports, (Huang *et al*., 2021) (251) showed significant risk of CRPC in men carrying variant allele but failed to show significant differences in progression-free survival PFS and overall survival OS. Thus, to our knowledge, for the first time in Asian individuals, the current study clearly shows significant detrimental PFS associated with variant allele. Superior quality of the sample origin might contribute to the successful demonstration of the significant results in this study, contrary to the results of (Huang *et al*., 2021) that used samples obtained from formalin-fixed archival tissues, which may cause errors in sequencing and may contain tumor-derived DNA. In addition, an imbalance in patients with no metastasis and metastasis between genotypes may explain the reason of failure to show prognostic significance (252).

Shiota *et al*. (253) found that the heterozygous genotype is associated with a lower HR of treatment failure (HR, 0.35; P 5 0.01) and all-cause mortality (HR, 0.40; P 5 0.04) compared with the homozygous adrenal restrictive genotype in 99 Japanese metastatic CRPC patients (254).The study confirmed the finding that HSD3B1 genetic variation may be associated with detrimental outcomes of ADT on prognosis done on Japanese men with prostate cancer, augmenting the robustness of previous findings and suggesting universal significance among different ethnicities (255).

This study done on Iraqi men with CaP to show if there are relationship present between AR gene mutation at rs6152 and HSD3B1 (rs1047303) with development of tumor. NO significant difference of AR gene between patients and

control (P value = 0.241) which is significant at $p < 0.05$ AR Dominant alleles. The analysis showed no association between prostate cancer and the A allele.

There was significant difference in expression of SNP between patients group and control group. where it was expressed more often in patients than in control. P value 0.015 which is significant at p value <0.05 where A allele changes to C allele.

Also the mean value of HSD3B1 serum level in the patients was 5.91 The mean value of HSD3B1 Serum Level in control 6.20. although the mean serum Level of HSD3B (mean 5.91) in patients group seems to be Less than that of the Control group (mean= 6.20) but the difference was not statically significant (P value 0.82).

212. Conclusions and Recommendations

a. Conclusions

From all data and correlations of different variables in the present study, it couldbe concluded that: -

1. Prostate cancer is more commune in elderly men than middle and young men.
2. Also this cancer is distributing in rural areas more than in urban areas as shown in result and discussion.
3. The genetic factors of HDS3B1 have main role in development of CaP.
4. The smoker is one of the factors that incidenceprostate cancer.
5. There was **no** significant difference in AR serum level between patients and control group.
6. Frequency of alleles of the AR-E211 G>A polymorphism in blood samples. The genotypes association of AR the model codominant was non-significant change when compared with healthy control. The all AR polymorphism not significant with CaP.
7. This study measures the serum level of HDS3B1 in PCa patient and noted if change and has role in disease or change with gene mutation. Although the mean serum level of HSD3B in patients group seems to be less than that of the control group but the difference was not statically significant.
8. HSD3B1 (rs1047303) genotyping was performed by sequencing technology. There was significant difference in expression of SNP between patients group and control group where A allele changes to C allele.

b. Recommendations:

1. This research study AR gene only at one snp AR-E211 gene (UCSC code: rs6152) for Iraqi men, so main other research need to different snp

2. There are many theories discuss the change that occur in AR will progress CaP in men, so main other study need to discuss these theories.

3. This study show the effect of HSD3B1 (rs1047303) on CaP, there are many other enzymes involve in prostate development and disease, many studies need to done on these enzymes for Iraqi men.

Reference:

1. Young, Barbara; O'Dowd, Geraldine; Woodford, Phillip (2013). Wheater's functional histology: a text and colour atlas (6th ed.). Philadelphia: Elsevier. pp. 347–8. ISBN 9780702047473.
2. Kristiansson, A., Örbom, A., Ahlstedt, J., Karlsson, H., Zedan, W., Gram, M., Åkerström, B., Strand, S. E., Altai, M., Strand, J., & Timmermand, O. V. (2021). 177 lu- psma- 617 therapy in mice, with or without the antioxidant α1- microglobulin (A1m), including kidney damage assessment using99m tc- mag3 imaging. Biomolecules, 11(2), 1–18. https://doi.org/10.3390/biom11020263
3. Standring, Susan, ed. (2016). "Prostate". Gray's anatomy : the anatomical basis of clinicalpractice(41st ed.). Philadelphia. pp. 1266–1270. ISBN 9780702052309. OCLC 920806541.
4. Goddard, Jonathan Charles (January 2019). "The history of the prostate, part one: say what you see". Trends in Urology & Men's Health. 10 (1): 28– 30. doi:10.1002/tre.676
5. Lin, C. H., Wu, W. J., Li, C. C., Ke, H. L., Jhan, J. H., & Wen, S. C. (2021). Comparison of different en bloc holmium laser enucleation of the prostate techniques to reduce the rate of postoperative transient urinary incontinence. Journal of International Medical Research, 49(8). https://doi.org/10.1177/03000605211037488
6. "The human proteome in prostate — The Human Protein Atlas". www.proteinatlas.org. Retrieved 2017-09-26.

7. Iacobas, S., & Iacobas, D. A. (2022). Personalized 3-Gene Panel for Prostate Cancer Target Therapy. Current Issues in Molecular Biology, 44(1), 360–382. https://doi.org/10.3390/cimb44010027
8. ^ O'Hurley, Gillian; Busch, Christer; Fagerberg, Linn; Hallström, Björn M.; Stadler, Charlotte; Tolf, Anna; Lundberg, Emma; Schwenk, Jochen M.; Jirström, Karin (2015-08-03). "Analysis of the Human Prostate- Specific Proteome Defined by Transcriptomics and Antibody-Based Profiling Identifies TMEM79 and ACOXL as Two Putative, Diagnostic Markers in Prostate Cancer". PLOS ONE. 10(8): e0133449. Bibcode:2015PLoSO..1033449O. doi:10.1371/journal.pon e.0133449. ISSN 1932-6203. PMC 4523174. PMID 26237329.
9. ^ Kong, HY; Byun, J (January 2013). "Emerging roles of human prostatic Acid phosphatase". Biomolecules & Therapeutics. 21(1): 10– 20. doi:10.4062/biomolther.2012.095. PMC 3762301. PMID 2400985 3.
10. Siegel, R. L., Miller, K. D. and Jemal, A. (2016). Cancer statistics, 2016. CA Cancer J. Clin. 66, 7-30.
11. Aaron L, Franco OE, Hayward SW. Review of Prostate Anatomy and Embryology and the Etiology of Benign Prostatic Hyperplasia. Urol Clin NorthAm. 2016 Aug;43(3):279-88. [PMC free article] [PubMed]
12. Hyuga, T., Alcantara, M., Kajioka, D., Haraguchi, R., Suzuki, K., Miyagawa, S., Kojima, Y., Hayashi, Y., & Yamada, G. (2020). Hedgehog signaling for urogenital organogenesis and prostate cancer: An implication for the epithelial–mesenchyme interaction (EMI). International Journal of Molecular Sciences, 21(1), 1–18. https://doi.org/10.3390/ijms21010058

13. Dhole, B., Gupta, S., Venugopal, S. K., & Kumar, A. (2018). Triiodothyronine stimulates VEGF expression and secretion via steroids and HIF-1α in murine Leydig cells. Systems Biology in Reproductive Medicine, 64(3), 191–201. https://doi.org/10.1080/19396368.2018.1433248
14. Sadley, TW (2019). "Bladder and urethra". Langman's medical embryology (14th ed.). Philadelphia: Wolters Kluwer. pp. 263– 66. ISBN 9781496383907.
15. ^ Jump up to:[a] [b] [c] Sadley, TW (2019). Langman's medical embryology(14th ed.). Philadelphia: Wolters Kluwer. pp. 265– 6. ISBN 9781496383907.
16. Tzelves, L., Protogerou, V., & Varkarakis, I. (2022). Denonvilliers' Fascia: The Prostate Border to the Outside World. Cancers, 14(3), 1–12. https://doi.org/10.3390/cancers14030688
17. Standring, Susan, ed. (2016). "Prostate". Gray's anatomy : the anatomical basis of clinical practice(41st ed.). Philadelphia. pp. 1266– 1270. ISBN 9780702052309. OCLC 920806541.
18. Barrett, Kim E. (2019). Ganong's review of medical physiology. Barman, Susan M.,, Brooks, Heddwen L.,, Yuan, Jason X.-J. (26th ed.). New York.pp. 411, 415. ISBN 9781260122404. OCLC 1076268769.
19. Standring, Susan, ed. (2016). "Prostate". Gray's anatomy : the anatomical basis of clinical practice(41st ed.). Philadelphia. pp. 1266– 1270. ISBN 9780702052309. OCLC 920806541.
20. ^ Rosenthal, Martha (2012). Human Sexuality: From Cells to Society. Cengage Learning. pp. 133–135. ISBN 978-0618755714. Retrieved September 17, 2012.

21. Otani, T. (2019). Clinical review of ejaculatory dysfunction. Reproductive Medicine and Biology, 18(4), 331–343. https://doi.org/10.1002/rmb2.12289

22. ^ Levin, R. J. (2018). "Prostate-induced orgasms: A concise review illustrated with a highly relevant case study". Clinical Anatomy. 31(1): 81–85. doi:10.1002/ca.23006. PMID 29265651.

23. ^ Hocaoglu Y, Roosen A, Herrmann K, Tritschler S, Stief C, Bauer RM (2012). "Real-time magnetic resonance imaging (MRI): anatomical changes during physiological voiding in men". BJU Int. 109 (2): 234– 9. doi:10.1111/j.1464-410X.2011.10255.x. PMID 21736694.

24. ^ Hocaoglu Y, Herrmann K, Walther S, Hennenberg M, Gratzke C, Bauer R; et al. (2013). "Contraction of the anterior prostate is required for the initiation of micturition". BJU Int. 111 (7): 1117–23. doi:10.1111/j.1464-410X.2012.11698.x. PMID 23356864.

25. Michael Schünke, Erik Schulte, Udo Schumacher: PROMETHEUS Innere Organe. LernAtlas Anatomie, vol 2: Innere Organe, Thieme Verlag, Stuttgart/Germany 2012, ISBN 9783131395337, p. 298, PDF.

26. ^ Lebdai S, Chevrot A, Doizi S, Pradere B, Delongchamps NB, Benchikh A; et al. (2019). "Do patients have to choose between ejaculation and miction? A systematic review about ejaculation preservation technics for benign prostatic obstruction surgical treatment" (PDF). World J Urol. 37 (2): 299–308. doi:10.1007/s00345-018-2368-6. PMID 29967947. S2CID 49556196.

27. Berry SJ, Coffey DS, Walsh PC, Ewing LL. The development of human benign prostatic hyperplasia with age. J Urol 1984; 132:474e9.

28. Söderholm, S., & Cantù, C. (2020). The WNT/β-catenin dependent transcription: A tissue-specific business. Wiley Interdisciplinary Reviews: Systems Biology and Medicine, September 2020, 1–41. https://doi.org/10.1002/wsbm.1511

29. Leach DA, Panagopoulos V, Nash C, Bevan C, Thomson AA, Selth LA, et al. Cell-lineage specificity and role of AP-1 in the prostate fibroblast androgen receptor cistrome. Mol Cell Endocrinol 2017;439:261e72.

30. Matsushita, S., Suzuki, K., Murashima, A., Kajioka, D., Acebedo, A. R., Miyagawa, S., Haraguchi, R., Ogino, Y., & Yamada, G. (2018). Regulation of masculinization: Androgen signalling for external genitalia development. Nature Reviews Urology, 15(6), 358–368. https://doi.org/10.1038/s41585-018-0008-y

31. Buskin, A., Singh, P., Lorenz, O., Robson, C., Strand, D. W., & Heer, R. (2021). A review of prostate organogenesis and a role for ipsc-derived prostate organoids to study prostate development and disease. International Journal of Molecular Sciences, 22(23). https://doi.org/10.3390/ijms222313097

32. Lemke, S. B., & Nelson, C. M. (2021). Dynamic changes in epithelial cell packing during tissue morphogenesis. Current Biology, 31(18), R1098–R1110. https://doi.org/10.1016/j.cub.2021.07.078.

33. Golden, E. J., Larson, E. D., Shechtman, L. A., Trahan, G. D., Gaillard, D., Fellin, T. J., Scott, J. K., Jones, K. L., & Barlow, L. A. (2021). Onset of taste bud cell renewal starts at birth and coincides with a shift in shh function. ELife, 10, 1–28. https://doi.org/10.7554/eLife.64013.

34. Makino, T., Izumi, K., & Mizokami, A. (2021). Undesirable status of prostate cancer cells after intensive inhibition of AR signaling: Post-AR era of CRPC treatment. Biomedicines, 9(4).https://doi.org/10.3390/biomedicines9040414.

35. Olson, A. W., Le, V., Wang, J., Hiroto, A., Kim, W. K., Lee, D. H., Aldahl, J., Wu, X., Kim, M., Cunha, G. R., You, S., & Sun, Z. (2021). Stromal androgen and hedgehog signaling regulates stem cell niches in pubertal prostate development. Development (Cambridge), 148(19).https://doi.org/10.1242/DEV.199738.

36. Donjacour AA, Cunha GR. Assessment of prostatic protein secretion in tissue recombinants made of urogenital sinus mesenchyme and urothelium from normal or androgeninsensitive mice. Endocrinology 1993;132:2342e420.

37. Pippione, A. C., Boschi, D., Pors, K., Oliaro-Bosso, S., & Lolli, M. L. (2017). Androgen-AR axis in primary and metastatic prostate cancer: chasing steroidogenic enzymes for therapeutic intervention. Journal of Cancer Metastasis and Treatment, 3(12), 328. https://doi.org/10.20517/2394- 4722.2017.44.

38. [26] Gao W, Bohl CE, Dalton JT. Chemistry and structural biology of androgenreceptor. Chem Rev 2005;105:3352e70.

39. Kopp, W. (2018). Diet-Induced Hyperinsulinemia as a Key Factor in the Etiology of Both Benign Prostatic Hyperplasia and Essential Hypertension? Nutrition and Metabolic Insights, 11, 117863881877307. https://doi.org/10.1177/1178638818773072

40. Shibata K, Hirasawa A, Moriyma N, et al (1996) alpha1a-Adrenoceptor polymorphism: pharmacological characterization and association with benign prostatic hypertrophy. Br J Pharm. 118: 1403-1408.

41. Siemińska, L., Borowski, A., Marek, B., Nowak, M., Kajdaniuk, D., Warakomski, J., & Kos-Kudła, B. (2018). Serum concentrations of adipokinesin men with prostate cancer and benign prostate hyperplasia. Endokrynologia Polska, 69(2), 120–127. https://doi.org/10.5603/EP

42. Evans-durán, B., Tripp, D. A., Campbell, J., Doiron, R. C., & Nickel, J. C.
(2022). on sexual functioning. 1–12.

43. Cangiano, M. (2022). Network based analysis to identify master regulators in prostate carcinogenesis

44. A.J. Schaeffer, Chronic prostatitis and the chronic pelvic pain syndrome, New Engl. J. Med. 355 (16) (2006) 1690–1698, doi:http://dx.doi.org/10.1056/ NEJMcp060423 (published Online First: Epub Date).

45. Bryk, D. J., & Shoskes, D. A. (2021). Using the UPOINT system to manage men with chronic pelvic pain syndrome. Arab Journal of Urology, 19(3), 387–393.https://doi.org/10.1080/2090598X.2021.1955546.

46. J.K. Tan, D.J. Png, L.C. Liew, et al., Prevalence of prostatitis-like symptoms in Singapore: a population-based study, Singapore Med. J. 43 (4) (2002) 189– 193.

47. Collins, A., Sundararajan, V., Millar, J., Burchell, J., Le, B., Krishnasamy, M., McLachlan, S. A., Hudson, P., Mileshkin, L., & Philip, J. (2019). The trajectory of patients who die from metastatic prostate cancer: a population-based study. BJU International, 123(S5), 19–26.

https://doi.org/10.1111/bju.14593

48. DeWitt-Foy, M. E., Nickel, J. C., & Shoskes, D. A. (2019). Management of Chronic Prostatitis/Chronic Pelvic Pain Syndrome(Figure presented.). European Urology Focus, 5(1), 2–4. https://doi.org/10.1016/j.euf.2018.08.027.

49. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA Cancer J Clin. 2019;69(1):7–34.

50. Ferlay J, Soerjomataram I, Ervik M, et al. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. International Agency for Research on Cancer, Lyon. 2013. http://globocan.iarc.fr. Accessed 24 May 2018.

51. Grossman DC, Curry SJ, Owens DK, et al. Screening for prostate cancer: US Preventive services task force recommendation statement. JAMA. 2018;319(18):1901–1913. doi: 10.1001/jama.2018.3710. [PubMed] [CrossRef] [Google Scholar]

52. NICE. Suspected cancer: recognition and referral. 2015. p. 1–95. https://www.nice.org.uk/guidance/ng12.

53. Østerø í Jákupsstovu J, Brodersen J. Do men with lower urinary tract symptoms have an increased risk of advanced prostate cancer? BMJ. 2018;361(1202):1–6. Available from https://www.bmj.com/content/bmj/361/bmj.k1202.full.pdf. [PubMed]

54. Jones D, Friend C, Dreher A, Allgar V, Macleod U. The diagnostic test accuracy of rectal examination for prostate cancer diagnosis in symptomatic patients: a systematic review. BMC Fam Pract. 2018;19:79. doi: 10.1186/s12875-018-0765-y. [PMC free article] [PubMed] [CrossRef] [Google Scholar]

55. Parekh DJ, Punnen S, Sjoberg DD, et al. A multi-institutional prospective trial in the USA confirms that the 4Kscore accurately identifies men with high-grade prostate cancer. Eur Urol. 2015;68(3):464–470. doi: 10.1016/j.eururo.2014.10.021. [PubMed] [CrossRef] [Google Scholar]

56. Schumacher FR, Al Olama AA, Berndt SI, et al. Association analyses of more than 140,000 men identify 63 new prostate cancer susceptibility loci. Nat Genet. 2018; 50:928–36. Available from http://www.nature.com/articles/s41588-018-0142-8.

57. Ahmed HU, Bosaily AE-S, Brown LC, et al. Diagnostic accuracy of multi-parametric MRI and TRUS biopsy in prostate cancer (PROMIS): a paired validating confirmatory study. Lancet. 2017; 380:1–8. [PubMed] [Google Scholar]

58. Giatti, S., Diviccaro, S., Maria, M., Caruso, D., Garcia-segura, L. M., Viviani, B., & Melcangi, R. C. (2020). Frontiers in Neuroendocrinology Sex di ff erences in steroid levels and steroidogenesis in the nervous system : Physiopathological role. 56(November 2019). https://doi.org/10.1016/j.yfrne.2019.100804.

59. Tyutyusheva, N., Mancini, I., Baroncelli, G. I., Elios, S. D., Peroni, D., Meriggiola, M. C., & Bertelloni, S. (2021). Complete Androgen Insensitivity Syndrome : From Bench to Bed. 1.

60. Barbagallo, F., Cannarella, R., Bertelli, M., Crafa, A., Vignera, S. La, Condorelli, R. A., & Calogero, A. E. (2021). Complete Androgen Insensitivity Syndrome : From the Relevance of an Accurate Genetic Diagnosis to the Challenge of Clinical Management . A Case Report.

61. Magrinelli, F. (2021). Challenges in Clinicogenetic Correlations : One Gene – Many Phenotypes Phenomena Contributing to Incomplete Penetrance. 8(March), 299–310. https://doi.org/10.1002/mdc3.13165.

62. Jeong, J., Kim, H., & Choi, J. (2019). In Silico Molecular Docking and In Vivo Validation with Caenorhabditis elegans to Discover Molecular Initiating Events in Adverse Outcome Pathway Framework : Case Study on Endocrine-Disrupting Chemicals with Estrogen and Androgen Receptors. https://doi.org/10.3390/ijms20051209

63. Hashizume, A., Fischbeck, K. H., Penuto, M., & Fratta, P. (n.d.). Disease mechanism , biomarker , and therapeutics for spinal and bulbar muscular atrophy (SBMA).

64. Zajac JD, Fui MN . Kennedy's disease: clinical significance of tandem repeats in the androgen receptor. Adv Exp Med Biol 2012; 769: 153–6

65. Finsterer J . Bulbar and spinal muscular atrophy (Kennedy's disease): a review. Eur J Neurol 2009; 16: 556–61.

66. Duffy, S. W., Smith, R. A., Chen, S. L., & Ahlgren, J. (2021). Beneficial Effect of Consecutive Screening Mammography Examinations on Mortality from Breast Cancer : 5.

67. Steenland, K., Winquist, A., & Highway, B. (2022). HHS Public Access. https://doi.org/10.1016/j.envres.2020.110690.PFAS.

68. Cao, M. (2020). Cancer burden of major cancers in China : A need for sustainable. March, 205–210. https://doi.org/10.1002/cac2.12025.

69. Steenland, K., Winquist, A., & Highway, B. (2022). HHS Public Access. https://doi.org/10.1016/j.envres.2020.110690.PFAS.

70. Makhaola, K., Moyo, S., & Kebaabetswe, L. P. (2020). Distribution and genetic variability of sapoviruses in Africa. Viruses, 12(5), 1–19. https://doi.org/10.3390/v12050490.

71. Ballout F, Habli Z, Monzer A, Rahal ON, Fatfat M, Gali-Muhtasib H. Anticancer Alkaloids: Molecular Mechanisms and Clinical Manifestations.

Bioactive Natural Products for the Management of Cancer: from Bench to Bedside: Springer; 2019. p. 1-35.

72. Kasprzak, A. (2021). Insulin-like growth factor 1 (Igf-1) signaling in glucose metabolism in colorectal cancer. In International Journal of Molecular Sciences (Vol. 22, Issue 12). https://doi.org/10.3390/ijms22126434.
73. Zheng, Y., Lin, S. X., Wu, S., Dahl, D. M., Blute, M. L., Zhong, W. De, Zhou, X., & Wu, C. L. (2020). Clinicopathological characteristics of localized prostate cancer in younger men aged ≤ 50 years treated with radical prostatectomy in the PSA era: A systematic review and meta-analysis. Cancer Medicine, 9(18), 6473–6484. https://doi.org/10.1002/cam4.3320.
74. Taitt, H. E. (2018). Global Trends and Prostate Cancer: A Review of Incidence, Detection, and Mortality as Influenced by Race, Ethnicity, and Geographic Location. American Journal of Men's Health, 12(6), 1807–1823. https://doi.org/10.1177/1557988318798279.
75. Clift, A. K., Coupland, C. A. C., & Hippisley-Cox, J. (2021). Prostate-specific antigen testing and opportunistic prostate cancer screening: A cohort study in England, 1998–2017. British Journal of General Practice, 71(703), E157– E165. https://doi.org/10.3399/bjgp20X713957.
76. Jain, B., Ng, K., Santos, P. M. G., Taparra, K., Muralidhar, V., Mahal, B. A., Vapiwala, N., Trinh, Q.-D., Nguyen, P. L., & Dee, E. C. (2022). Prostate Cancer Disparities in Risk Group at Presentation and Access to Treatment for Asian Americans, Native Hawaiians, and Pacific Islanders: A Study With Disaggregated Ethnic Groups. JCO Oncology Practice, 18(1), e204–e218. https://doi.org/10.1200/op.21.00412.

77. Lloyd T, Hounsome L, Mehay A, Mee S, Verne J, Cooper A. Lifetime risk of being diagnosed with, or dying from, prostate cancer by major ethnic group in England 2008–2010. BMC medicine. 2015;13(1):171.

78. Kwon, D. H. M., Borno, H. T., Cheng, H. H., Zhou, A. Y., & Small, E. J. (2020). Ethnic disparities among men with prostate cancer undergoing germline testing. Urologic Oncology: Seminars and Original Investigations, 38(3), 80. e1-80. e7. https://doi.org/10.1016/j.urolonc.2019.09.010.

79. Chinegwundoh F, Enver M, Lee A, Nargund V, Oliver T, Ben- Shlomo Y. Risk and presenting features of prostate cancer amongst African-Caribbean, South Asian and European men in North- east London. BJU international. 2006;98(6):1216-20.

80. Jack RH, Davies EA, Møller H. Prostate cancer incidence, stage at diagnosis, treatment and survival in ethnic groups in South- East England.BJU international. 2010;105(9):1226-30.

81. Walsh-Childers, K., Odedina, F., Poitier, A., Kaninjing, E., & Taylor, G. (2018). Choosing Channels, Sources, and Content for Communicating Prostate Cancer Information to Black Men: A Systematic Review of the Literature. American Journal of Men's Health, 12(5), 1728–1745. https://doi.org/10.1177/1557988318786669.

82. Islami, F., Guerra, C. E., Minihan, A., Yabroff, K. R., Fedewa, S. A., Sloan, K., Wiedt, T. L., Thomson, B., Siegel, R. L., Nargis, N., Winn, R. A., Lacasse, L., Makaroff, L., Daniels, E. C., Patel, A. V., Cance, W. G., & Jemal, A. (2022). American Cancer Society's report on the status of cancer disparities in the United States, 2021. CA: A Cancer Journal for Clinicians, 72(2), 112–143. https://doi.org/10.3322/caac.21703.

83. Mutuku, S. M., Spotbeen, X., Trim, P. J., Snel, M. F., Butler, L. M., & Swinnen, J. V. (2022). Approaches to Lipidomics and Transcriptomics. 1–16.

84. Lewis, D. D., & Cropp, C. D. (2020). The impact of African ancestry on prostate cancer disparities in the era of precision medicine. Genes, 11(12), 1–28. https://doi.org/10.3390/genes11121471.

85. Miller, J. S., Rodriguez-Saona, L., & Hackshaw, K. V. (2020).

Metabolomics in central sensitivity syndromes. Metabolites, 10(4). https://doi.org/10.3390/metabo10040164.

86. Daram, S., Syeda, Z., Poornima, S., Boppana, S., Prabhala, S., Sandhya, A., Ramakrishna, D., & Hasan, Q. (2019). Association of Androgen Receptor (AR) CAG Repeats and Cytochrome P450 3A5*3 (CYP3A5*3) Gene Polymorphisms in South Indian Men with Prostate Cancer. Journal of Clinical and Diagnostic Research, 3(August), 1–5. https://doi.org/10.7860/jcdr/2019/41564.13077.

87. Kim, T. J., Lee, Y. H., & Koo, K. C. (2021). Current status and future perspectives of androgen receptor inhibition therapy for prostate cancer: A comprehensive review. Biomolecules, 11(4). https://doi.org/10.3390/biom11040492

88. Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. Nature Reviews Cancer. 2001;1(1):34-45.

89. Afify, S. M., & Seno, M. (2019). Conversion of stem cells to cancer stem cells: Undercurrent of cancer initiation. Cancers, 11(3), 1–19. https://doi.org/10.3390/cancers11030345.

90. Li, Y., Deng, S.-L., Lian, Z.-X., & Yu, K. (2019). Roles of Toll-Like Receptors in Nitroxidative Stress in Mammals. Cells, 8(6), 576. https://doi.org/10.3390/cells8060576.

91. Saitoh, T., & Oda, T. (2021). Dna damage response in multiple myeloma: The role of the tumor microenvironment. Cancers, 13(3), 1–22. https://doi.org/10.3390/cancers13030504.

92. Tamminga, M., Hiltermann, T. J. N., Schuuring, E., Timens, W., Fehrmann, R. S. N., & Groen, H. J. M. (2020). Immune microenvironment composition in non-small cell lung cancer and its association with survival. Clinical and Translational Immunology, 9(6), 1–13. https://doi.org/10.1002/cti2.1142.

93. Mydin, R. B. S. M. N., & Okekpa, S. I. (2019). Molecular Pathways for Nasopharyngeal Carcinoma focused on Acetaldehyde, Nitrosamines and Nicotine Exposures. Malaysian Journal of Medicine and Health Sciences, 15(July), 64–70.

94. Simou, E., Britton, J., & Leonardi-Bee, J. (2018). Alcohol and the risk of pneumonia: A systematic review and meta-analysis. BMJ Open, 8(8), 1–10. https://doi.org/10.1136/bmjopen-2018-022344.

95. Agalliu, I., Lin, W. K. J., Zhang, J. S., Jacobson, J. S., Rohan, T. E., Adusei, B., Snyper, N. Y. F., Andrews, C., Sidahmed, E., Mensah, J. E., Biritwum, R., Adjei, A. A., Okyne, V., Ainuson-Quampah, J., Fernandez, P., Irusen, H., Odiaka, E., Folasire, O. F., Ifeoluwa, M. G., … Hsing, A. W. (2022). Overall and central obesity and prostate cancer risk in African men. Cancer Causes and Control, 33(2), 223–239. https://doi.org/10.1007/s10552-021-01515-0.

96. Andò, S., Gelsomino, L., Panza, S., Giordano, C., Bonofiglio, D., Barone, I., & Catalano, S. (2019). Obesity, leptin and breast cancer: Epidemiological

evidence and proposed mechanisms. Cancers, 11(1), 1–27. https://doi.org/10.3390/cancers11010062.

97. VALERIU, H. M. (2019). Anatomy Without Frontiers, a Systemic Morpho-Functional Approach.

98. Travis, R. C., Appleby, P. N., Martin, R. M., Holly, J. M. P., Albanes, D., Black, A., Bueno-De-Mesquita, H. B., Chan, J. M., Chen, C., Chirlaque, M. D., Cook, M. B., Deschasaux, M., Donovan, J. L., Ferrucci, L., Galan, P., Giles, G. G., Giovannucci, E. L., Gunter, M. J., Habel, L. A., … Allen, N. E. (2016). A meta-analysis of individual participant data reveals an association between circulating levels of IGF-I and prostate cancer risk. Cancer Research, 76(8), 2288–2300. https://doi.org/10.1158/0008-5472.CAN-15-1551.

99. Maitland, N. J. (2021). Resistance to antiandrogens in prostate cancer: Is it inevitable, intrinsic or induced? Cancers, 13(2), 1–32. https://doi.org/10.3390/cancers13020327.

100. Zaidi, S., Gandhi, J., Joshi, G., Smith, N. L., & Khan, S. A. (2019). The anticancer potential of metformin on prostate cancer. Prostate Cancer and Prostatic Diseases, 22(3), 351–361. https://doi.org/10.1038/s41391-018-0085-2.

101. Hormones E, Group PCC. Endogenous sex hormones and prostate cancer: a collaborative analysis of 18 prospective studies. JNCI: Journal of the National Cancer Institute. 2008;100(3):170-83.

102. Estébanez-Perpiñá, E., Bevan, C. L., & McEwan, I. J. (2021). Eighty years of targeting androgen receptor activity in prostate cancer: The fight goes on. Cancers, 13(3), 1–19. https://doi.org/10.3390/cancers13030509.

103. Maitland, N. J. (2021). Resistance to antiandrogens in prostate cancer: Is it inevitable, intrinsic or induced? Cancers, 13(2), 1–32. https://doi.org/10.3390/cancers13020327.

104. Federici, S., Goggi, G., Quinton, R., Giovanelli, L., Persani, L., Cangiano, B., & Bonomi, M. (2021). New and Consolidated Therapeutic Options for Pubertal Induction in Hypogonadism: In-depth Review of the Literature. Endocrine Reviews, January 2022. https://doi.org/10.1210/endrev/bnab043.

105. Ban, F., Leblanc, E., Cavga, A. D., Huang, C. C. F., Flory, M. R., Zhang, F., Chang, M. E. K., Morin, H., Lallous, N., Singh, K., Gleave, M. E., Mohammed, H., Rennie, P. S., Lack, N. A., & Cherkasov, A. (2021). Development of an androgen receptor inhibitor targeting the n-terminal domain of androgen receptor for treatment of castration resistant prostate cancer. Cancers, 13(14). https://doi.org/10.3390/cancers13143488

106. Genazzani, A. R., Monteleone, P., Giannini, A., & Simoncini, T. (2021). Hormone therapy in the postmenopausal years: Considering benefits and risks in clinical practice. Human Reproduction Update, 27(6), 1115–1150. https://doi.org/10.1093/humupd/dmab026.

107. Amani, N., Shariati, M., Ahmadi, R., Mokhtari, M., & Khatamsaz, S. (2021). i l n e n s r i t i t r e l c i l n e n s r i t i. 1–9.

108. Sun, J., Pan, Y., Li, X., Wang, L., Liu, M., & Tu, P. (2022). Quercetin Attenuates Osteoporosis in Orchiectomy Mice by Regulating Glucose and Lipid Metabolism via the GPRC6A / AMPK / mTOR Signaling Pathway. 13(April), 1–13. https://doi.org/10.3389/fendo.2022.849544.

109. Miyoshi Y, Uemura H, Umemoto S, Sakamaki K, Morita S, Suzuki K, et al. High testosterone levels in prostate tissue obtained by needle biopsy

correlate with poor-prognosis factors in prostate cancer patients. BMC cancer. 2014;14(1):717.

110. Zhang, Z., Cheng, L., Zhang, Q., Kong, Y., He, D., Li, K., Rea, M., Wang, J., Wang, R., Liu, J., Li, Z., Yuan, C., Liu, E., Fondufe-Mittendorf, Y. N., Li, L., Han, T., Wang, C., & Liu, X. (2021). Co-Targeting Plk1 and DNMT3a in Advanced Prostate Cancer. Advanced Science, 8(13), 1–16. https://doi.org/10.1002/advs.202101458.

111. Huang, L., Labonte, M. J., Craig, S. G., Finn, S. P., & Allott, E. H. (2022). Inflammation and Prostate Cancer: A Multidisciplinary Approach to Identifying Opportunities for Treatment and Prevention. Cancers, 14(6), 1–18. https://doi.org/10.3390/cancers14061367.

112. Xia, C., Dong, X., Li, H., Cao, M., Sun, D., He, S., Yang, F., Yan, X., Zhang, S., Li, N., & Chen, W. (2022). Cancer statistics in China and United States, 2022: Profiles, trends, and determinants. Chinese Medical Journal, 135(5), 584–590. https://doi.org/10.1097/CM9.0000000000002108.

113. Estébanez-Perpiñá, E., Bevan, C. L., & McEwan, I. J. (2021). Eighty years of targeting androgen receptor activity in prostate cancer: The fight goes on. Cancers, 13(3), 1–19. https://doi.org/10.3390/cancers13030509.

114. Messner, E. A., Steele, T. M., Tsamouri, M. M., Hejazi, N., Gao, A. C., Mudryj, M., & Ghosh, P. M. (2020). The androgen receptor in prostate cancer: Effect of structure, ligands and spliced variants on therapy. Biomedicines, 8(10), 1–19. https://doi.org/10.3390/biomedicines8100422.

115. Figg, W. D., Chau, C. H., Price, D. K., Till, C., Goodman, P. J., Cho, Y., Varella-garcia, M., Reichardt, J. K. V, Tangen, C. M., Leach, R. J., Bokhoven, A. Van, Thompson, I. M., & Lucia, M. S. (2014). Prevention Trial. Denmeade SR, Lin XS, Isaacs JT . Role of programmed (apoptotic) cell death

during the progression and therapy for prostate cancer. Prostate 1996; 28: 251–65.

116. Melekhova, A., & Baniahmad, A. (2021). Ing tumour suppressors and ing splice variants as coregulators of the androgen receptor signalling in prostate cancer. Cells, 10(10). https://doi.org/10.3390/cells10102599.

117. Denmeade, S., Antonarakis, E. S., & Markowski, M. C. (2022). Bipolar androgen therapy (BAT): A patient's guide. The Prostate, February, 753–762. https://doi.org/10.1002/pros.24328.

118. Peter, M. R., & Peter, M. R. (2021). Discovery of Novel Circulating Biomarkers in Metastatic Castration Resistant Prostate Cancer to Guide Optimal Treatment Decisions by Discovery of Novel Circulating Biomarkers in Metastatic Castration Resistant Prostate Cancer to Guide Optimal Treatment Decisions.

119. Ferraro, S., Bussetti, M., & Panteghini, M. (2020). Serum prostate-specific antigen testing for early detection of prostate cancer: Managing the gap between clinical and laboratory practice. Clinical Chemistry, 67(4). https://doi.org/10.1093/clinchem/hvab002.

120. Marx J . Medicine. Fused genes may help explain the origins of prostate cancer. Science 2005; 310: 603.

121. Tomlins SA, Rhodes DR, Perner S, Dhanasekaran SM, Mehra R, Sun XW, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005; 310: 644–8.

122. Lin C, Yang L, Tanasa B, Hutt K, Ju BG, Ohgi K, et al. Nuclear receptor-induced chromosomal proximity and DNA breaks underlie specific translocations in cancer. Cell 2009; 139: 1069–83.

123. Haffner MC, Aryee MJ, Toubaji A, Esopi DM, Albadine R, Gurel B, et al. Androgen-induced TOP2B-mediated double-strand breaks and prostate cancer gene rearrangements. Nat Genet 2010; 42: 668–75.

124. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, et al. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010; 18: 11–128.

125. Dovey, Z. S., Nair, S. S., Chakravarty, D., & Tewari, A. K. (2021). Racial disparity in prostate cancer in the African American population with actionable ideas and novel immunotherapies. Cancer Reports, 4(5), 1–28. https://doi.org/10.1002/cnr2.1340.

126. Melloni, C., & Roe, M. T. (2020). Androgen deprivation therapy and cardiovascular disease. Urologic Oncology: Seminars and Original Investigations, 38(2), 45–52. https://doi.org/10.1016/j.urolonc.2019.02.010.

127. Ahow, M. R., & Babwah, A. (2014). Pathway-Specific Signaling and its Impact on Fertility: A Focus on the Kisspeptin Receptor, Kiss1r Part of the Molecular and Cellular Neuroscience Commons. https://ir.lib.uwo.ca/etdhttps://ir.lib.uwo.ca/etd/2537.

128. Andela, C. D., Matte, R., Jazet, I. M., Zonneveld, W. C. G., Schoones, J. W., & Meinders, A. E. (2021). Effect of androgen deprivation therapy on cognitive functioning in men with prostate cancer: A systematic review. International Journal of Urology, 28(8), 786–798. https://doi.org/10.1111/iju.14596.

129. Lim, J., Onozawa, M., Saad, M., Ong, T. A., Malek, R., & Akaza, H. (2021). Recent trend of androgen deprivation therapy in newly diagnosed prostate cancer patients: Comparing between high- and middle-income Asian countries. Cancer Science, 112(6), 2071–2080. https://doi.org/10.1111/cas.14889.

130. Bonollo, F., Thalmann, G. N., Julio, M. K. De, & Karkampouna, S. (2020). The role of cancer-associated fibroblasts in prostate cancer tumorigenesis. Cancers, 12(7), 1–28. https://doi.org/10.3390/cancers12071887.

131. Dariane, C., Clairefond, S., Péant, B., Communal, L., Thian, Z., Ouellet, V., Trudel, D., Benzerdjeb, N., Azzi, F., Méjean, A., Timsit, M.-O., Baurès, M., Guidotti, J.-E., Goffin, V., Karakiewicz, P. I., Mes-Masson, A.-M., & Saad, F. (2022). High Keratin-7 Expression in Benign Peri-Tumoral Prostatic Glands Is Predictive of Bone Metastasis Onset and Prostate Cancer-Specific Mortality. Cancers, 14(7), 1623. https://doi.org/10.3390/cancers14071623

132. Archer, M., Dogra, N., & Kyprianou, N. (2020). Inflammation as a driver of prostate cancer metastasis and therapeutic resistance. Cancers, 12(10), 1–24. https://doi.org/10.3390/cancers12102984.

133. Maitland, N. J. (2021). Resistance to antiandrogens in prostate cancer: Is it inevitable, intrinsic or induced? Cancers, 13(2), 1–32. https://doi.org/10.3390/cancers13020327.

134. Christenson, M., Song, C. S., Liu, Y. G., & Chatterjee, B. (2022). Precision Targets for Intercepting the Lethal Progression of Prostate Cancer: Potential Avenues for Personalized Therapy. Cancers, 14(4). https://doi.org/10.3390/cancers14040892.

135. Perry, M. (n.d.). Molecular and Genetic Crosstalk Between ERBB2 and ERR α in Heart and Muscle : Implications for Targeted Therapies in Breast Cancer.

136. Messner, E. A., Steele, T. M., Tsamouri, M. M., Hejazi, N., Gao, A. C., Mudryj, M., & Ghosh, P. M. (2020). The androgen receptor in prostate

cancer: Effect of structure, ligands and spliced variants on therapy. Biomedicines, 8(10), 1–19. https://doi.org/10.3390/biomedicines8100422.

137. Porter, B. A., Ortiz, M. A., Bratslavsky, G., & Kotula, L. (2019). Structure and function of the nuclear receptor superfamily and current targeted therapies of prostate cancer. Cancers, 11(12).https://doi.org/10.3390/cancers11121852.

138. Skowron, K. J., Booker, K., Cheng, C., Creed, S., Brian, P., Lazzara, P. R., Lian, A., Siddiqui, Z., Speltz, T. E., & Terry, W. (1846). Version of Record: https://www.sciencedirect.com/science/article/pii/S030372071930173X.

139. Tan, H., Wang, X., Hong, H., Benfenati, E., Giesy, J. P., Gini, G. C., Kusko, R., Zhang, X., Yu, H., & Shi, W. (2020). Structures of Endocrine-Disrupting Chemicals Determine Binding to and Activation of the Estrogen Receptor α and Androgen Receptor. Environmental Science and Technology, 54(18), 11424–11433. https://doi.org/10.1021/acs.est.0c02639.

140. Zhao, J., Liu, X., Blayney, A., Zhang, Y., Gandy, L., Mirsky, P. O., Smith, N., Zhang, F., Linhardt, R. J., Chen, J., Baines, C., Loh, S. N., & Wang, C. (2022). Intrinsically Disordered N-terminal Domain (NTD) of p53 Interacts with Mitochondrial PTP Regulator Cyclophilin D. Journal of Molecular Biology, 434(9), 167552. https://doi.org/10.1016/j.jmb.2022.167552.

141. Jafari, H., & Hussain, S. (2022). Nuclear Receptor Coregulators in Hormone-Dependent Cancers. 1–30.

142. Hu R, Dunn TA, Wei S, Isharwal S, Veltri RW, Humphreys E, et al. Ligand-independent androgen receptor variants derived from splicing of cryptic exons signify hormonerefractory prostate cancer. Cancer Res 2009;69:16e22.

143. Porter, B. A., Ortiz, M. A., Bratslavsky, G., & Kotula, L. (2019). Structure and function of the nuclear receptor superfamily and current targeted therapies of prostate cancer. Cancers, 11(12).https://doi.org/10.3390/cancers11121852.

144. Mazaira, G. I., Piwien Pilipuk, G., & Galigniana, M. D. (2021). Corticosteroid receptors as a model for the Hsp90•immunophilin-based transport machinery. Trends in Endocrinology and Metabolism, 32(10), 827–838. https://doi.org/10.1016/j.tem.2021.07.005.

145. Maeda, K., Habara, M., Kawaguchi, M., Matsumoto, H., Hanaki, S., Masaki, T., Sato, Y., Matsuyama, H., Kunieda, K., Nakagawa, H., & Shimada, M. (2022). FKBP51 and FKBP52 regulate androgen receptor dimerization and proliferation in prostate cancer cells. Molecular Oncology, 16(4), 940–956. https://doi.org/10.1002/1878-0261.13030.

146. Cheung, S. (2020). p38 Mitogen-Activated Protein Kinase Inhibition in Castration-Resistant Prostate Cancer p38 Mitogen-Activated Protein Kinase Inhibition in Castration- Resistant Prostate Cancer.

147. Nicolet, B. P., Zandhuis, N. D., Lattanzio, V. M., & Wolkers, M. C. (2021). Sequence determinants as key regulators in gene expression of T cells. Immunological Reviews, 304(1), 10–29. https://doi.org/10.1111/imr.13021.

148. Pfleger, J., Coleman, R. C., Ibetti, J., Roy, R., Kyriazis, I. D., Gao, E., Drosatos, K., & Koch, W. J. (2020). Genomic binding patterns of forkhead box protein O1 reveal its unique role in cardiac hypertrophy. Circulation, 882– 898. https://doi.org/10.1161/CIRCULATIONAHA.120.046356.

149. Nash C, Boufaied N, Mills IG, Franco OE, Hayward SW, Thomson AA. Genome-wide analysis of AR binding and comparison with transcript expression in primary human fetal prostate fibroblasts and cancer associated fibroblasts. Mol Cell Endocrinol 2018;471:1e14. [

150. Duque, M., Amorim, J. P., & Bessa, J. (2021). Ptf1a function and transcriptional cis-regulation, a cornerstone in vertebrate pancreas development. FEBS Journal, 1–16. https://doi.org/10.1111/febs.16075

151. Erdmann, É., Ould Madi Berthélémy, P., Cottard, F., Angel, C. Z., Schreyer, E., Ye, T., Morlet, B., Negroni, L., Kieffer, B., & Céraline, J. (2021). Androgen receptor-mediated transcriptional repression targets cell plasticity in prostate cancer. Molecular Oncology, 1–19. https://doi.org/10.1002/1878-0261.13164Gao J, Arnold JT, Isaacs JT (2001) Conversion from a paracrine to an autocrine mechanism of androgen-stimulated growth during malignant transformation of prostatic epithelial cells. Cancer Res. 61(13): 5038-44

152. Oduwole, O. O., Huhtaniemi, I. T., & Misrahi, M. (2021). The roles of luteinizing hormone, follicle-stimulating hormone and testosterone in spermatogenesis and folliculogenesis revisited. International Journal of Molecular Sciences, 22(23).https://doi.org/10.3390/ijms222312735

153. Waszkiewicz, E. M., Kozlowska, W., Zmijewska, A., & Franczak, A. (2020). Expression of insulin-like growth factor 1 (IGF-1) and epidermal growth factor (EGF) receptors and the effect of IGF-1 and EGF on androgen and estrogen release in the myometrium of pigs—in vitro study. Animals, 10(5), 1–17. https://doi.org/10.3390/ani10050915

154. Olson, A. W., Le, V., Wang, J., Hiroto, A., Kim, W. K., Lee, D. H., Aldahl, J., Wu, X., Kim, M., Cunha, G. R., You, S., & Sun, Z. (2021). Stromal androgen and hedgehog signaling regulates stem cell niches in pubertal prostate development. Development (Cambridge), 148(19). https://doi.org/10.1242/DEV.199738.

155. Rohayem, J., Zitzmann, M., Laurentino, S., Kliesch, S., Nieschlag, E., Holterhus, P. M., & Kulle, A. (2021). The role of gonadotropins in testicular and adrenal androgen biosynthesis pathways—Insights from males with congenital hypogonadotropic hypogonadism on hCG/rFSH and on testosterone replacement. Clinical Endocrinology, 94(1), 90–101. https://doi.org/10.1111/cen.14324.

156. Messner, E. A., Steele, T. M., Tsamouri, M. M., Hejazi, N., Gao, A. C., Mudryj, M., & Ghosh, P. M. (2020). The androgen receptor in prostate cancer: Effect of structure, ligands and spliced variants on therapy. Biomedicines, 8(10), 1–19. https://doi.org/10.3390/biomedicines8100422.

157. Sawal, N., Kaur, J., Kaur, K., & Gombar, S. (2020). Dihydrotestosterone in Amyotrophic lateral sclerosis—The missing link? Brain and Behavior, 10(11), 1–10. https://doi.org/10.1002/brb3.1645

158. Kur, P., Kolasa-Wołosiuk, A., Grabowska, M., Kram, A., Tarnowski, M., Baranowska-Bosiacka, I., Rzeszotek, S., Piasecka, M., & Wiszniewska, B. (2021). The postnatal offspring of finasteride- treated male rats shows hyperglycaemia, elevated hepatic glycogen storage and altered GLUT2, IR, and AR expression in the liver. International Journal of Molecular Sciences, 22(3), 1–20. https://doi.org/10.3390/ijms22031242

159. La Vignera, S., Aversa, A., Cannarella, R., Condorelli, R. A., Duca, Y.,

Russo, G. I., & Calogero, A. E. (2021). Pharmacological treatment of lower urinary tract symptoms in benign prostatic hyperplasia: consequences on sexual function and possible endocrine effects. Expert Opinion on Pharmacotherapy, 22(2), 179–189. https://doi.org/10.1080/14656566.2020.1817382.

160. Page ST, Lin DW, Mostaghel EA, Hess DL, True LD, Amory JK, Nelson PS, Matsumoto AM, Bremner WJ (2006) Persistent intraprostatic androgen concentrations after medical castration in healthy men. J Clin Endocrinol Metab. 91(10): 3850-6

161. growth and development Ashley Scott Meakin A thesis submitted for the degree of Doctor of Philosophy at The University of Queensland in 2021 Faculty of Medicine. (2021)

162. Doultsinos, D., & Mills, I. G. (2021). Derivation and application of molecular signatures to prostate cancer: Opportunities and challenges. Cancers, 13(3), 1–25. https://doi.org/10.3390/cancers13030495

163. Doultsinos, D., & Mills, I. G. (2021). Derivation and application of molecular signatures to prostate cancer: Opportunities and challenges. Cancers, 13(3), 1–25. https://doi.org/10.3390/cancers13030495.

164. Cooper, P. (2020). THE IMPACTS OF INFLAMMATION ON ADULT PROSTATE STEM by. August.

165. Nikolaev, G., Robeva, R., & Konakchieva, R. (2022). Membrane melatonin receptors activated cell signaling in physiology and disease. International Journal of Molecular Sciences, 23(1). https://doi.org/10.3390/ijms23010471.

166. Lao-Pérez, M., Massoud, D., Real, F. M., Hurtado, A., Ortega, E., Burgos, M., Jiménez, R., & Barrionuevo, F. J. (2021). Mediterranean pine

vole, microtus duodecimcostatus: A paradigm of an opportunistic breeder. Animals, 11(6). https://doi.org/10.3390/ani11061639.

167. Olson, A. W., Le, V., Wang, J., Hiroto, A., Kim, W. K., Lee, D. H., Aldahl, J., Wu, X., Kim, M., Cunha, G. R., You, S., & Sun, Z. (2021). Stromal androgen and hedgehog signaling regulates stem cell niches in pubertal prostate development. Development (Cambridge), 148(19). https://doi.org/10.1242/DEV.199738.

168. Ali, A., & Kulik, G. (2021). Signaling pathways that control apoptosis in prostate cancer. Cancers, 13(5), 1–35. https://doi.org/10.3390/cancers13050937.

169. Krolewski, J. J., Singh, S., Sha, K., Agrawal, N. J., & Turowski, S. G. (2022). TNF signaling is required for castration-induced vascular damage preceding prostate cancer regression SIGNIFICANCE : These studies define TNF as the mediator of androgen deprivation therapy- induced functional and structural vascular damage in prostate tumors . 0378, 1–27.

170. Kuwahara, N., Nicholson, K., Isaacs, L., & MacLusky, N. J. (2021). Androgen Effects on Neural Plasticity. Androgens: Clinical Research and Therapeutics, 2(1), 216–230. https://doi.org/10.1089/andro.2021.0022.

171. Federici, S., Goggi, G., Quinton, R., Giovanelli, L., Persani, L., Cangiano, B., & Bonomi, M. (2021). New and Consolidated Therapeutic Options for Pubertal Induction in Hypogonadism: In-depth Review of the Literature. Endocrine Reviews, January 2022. https://doi.org/10.1210/endrev/bnab043.

172. arakas, M., Schäfer, S., Appelbaum, S., Ojeda, F., Kuulasmaa, K., Brückmann, B., Berisha, F., Schulte-Steinberg, B., Jousilahti, P., Blankenberg, S., Palosaari, T., Salomaa, V., & Zeller, T. (2018). Testosterone levels and type 2 diabetes—No correlation with age, differential predictive

value in men and women. Biomolecules, 8(3). https://doi.org/10.3390/biom8030076.

173. Facondo, P., Di Lodovico, E., Pezzaioli, L. C., Cappelli, C., Ferlin, A., & Delbarba, A. (2022). Usefulness of routine assessment of free testosterone for the diagnosis of functional male hypogonadism. Aging Male, 25(1), 65–71. https://doi.org/10.1080/13685538.2022.2046727.

174. Discovery and Validation of a Panel of Methylation Biomarkers for the Detection of Aggressive Prostate Cancer By Discovery and Validation of a Panel of Methylation Biomarkers for the Detection of Aggressive Prostate Cancer Renu Jeyapala, 2021.

175. Wu CT, Altuwaijri S, Ricke WA, Huang SP, Yeh S, Zhang C, et al. Increased prostate cell proliferation and loss of cell differentiation in mice lacking prostate epithelial androgen receptor. Proc Natl Acad Sci U S A 2017;104:12679e84.

176. Toivanen, R., & Shen, M. M. (2017). Prostate organogenesis: Tissue induction, hormonal regulation and cell type specification. Development (Cambridge), 144(8), 1382–1398. https://doi.org/10.1242/dev.148207.

177. Haston, S. (2019). INVESTIGATION OF THE TUMOUR PROMOTING EFFECTS OF CELLULAR SENESCENCE DURING PITUITARY AND by. 1–318.

178. Vigneswara, V., & Ahmed, Z. (2020). The Role of Caspase-2 in Regulating Cell Fate. Cells, 9(5). https://doi.org/10.3390/cells9051259.

179. Mehrabadi, Y. (2018). Regulation of cytotoxic drug-induced apoptosis by death receptor 5 in human colon cancer cells. April.

180. Rossi R, Zatelli MC, Valentini A, Cavazzini P, Fallo F, del Senno L, et al. Evidence for androgen receptor gene expression and growth inhibitory

effect of dihydrotestosterone on human adrenocortical cells. J Endocrinol 1998;159:373e80.

181. Abbastabar, M., Kheyrollah, M., Azizian, K., Bagherlou, N., Tehrani, S. S., Maniati, M., & Karimian, A. (2018). Multiple functions of p27 in cell cycle, apoptosis, epigenetic modification and transcriptional regulation for the control of cell growth: A double-edged sword protein. DNA Repair, 69(July), 63–72. https://doi.org/10.1016/j.dnarep.2018.07.008.

182. Wang, J., Li, D., Cang, H., & Guo, B. (2019). Crosstalk between cancer and immune cells: Role of tumor-associated macrophages in the tumor microenvironment. Cancer Medicine, 8(10), 4709–4721. https://doi.org/10.1002/cam4.2327.

183. Wyld, L., Bellantuono, I., Tchkonia, T., Morgan, J., Turner, O., Foss, F., George, J., Danson, S., & Kirkland, J. L. (2020). Senescence and cancer: A review of clinical implications of senescence and senotherapies. Cancers, 12(8), 1–20. https://doi.org/10.3390/cancers12082134.

184. Chua, C. W., Epsi, N. J., Leung, E. Y., Xuan, S., Lei, M., Li, B. I., Bergren, S. K., Hibshoosh, H., Mitrofanova, A., & Shen, M. M. (2018). Differential requirements of androgen receptor in luminal progenitors during prostate regeneration and tumor initiation. ELife, 7, 1–26. https://doi.org/10.7554/elife.28768.

185. Duffy, C. P., & McCoy, C. E. (2020). The Role of MicroRNAs in Repair Processes in Multiple Sclerosis. Cells, 9(7). https://doi.org/10.3390/cells9071711.

186. Yang Q, Fung KM, Day WV, Kropp BP, Lin HK. Androgen receptor signaling is required for androgen-sensitive human prostate cancer cell proliferation and survival. Cancer Cell Int 2015;5:8.

187. Wang, Y., Chen, J., Wu, Z., Ding, W., Gao, S., Gao, Y., & Xu, C. (2021). Mechanisms of enzalutamide resistance in castration-resistant prostate cancer and therapeutic strategies to overcome it. British Journal of Pharmacology, 178(2), 239–261. https://doi.org/10.1111/bph.15300.

188. Endo, H., & Inoue, M. (2019). Dormancy in cancer. Cancer Science, 110(2), 474–480. https://doi.org/10.1111/cas.13917.

189. Wang, X., Yang, X., Zhang, C., Wang, Y., Cheng, T., Duan, L., Tong, Z., Tan, S., Zhang, H., Saw, P. E., Gu, Y., Wang, J., Zhang, Y., Shang, L., Liu, Y., Jiang, S., Yan, B., Li, R., Yang, Y., … Gao, S. (2020). Tumor cell-intrinsic PD-1 receptor is a tumor suppressor and mediates resistance to PD-1 blockade therapy. Proceedings of the National Academy of Sciences of the United States of America, 117(12), 6640–6650. https://doi.org/10.1073/pnas.1921445117Shiota M, EtoM. Current status of primary pharmacotherapy and future perspectives toward upfront therapy formetastatic hormone-sensitive prostate cancer. Int J Urol. 2016;23(5):360-369. doi:10.1111/iju.13091

190. Montgomery RB, Mostaghel EA, Vessella R, et al. Maintenance of intratumoral androgens in metastatic prostate cancer: a mechanism for castration-resistant tumor growth. Cancer Res. 2008;68(11):4447-4454. doi:10.1158/ 0008-5472.CAN-08-0249

191. Ehsani, M., David, F. O., & Baniahmad, A. (2021). Androgen receptor-dependent mechanisms mediating drug resistance in prostate cancer. Cancers, 13(7). https://doi.org/10.3390/cancers13071534.

192. Moll, J. M., Hofland, J., Teubel, W. J., de Ridder, C. M. A., Taylor, A. E., Graeser, R., Arlt, W., Jenster, G. W., & van Weerden, W. M. (2022). Abiraterone switches castration-resistant prostate cancer dependency from adrenal androgens towards androgen receptor variants and glucocorticoid

receptor signalling. Prostate, 82(5), 505–516. https://doi.org/10.1002/pros.24297

193. Pandey, M., Cuddihy, G., Gordon, J. A., Cox, M. E., & Wasan, K. M. (2021). Inhibition of scavenger receptor class b type 1 (Sr-b1) expression and activity as a potential novel target to disrupt cholesterol availability in castration-resistant prostate cancer. Pharmaceutics, 13(9), 1–37. https://doi.org/10.3390/pharmaceutics13091509

194. McKay RR .Ye H. Xie W et al. Evaluation of intense androgen deprivation before prostatectomy: a randomized phase II trial of enzalutamide and leuprolide with or without abiraterone. J Clin Oncol. 2019; 37: 923-931.

195. Fizazi K, Tran N, Fein L, et al; LATITUDE Investigators. Abiraterone plus prednisone in metastatic, castrationsensitive prostate cancer. N Engl J Med. 2017;377(4):352-360. doi:10.1056/NEJMoa1704174

196. Gao, M., Nie, K., Qin, M., Xu, H., Wang, F., & Liu, L. (2021). Molecular mechanism study on stereo- selectivity of α or β hydroxysteroid dehydrogenases. Crystals, 11(3), 1–25. https://doi.org/10.3390/cryst11030224

197. Zein, J., Gaston, B., Bazeley, P., DeBoer, M. D., Igo, R. P., Bleecker, E. R., Meyers, D., Comhair, S., Marozkina, N. V., Cotton, C., Patel, M., Alyamani, M., Xu, W., Busse, W. W., Calhoun, W. J., Ortega, V., Hawkins, G. A., Castro, M., Chung, K. F., … Sharifi, N. (2020). HSD3B1 genotype identifies glucocorticoid responsiveness in severe asthma. Proceedings of the National Academy of Sciences of the United States of America, 117(4), 2187–2193. https://doi.org/10.1073/pnas.1918819117

198. Asif, S., & Teply, B. A. (2021). Biomarkers for treatment response in advanced prostate cancer. Cancers, 13(22). https://doi.org/10.3390/cancers13225723

199. Mori, K., Mostafaei, H., Sari Motlagh, R., Pradere, B., Quhal, F., Laukhtina, E., Schuettfort, V. M., Kramer, G., Abufaraj, M., Karakiewicz, P. I., Kimura, T., Egawa, S., & Shariat, S. F. (2022). Systemic therapies for metastatic hormone-sensitive prostate cancer: network meta-analysis. BJU International, 129(4), 423–433. https://doi.org/10.1111/bju.15507

200. Hearn JWD, XieW, Nakabayashi M, et al. Association of HSD3B1 genotype with response to androgendeprivation therapy for biochemical recurrence after radiotherapy for localized prostate cancer. JAMA Oncol. 2018;4(4):558-562. doi:10.1001/jamaoncol.2017.3164.

201. Poon, D. M. C., Chan, K., Chan, T., Cheung, F. Y., Lam, D., Lam, M., Law,

K. S., Lee, C., Lee, E. K. C., Leung, A., Sze, H., Tong, C. C., Wong, K. C. W., & Kwong, P. (2022). Ethnic Pharmacogenomic Differences in the Management of Asian Patients with Metastatic Prostate Cancer. Cancers, 14(2), 1–13. https://doi.org/10.3390/cancers14020407

202. Aurilio, G., Cimadamore, A., Mazzucchelli, R., Lopez-Beltran, A., Verri, E., Scarpelli, M., Massari, F., Cheng, L., Santoni, M., & Montironi, R. (2020). Androgen Receptor Signaling Pathway in Prostate Cancer: From Genetics to Clinical Applications. Cells, 9(12), 1–14. https://doi.org/10.3390/cells9122653

203. Reptova, S., Trtkova, K. S., & Kolar, Z. (2014). Direct detection of the AR-E211 G∈>∈A gene polymorphism from blood and tissue samples without DNA Isolation. Pathology and Oncology Research, 20(2), 223–227. https://doi.org/10.1007/s12253-013-9671-8

204. Eiholzer, R. A., Mehta, S., Kazantseva, M., Drummond, C. J., McKinney, C., Young, K., Slater, D., Morten, B. C., Avery-Kiejda, K. A., Lasham, A., Fleming, N., Morrin, H. R., Reader, K., Royds, J. A., Landmann, M., Petrich, S., Reddel, R., Huschtscha, L., Taha, A., … Braithwaite, A. W. (2020). Intronic TP53 polymorphisms are associated with increased Δ133TP53 transcript, immune infiltration and cancer risk. Cancers, 12(9), 1–18. https://doi.org/10.3390/cancers12092472.

205. Youness, A., Miquel, C. H., & Guéry, J. C. (2021). Escape from x chromosome inactivation and the female predominance in autoimmune diseases. International Journal of Molecular Sciences, 22(3), 1–12. https://doi.org/10.3390/ijms22031114.

206. Deuker, M. et al. (2021) 'PSA, stage, grade and prostate cancer specific mortality in Asian American patients relative to Caucasians according to the United States Census Bureau race definitions', World Journal of Urology, 39(3), pp. 787–796. doi: 10.1007/s00345-020-03242-8.

207. Cunningham, M. et al. (2021) 'Patient reported factors influencing the decision-making process of men with localised prostate cancer when considering Active Surveillance—A systematic review and thematic synthesis', Psycho-Oncology, (May), pp. 1–17. doi: 10.1002/pon.5832.

208. Freeland, J. et al. (2021) 'Aging of the progenitor cells that initiate prostate cancer', Cancer Letters, 515, pp. 28–35. doi: 10.1016/j.canlet.2021.05.014.

209. Lassalle P, Molet S, Janin A, Heyden JV, Tavernier J. et al. ESM-1 is a novel human endothelial cell-specific molecule expressed in lung and regulated by cytokines. J Biol Chem. 1996;271:20458-20464.

210. Zegarra-Moro OL, Schmidt LJ, Huang H, Tindall DJ. Disruption of androgen receptor function inhibits proliferation of androgen-

refractory prostate cancer cells. Cancer Res. 2002;62:1008-1013.

211. Park SW, Kim JH, Lee HJ, Shin DH, Lee SD, Yoon S. The expression of androgen receptor and its variants in human prostate cancer tissue according to disease status, and its prognostic significance. World J Mens Health. 2018.1-10.

212. Husain I, Shukla S, Soni P, Husain N. Role of androgen receptor in prostatic neoplasia versus hyperplasia. J Can Res Ther. 2016; 12: 112-6.

213. Taha, Z. N. et al. (2020) 'RISK FACTORS INVOLVED IN ELEVATION OF PROSTATE CANCER AND BENIGN PROSTATIC HYPERPLASIA AMONG MEN IN KIRKUK , IRAQ', (October).

214. Ni Raghallaigh, H. and Eeles, R. (2021) 'Genetic predisposition to prostate cancer: an update', Familial Cancer, (0123456789). doi: 10.1007/s10689-021-00227-3.

215. Leão, R. R. N., Price, A. J. and James Hamilton, R. (2018) 'Germline BRCA mutation in male carriers - Ripe for precision oncology?', Prostate Cancer and Prostatic Diseases, 21(1), pp. 48–56. doi: 10.1038/s41391-017-0018-5.

216. Deuker, M. et al. (2021) 'PSA, stage, grade and prostate cancer specific mortality in Asian American patients relative to Caucasians according to the United States Census Bureau race definitions', World Journal of Urology, 39(3), pp. 787–796. doi: 10.1007/s00345-020-03242-8.

217. Factors, R. (2020) 'ASSOCIATION BETWEEN SOME RISK FACTORS AND PROSTATE', 19(2), pp. 50–61.

218. Jiménez-Mendoza, E. et al. (2018) 'Smoking and prostate cancer: A life course analysis', BMC Cancer, 18(1), pp. 1–11. doi: 10.1186/s12885-018-4065-7.

219. Amir, S., Shah, S. T. A., Mamoulakis, C., Docea, A. O., Kalantzi, O. I., Zachariou, A., Calina, D., Carvalho, F., Sofikitis, N., Makrigiannakis, A., & Tsatsakis, A. (2021). Endocrine disruptors acting on estrogen and androgen pathways cause reproductive disorders through multiple mechanisms: A review. International Journal of Environmental Research and Public Health, 18(4), 1–20. https://doi.org/10.3390/ijerph18041464.

220. Yang, Q., Ciebiera, M., Bariani, M. V., Ali, M., Elkafas, H., Boyer, T. G., & Al-Hendy, A. (2021). Comprehensive Review of Uterine Fibroids: Developmental Origin, Pathogenesis, and Treatment. In Endocrine Reviews: Vol. XX (Issue Xx). https://doi.org/10.1210/endrev/bnab039.

221. Estébanez-Perpiñá, E., Bevan, C. L., & McEwan, I. J. (2021). Eighty years of targeting androgen receptor activity in prostate cancer: The fight goes on. Cancers, 13(3), 1–19. https://doi.org/10.3390/cancers13030509.

222. HÍVEŠ, M., JUREČEKOVÁ, J., KLIMENT, J., GRENDÁR, M., KAPLÁN, P., DUŠENKA, R., EVIN, D., VILČKOVÁ, M., HOLEČKOVÁ, K. H., & SIVOŇOVÁ, M. K. (2022). Role of Genetic Variations in CDK2 , CCNE1 and p27 KIP1 in Prostate Cancer . Cancer Genomics - Proteomics, 19(3), 362–371. https://doi.org/10.21873/cgp.20326

223. Chen, Y., Wang, D., Song, Y., Zhang, X., Jiao, Z., Dong, J., Lin, L., Zou, Z., Du, W., & Qu, F. (2019). Functional polymorphisms in circadian positive feedback loop genes predict postsurgical prognosis of gastric cancer. Cancer Medicine, 8(4), 1919–1929. https://doi.org/10.1002/cam4.2050

224. Fayyad, N. (2021). Deciphering the role of xeroderma pigmentosum C (XPC) protein in base excision repair (BER) and oxidative stress Nour

Fayyad To cite this version : HAL Id : tel-03405199 Déchiffrage du rôle de la protéine XPC dans la réparation par excision de base (BER) et le stress oxydant.

225. Mononen N, Ikonen T, Autio V, Rökman A, Matikainen MP, Teuvo L et al (2002) Androgen receptor CAG polymorphism and prostate cancer risk. Hum Genet 111:166–171

226. Silva NB, Koff WJ, Biolchi V, Brenner C, Biolo KD, Spritzer PM et al (2008) Polymorphic CAG and GGC repeat lengths in the androgen receptor gene and prostate cancer risk: analysis of a Brazilian population. Cancer Invest 26:74–80.

227. Rutkowski, R. (2016). New Roles for Ephb4 in Prostate Cancer.

228. Guilherme, J. P. L. F., V. Shikhova, Y., R. Dondukovskaya, R., A. Topanova, A., A. Semenova, E., V. Astratenkova, I., & Ahmetov, I. I. (2021). Androgen receptor gene microsatellite polymorphism is associated with muscle mass and strength in bodybuilders and power athlete status. Annals of Human Biology, 48(2), 142–149. https://doi.org/10.1080/03014460.2021.1919204

229. Holgersson, M. B., Giwercman, A., Bjartell, A., Wu, F. C. W., Huhtaniemi, I. T., O'Neill, T. W., Pendleton, N., Vanderschueren, D., Lean, M. E. J., Han, T. S., Finn, J. D., Kula, K., Forti, G., Casanueva, F. F., Bartfai, G., Punab, M., & Giwercman, Y. L. (2014). Androgen receptor polymorphism-dependent variation in prostate-specific antigen concentrations of European men. Cancer Epidemiology Biomarkers and Prevention, 23(10), 2048–2056. https://doi.org/10.1158/1055-9965.EPI-14-0376

230. Voronkova, V. N., Piskunov, A. K., Nikolaeva, E. A., Semina, M. T., Konorov, E. A., & Stolpovsky, Y. A. (2021). Haplotype Diversity of

Mongolian and Tuvan Goat Breeds (Capra hircus) Based on mtDNA and Y-Chromosome Polymorphism. Russian Journal of Genetics, 57(10), 1170–1178. https://doi.org/10.1134/S102279542110015X

231. Hauert, C., & Doebeli, M. (2021). Spatial social dilemmas promote diversity. Proceedings of the National Academy of Sciences of the United States of America, 118(42). https://doi.org/10.1073/pnas.2105252118

232. Lolli, F., Pallotti, F., Rossi, A., Fortuna, M. C., Caro, G., Lenzi, A., Sansone, A., & Lombardo, F. (2017). Androgenetic alopecia: a review. Endocrine, 57(1), 9–17. https://doi.org/10.1007/s12020-017-1280-y.

233. Raspin, K., FitzGerald, L. M., Marthick, J. R., Field, M. A., Malley, R. C., Banks, A., Donovan, S., Thomson, R. J., Foley, G. R., Stanford, J. L., & Dickinson, J. L. (2021). A rare variant in EZH2 is associated with prostate cancer risk. International Journal of Cancer, 149(5), 1089–1099. https://doi.org/10.1002/ijc.33584.

234. Lolli, C., De Lisi, D., Conteduca, V., Gurioli, G., Scarpi, E., Schepisi, G., Ravaglia, G., Menna, C., Farolfi, A., Altavilla, A., Burgio, S. L., Tonini, G., Santini, D., & De Giorgi, U. (2019). Testosterone levels and androgen receptor copy number variations in castration-resistant prostate cancer treated with abiraterone or enzalutamide. Prostate, 79(11), 1211–1220. https://doi.org/10.1002/pros.23804.

235. Moll, J. M., Hofland, J., Teubel, W. J., de Ridder, C. M. A., Taylor, A. E., Graeser, R., Arlt, W., Jenster, G. W., & van Weerden, W. M. (2022). Abiraterone switches castration-resistant prostate cancer dependency from adrenal androgens towards androgen receptor variants and glucocorticoid receptor signalling. Prostate, 82(5), 505–516. https://doi.org/10.1002/pros.24297

236. McManus, J. M., Sabharwal, N., Bazeley, P., & Sharifi, N. (2021).

Inheritance of a common androgen synthesis variant allele is associated with female COVID susceptibility in UK Biobank. MedRxiv, 2020.08.27.20183004. http://medrxiv.org/content/early/2021/08/27/2020.08.27.20183004.abstract.

237. Schuh, K. (n.d.). Establishment of an experimental model for dairy cows of divergent peripartal mobilization of body reserves: Profiling circulating steroid hormones and the mRNA abundance of steroid metabolizing enzymes in liver and in adipose tissue.

238. Prizment, A. E., McSweeney, S., Pankratz, N., Joshu, C. E., Hwang, J. H., Platz, E. A., & Ryan, C. J. (2021). Prostate cancer mortality associated with aggregate polymorphisms in androgen-regulating genes: The atherosclerosis risk in the communities (aric) study. Cancers, 13(8). https://doi.org/10.3390/cancers13081958.

239. Chowdhury, N. U., Guntur, V. P., Newcomb, D. C., & Wechsler, M. E. (2021). Sex and gender in asthma. European Respiratory Review, 30(162). https://doi.org/10.1183/16000617.0067-2021.

240. Visakorpi T, Hyytinen E, Koivisto P, Tanner M, Keinanen R, Palmberg C, et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. Nature Genet 1995;9:401–6

241. National Center for Biotechnology Information. Homo sapiens hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid deltaisomerase 1 (HSD3B1), RefSeqGene on chromosome 1. https:// www.ncbi.nlm.nih.gov/nuccore/1039673932. Accessed 5 July 2019.

242. National Center for Biotechnology Information. NM_000862. 3(HSD3B1):c.1100C5 (p.Thr3675). https://www.ncbi.nlm.nih.gov/ clinvar/variation/620577/#summary-evidence. Accessed 5 July 2019.

243. IGSR: The International Genome Sample Resource. Genome browsers. http://www.internationalgenome.org/1000-genomesbrowsers/. Accessed 5 July 2019.

244. Shiota M, Narita S, Akamatsu S, Fujimoto N, Sumiyoshi T, Fujiwara M, Uchiumi T, Habuchi T, Ogawa O, Eto M. Association of missense polymorphism in HSD3B1 with outcomes among men with prostate cancer treated with androgen-deprivation therapy or abiraterone. JAMA Netw Open. 2019;2(2):e190115.

245. Gil SG, Rodr´ıguez RR, Bello AP, Nazco-Casariego GJ, Marrero RG, Jurado JC, Batista L ´opez JN, Garc´ıa JG, Nicol´as FG. Relationship between mutations in the HSD3B1 gene and response time to androgen deprivation therapy in the treatment of prostate cancer. In Proceedings of the 4th European

Conference of Oncology Pharmacy; 25–27 October 2018; Nantes, France. Abstract 107.

246. Sweeney CJ, Chen YH, Carducci M, Liu G, Jarrard DF, Eisenberger M, Wong YN, Hahn N, Kohli M, Cooney MM, Dreicer R, Vogelzang NJ, Picus J, Shevrin D, Hussain M, Garcia JA, DiPaola RS. Chemohormonal therapy in metastatic hormone-sensitive prostate cancer. N Engl J Med. 2015;373(8):737–746.

247. Hearn JW, Sweeney CJ, Almassi N, Reichard CA, Reddy C, Hobbs B, Jarrard DF, Chen Y-H, Dreicer R, Garcia J, Carducci MA, DiPaola RS, Sharifi N. HSD3B1 and overall survival (OS) in men with low-volume (LV) metastatic prostate cancer (PCa) treated with androgen deprivation therapy (ADT) or chemohormonal therapy in the CHAARTED Randomized trial. In Proceedings of the ASCO Annual Meeting 2019; 31 May–4 June 2019; Chicago, IL. Abstract 5020.

248. Prizment, A. E., McSweeney, S., Pankratz, N., Joshu, C. E., Hwang, J. H., Platz, E. A., & Ryan, C. J. (2021). Prostate cancer mortality associated with aggregate polymorphisms in androgen-regulating genes: The atherosclerosis risk in the communities (aric) study. Cancers, 13(8). https://doi.org/10.3390/cancers13081958.

249. Hearn JWD, Xie W, Nakabayashi M, et al. Association of HSD3B1 genotype with response to androgendeprivation therapy for biochemical recurrence after radiotherapy for localized prostate cancer.JAMA Oncol. 2018;4(4):558-562. doi:10.1001/jamaoncol.2017.316.

250. Agarwal N, Hahn AW, Gill DM, Farnham JM, Poole AI, Cannon-Albright L. Independent validation of effect of HSD3B1 genotype on response to androgen-deprivation therapy in prostate cancer.JAMA Oncol. 2017;3(6): 856-857. doi:10.1001/jamaoncol.2017.0147.

251. Huang, S. P., Chen, Y. T., Chen, L. C., Lee, C. H., Huang, C. Y., Yu, C. C., Lin, V. C., Lu, T. L., & Bao, B. Y. (2021). NRG1 genetic variant influences the efficacy of androgen-deprivation therapy in men with prostate cancer. Biomedicines, 9(5). https://doi.org/10.3390/biomedicines9050528.

252. Mpakali, A., & Stratikos, E. (2021). The role of antigen processing and presentation in cancer and the efficacy of immune checkpoint inhibitor immunotherapy. Cancers, 13(1), 1–30. https://doi.org/10.3390/cancers13010134

253. Shiota M, Narita S, Akamatsu S, Fujimoto N, Sumiyoshi T, Fujiwara M, Uchiumi T, Habuchi T, Ogawa O, Eto M. Association of missense polymorphism in HSD3B1 with outcomes among men with prostate cancer treated with androgen-deprivation therapy or abiraterone. JAMA Netw Open. 2019;2(2): e190115.

254. Hearn JWD, AbuAli G, Reichard CA, et al. HSD3B1 and resistance to androgen-deprivation therapy in prostate cancer: a retrospective, multicohort study. Lancet Oncol. 2016;17(10):1435-1444. doi:10.1016/S1470-2045(16) 30227-3.

255. Association of Missense Polymorphism in HSD3B1 with Outcomes Among Men with Prostate Cancer Treated with Androgen-Deprivation Therapy or Abiraterone Masaki Shiota, MD, PhD; Shintaro Narita, MD, PhD; Shusuke Akamatsu, MD, PhD; Naohiro Fujimoto, MD, PhD; Takayuki Sumiyoshi, MD; Maki Fujiwara, MD; Takeshi Uchiumi, MD, PhD; Tomonori Habuchi, MD, PhD; Osamu Ogawa, MD, PhD; Masatoshi Eto, MD, PhD JAMA Network Open. 2019.