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# Association Between *MDM2* del1518 Polymorphism and Prostate Cancer in Algerian East

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# Dedication

I would like to dedicate my work to

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The most tender, sweet and warm-hearted and yet strong-willed soul, who taught me to trust Allah, believe in hard work and that so much could be done with little. The one who made me the man I am today. The only one in the world whom truly love and care so much about me unconditionally without limit. You are the best ever mom.

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Thank you for your friendship, I am very proud to be friends with such great people my bros and sisters

To my partner in crime **Sabah**

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OKBA

# Dedication

"I don't know what your destiny will be, but one thing I know: the only ones among you who will be really happy are those who have sought and found how to serve."

-Albert Schweitzer

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I have to thank you for your love, for the noble values, education and support throughout my life. Thank you both for giving me strength to reach for the stars and chase my dreams. You can be proud and find here the result of long years of sacrifices. I love you too much.

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# List of Abbreviations

| | |
|---|---|
| **5AR:** | 5α-Reductase |
| **AAH:** | Atypical Adenomatous Hyperplasia |
| **ADT:** | Androgen Deprivation Therapy |
| **AR:** | Androgen Receptor |
| **ARID1A:** | AT-Rich Interaction Domain 1A |
| **ARID2:** | AT-Rich Interaction Domain 2 |
| **ARID4A:** | AT-Rich Interaction Domain 4A |
| **AS:** | Active Surveillance |
| **ASXL1:** | Additional SeX combs Like 1 |
| **BALB/c:** | Bagg ALBino c |
| **BPH:** | Benign Prostatic Hyperplasia |
| ***BRCA*2:** | BReast CAncer 2 |
| ***CAPB*:** | CAncer Prostate and Brain |
| **CHU:** | Centre Hospitalier Universitaire |
| **CI:** | Confidence Intervals |
| **CpG:** | Cytosine-phosphate-Guanine |
| **CRPC:** | Castration-Resistant Prostate Cancer |
| ***CYP* 17:** | Cytochrome P450 17 |
| **CZ:** | Central Zone |
| ***DAB2IP*:** | DisABled homolog 2-Interacting Protein |
| **DHT:** | 5α-DiHydroTestosterone |
| **DRE:** | Digital Rectal Examination |
| **EDTA:** | Ethylene Diamine Tetra-acetic Acid |
| **EHS:** | Etablissement Hospitalier Spécialisé |
| ***ELAC*2:** | elaC Ribonuclease Z 2 |
| **EtBr:** | Ethidium Bromide |
| **EZH2:** | Enhancer of Zeste Homolog 2 |
| **FPC:** | Familial Prostate Cancer |
| **GS:** | Gleason Score |
| **HG-PIN:** | High Grade Prostatic Intraepithelial Neoplasia |
| ***HOXB*13:** | HOmeoboX B13 |

**HPC:** Hereditary Prostate Cancer

***HPC*1:** Hereditary Prostate Cancer 1

***HPC*2:** Hereditary Prostate Cancer 2

***HPC*20:** Hereditary Prostate Cancer 20

***HPCX*:** Hereditary Prostate Cancer X-Linked

**IGF-1:** Insulin-Like Growth Factor 1

**IHC:** ImmunoHistoChemistry

**KDM6A:** lysine(K) DeMethylase 6A

**KMT2C:** lysine(K) MethylTransferase 2C

**LHRH:** Luteinizing Hormone-Releasing Hormone

**LUTS:** Lower Urinary Tract Symptoms

***MDM*2:** Murine Double Minute 2

***MDM*4:** Murine Double Minute 4

**MRI:** Magnetic Resonance Imaging

***MSR*1:** Macrophage Scavenger Receptor 1

***MYC*:** MYeloCytomatosis

**NES:** Nuclear Export Signal

***NKX*3-1:** NK3 homeoboX 1

**NLS:** Nuclear Localization Sequence

**OR:** Odds Ratio

**PAP:** Prostatic Acid Phosphatase

**PCa:** Prostate Cancer

***PCA*3:** Prostate CAncer gene 3

***PCAP*:** Prostate CAncer Predisposing

**PCR:** Polymerase Chain Reaction

**PIN:** Prostatic Intraepithelial Neoplasia

**RP:** Radical Prostatectomy

**Proteinase K:** Proteinase Keratin

**PSA:** Prostate-Specific Antigen

***PTEN*:** Phosphatase and TENsin homolog

**PZ:** Peripheral Zone

**P-value:** Probability Value

***RB*1:** RetinoBlastoma transcriptional corepressor 1

| | |
|---|---|
| **RING:** | Really Interesting New Gene |
| ***RNASEL*:** | RiboNucleASE L |
| **SDS:** | Sodium Dodecyl Sulfate |
| **SNP:** | Single-Nucleotide Polymorphisms |
| **SP1:** | Specificity Protein 1 |
| **TBE:** | Tris Borate EDTA |
| **TNM:** | Tumor Nodes Metastasis |
| ***TP*53:** | Tumor protein P53 |
| **TRUS:** | TransRectal UltraSound |
| **TZ:** | Transition Zone |

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Prostate cancer (PCa) is the second most commonly diagnosed cancer and the fifth cause of death by cancer in men. In 2018, almost 1.3 million new cases of prostate cancer and 359,000 associated deaths worldwide (Bray et al., 2018). Carcinoma of the prostate is a common cancer with a large spectrum of clinical behavior that ranges from decades of indolence to rapid metastatic progression and lethality (Pritchard et al., 2016).

In Algeria, the cancer registry places prostate cancer in 4th place. It is the first urologic cancer, and virtually the first cancer in the subject after the age of 50 years. In 2014, PCa represents 10% of cancers in men, with 1645 new cases (Hamdi Cherif et al., 2014; 2015).

Although human prostate cancer displays significant phenotypic heterogeneity, >95% of prostate cancers are classified pathologically as adenocarcinoma (Shen and Abate-Shen, 2010). Prostate cancer is graded according to the Gleason grading system based on the pattern of growth. There are five grades (from 1 to 5) based upon the architectural patterns (Moreira and Abern, 2018).

The etiology of prostate cancer still unclear. Genetic, life style, environmental, and behavioral factors have been associated with an increased risk of prostate cancer (Sierra et al., 2016). The murine double minute 2 (MDM2) is an important negative regulator of the Tumor Protein 53 (TP53) tumor suppressor protein, because of many chromosomal and epigenetic alterations. Overexpression of *MDM*2 may leads to the repression of *TP53* that allow the initiation and progression of PCa (Mendoza et al., 2014).

The purposes of this work are to:

- Describe the epidemiological, clinical, pathological and genetical profile of prostate cancer.
- Study different genetical and environmental risk factors and their relation to prostate cancer.
- Explore the possible association between Del1518 (rs3730485) polymorphism of *MDM*2 gene and the risk of prostate cancer in Algerian sample

The prostate is the biggest accessory gland of the male reproductive system (Lee et al., 2011), a walnut-sized cone-shaped truncated gland surrounding the urethra at the base of the bladder (Shen and Abate-Shen, 2010; Vakar-Lopez and True, 2019).

## 1- Prostate anatomy

The prostate is a compound tubuloalveolar exocrine gland, consists of both glandular and muscular (non-glandular) tissues tightly fused together within a common capsule of fibrous collagenous connective tissue (Kuehnel, 2003; Faller et al., 2004). It has the size and shape of walnut, measures about 3cm long, 4cm wide and 2cm thick (Mcneal, 1988; Mader, 2004; Scanlon and Sanders, 2007).

The prostate of adult male weigh about 20g to 25g (Giles, 2017; Farrant and Page, 2018). Its development is stimulated initially by the androgen hormone: Testosterone secreted from fetal testes, this development remain incomplete until puberty were he grown to its most complete and mature form: the adult prostate (Hricak and Scardino, 2008; Giles, 2017).

The prostate is located at the base of the pelvis beneath the urinary bladder which encircles urethra (Giles, 2017; Ricke et al., 2018). Its posterior aspect abuts directly on the rectum (Faller et al., 2004). The prostatic urethra is encircled by sphincteric muscles (Fig. 1) (Selman, 2011).

The prostate gland in adult is composed of a base, an apex, anterior, posterior, and inferolateral surfaces:

- The base of the prostate is attached to the neck of the bladder and the prostatic urethra enters the middle of it near the anterior surface, which is narrow and convex.
- The apex rests on the superior surface of the urogenital diaphragm and contacts the medial surface of the levator ani muscles.
- The posterior surface is triangular and flat, and rests on the anterior wall of the rectum.
- The inferolateral surface joins the anterior surface and rests on the levator ani fascia above the urogenital diaphragm (Gray, 1918; Feneis and Dauber, 2000).

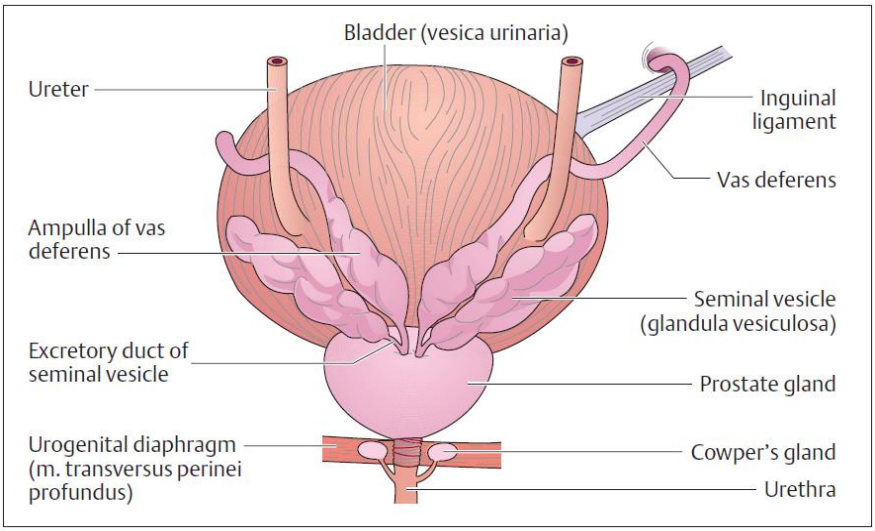


**Fig. 1:** Prostate gland and urinary bladder (Faller et al., 2004)**.**

### 1-1 Zonal architecture of human prostate

The prostate gland contains four (4) regions or zones whom differs histologically and biologically (Fig. 2): the peripheral zone (PZ), the central zone (CZ), the transitional zone (TZ), and the interior fibromuscular stroma (Mcneal, 1988). These zones have different embryologic origins (Lee et al., 2011).

- **The peripheral zone (PZ)** is located outside the posterior region of the central zone, its ducts extend from the urethra below the intersection of the ejaculatory ducts. The PZ forms the postero-inferior aspect of the gland and constitutes approximately 70% of the normal prostate volume and increases with age due primarily to the increased incidence of prostate cancer (PCa); the prostatic ducts in the PZ are the most prone to cancer, which made this zone have major occurrence of prostate adenocarcinomas (Mcneal, 1988; Selman, 2011). This zone is accessible via digital rectal examination (DRE) (Giles, 2017).

- **The central zone (CZ)** surrounds the transition zone just under the urinary bladder, it is the zone through which the ejaculatory ducts pass and connect with the urethra. The CZ constitutes approximately 25% of the normal prostate, and in general is not associated with benign prostatic hyperplasia (BPH) but most commonly affected by inflammatory processes such as prostatitis (Mcneal, 1988; Selman, 2011).

- **The transition zone (TZ)** is composed of short glandular ducts, which extend from the urethra above the intersection of the ejaculatory ducts. In the normal prostate the TZ consists of approximately 5% of prostate volume, but can increase in size significantly with age, mainly due to benign prostatic hyperplasia, however the TZ has been reported to account for up to a quarter of prostate cancer cases (Mcneal, 1988; Selman, 2011).

Finally, the smallest zone that is less commonly described in men, is the **anterior zone** or the fibromuscular stroma, which is mostly fibromuscular without glandular structures (Mcneal, 1988; Selman, 2011; Ricke et al., 2018).

The peripheral zone with central zones altogether referred to as **outer prostate**, while the transition zone and the anterior zone termed as **inner prostate** (Shah and Zhou, 2012).

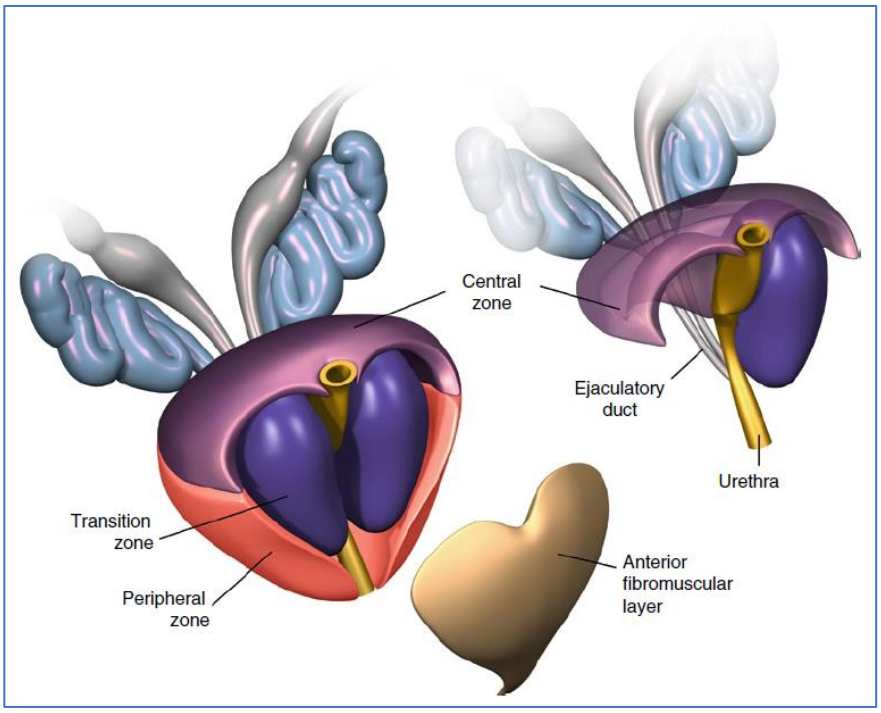


**Fig. 2:** Prostate zonal architecture (Shah and Zhou, 2012)**.**

## 2- Prostate histology

The adult prostate gland consists of 30−50 tubuloalveolar glands resides in the fibromuscular stroma, each one of these glands has 15–30 secretory ducts (Kuehnel, 2003; Faller et al., 2004; Nehikhare et al., 2018). Their secretory ducts drain into the urethra, which runs vertically through the prostate (Faller et al., 2004). The adult prostate consists of both stromal and glandular (epithelial) elements, tightly fused within a capsule and a basal lamina that surrounds the epithelium and forms a barrier between the glandular epithelium and the stroma (Fig. 3) (Lee et al., 2011; Gevaert et al., 2014; Farrant and Page, 2018). The interior layer of the prostate capsule is composed of smooth muscle while its exterior layer is covered with collagen (Faller et al., 2004; Lee et al., 2011). There aren't any adipose tissue present in the prostate gland (Ittmann, 2018).

### 2-1 Glandular epithelium tissue

The glandular epithelium is organized as acini and ducts, this glandular acini secrete into a lumen that converges into a duct, and then into the urethra (Farrant and Page, 2018; Ittmann, 2018). The glandular epithelium, or parenchyma of the prostate gland is lined by three types of differentiated cells that are distinct phenotypically and functionally: luminal, basal, and neuroendocrine cells (Gauntner and Prins, 2018; Ittmann, 2018). The glandular epithelial tissue contains also a specific type of stem cells, with the ability to differentiate into basal, luminal or neuroendocrine cells (Kurita, 2004; Gauntner and Prins, 2018).

### 2-2 Prostatic stroma

The prostatic stroma is fibromuscular, with abundant AR (Androgen Receptor) positive smooth muscle cells intermixed with fibroblasts, blood vessels, nerves, mesenchymal stem cells and immune cells (Farrant and Page, 2018; Gauntner and Prins, 2018; Ittmann, 2018).
The stromal cells had an important role in growth, development, support and structure to the prostate gland. The prostatic growth and development depend upon androgen. In case of androgen withdrawal, the prostate regresses, and regain its regeneration upon restoration of systemic androgen (Kurita, 2004; Gauntner and Prins, 2018).

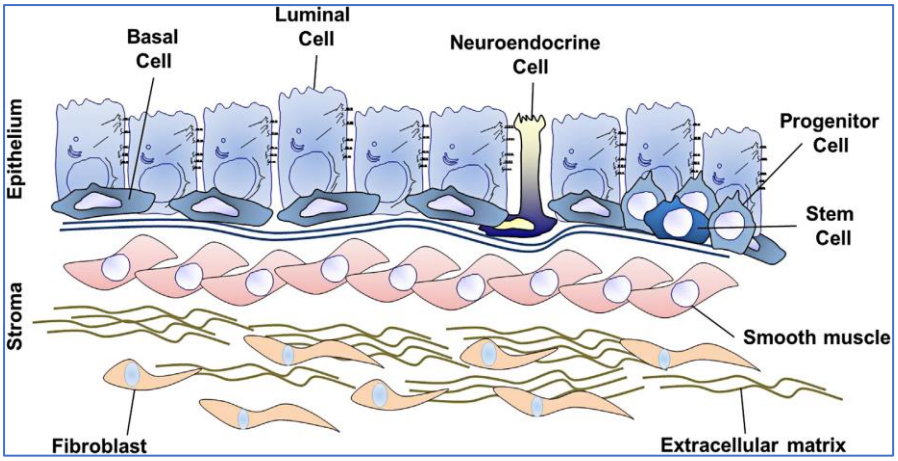


**Fig. 3:** Cell types and organization in the adult prostate gland (Gauntner and Prins, 2018)**.**

The normal human prostate commonly shows a wide variety of alterations in the epithelium and stroma. The most common alterations in the epithelium and stroma of normal prostate are atrophy of the epithelium with basal cell hyperplasia (Shah and Zhou, 2012; Ittmann, 2018).

## 3- Prostate physiology

The adult prostate is an accessory reproductive gland which have a major role in fertility, its principal function is to fabricate, stores, and, upon demand, secretes the proteins and electrolytes that form the ejaculate in combination with the proteins expressed in the seminal vesicle. It have also a role in maintaining involuntary urinal continence through autonomic control of the internal urethral sphincter (Hayward and Cunha, 2000; Nehikhare et al., 2018). The smooth muscle of the prostate gland contracts during ejaculation so the semen get expulsed from the urethra (Scanlon and Sanders, 2007).

The secreted proteins and electrolytes secretions adjust the pH of seminal fluid so can maintains sperm viability in their passage through vagina and enhances the motility of sperm (De Graaff, 2001; Faller et al., 2004; Gauntner and Prins, 2018). These secretions include prostaglandins which stimulate the uterus, spermine, a protein found in prostatic secretion which promotes the motility and fertility of the spermatozoa, proteolytic enzymes include PSA (Prostate-Specific Antigen) and PAP (Prostatic Acid Phosphatase) (which used generally in PCa screening and diagnostic), zinc, and citric acid, which contributes to 30% of volume of the seminal fluid (Frick and Aulitzky, 1991; Faller et al., 2004; Farrant and Page, 2018).

Androgens plays a vital role in prostate physiology which demonstrate functional relation between prostate and testis (Hayward and Cunha, 2000). Among these androgens, testosterone control cell proliferation within prostate, thus the testosterone stimulate prostate growth, and maintain its size and function (Nehikhare et al., 2018). Adrenal androgens can also stimulate the growth of the prostate gland, but these androgens don’t have the same effect on prostate function as testicular androgens (Frick and Aulitzky, 1991; Farrant and Page, 2018).

The AR is one of 48 human nuclear receptors, which have a dual function, an intracellularly located receptors and a ligand-activated transcription factors (Farrant and Page, 2018).

## 4- Prostate pathologies

The human prostate gland can be affected by 3 different conditions: Prostatitis, Benign Prostatic Hyperplasia (BPH) or prostate adenoma and Prostate Cancer (PCa).

### 4-1 Prostatitis

Prostatitis are the inflammations and infections of the prostate gland, refers to several clinical syndromes, including acute and chronic bacterial infections, chronic pelvic pain syndrome and asymptomatic inflammation in the prostatic gland (Pontari et al., 2007;

Kyriazis et al., 2014) . It is a very common condition in men but is difficult to treat effectively (Domingue, Sr and Hellstrom, 1998). It affects men of all ages but in most cases happens to those in their 40s (Pluta, 2012).

The prostatitis can be generally classified as acute or chronic based on the duration of condition and the severity of symptoms (Kyriazis et al., 2014).

- Acute prostatitis is a serious infection caused by bacterial infections such as *Trichomonas vaginalis, Chlamydia trachomatis* and *Escherichia coli* which are in most cases sexually transmitted, and can progress rapidly to urosepsis if remain untreated, especially in elderly or diabetic (Domingue, Sr and Hellstrom, 1998; Kyriazis et al., 2014; Cai and Bjerklund Johansen, 2016).
- Chronic prostatitis is a clinical condition in which an acute prostatitis infection last more than 3 months after initial infection (Hricak and Scardino, 2008), It is considerated the most common prostatitis form in men (Rees and Doble, 2015). It can be bacterial, inflammatory or non-inflammatory (Schaeffer, 2002; Rees and Doble, 2015).

### 4-2 Benign prostatic hyperplasia (BPH):

It is known that the human prostate gland is one of the only internal organs that continue to enlarge from early ages of development, during adolescence and even in adulthood (Roehrborn, 2005). So as the hormonal milieu changes with middle age, the prostate grows and enlarges gradually with aging in normal male from 25–30 g for men in their 40s reaching about 30-45 grams at the age of 60 (Giles, 2017; Farrant and Page, 2018).

BPH is a non-cancerous benign increase in the size (hypertrophy) and number (hyperplasia) of prostate cells which result in an abnormal growth of prostate gland (McNicholas and Mitchell, 2008; Langan, 2019). This condition is very common in middle-aged and older men, while occur very rarely in young men (Roehrborn, 2005; Langan, 2019).

BPH is characterized by a hyperplasia of both stromal and to lesser extent epithelial cells of the transitional and the periurethral zones of the prostate, this enlargement is considerably variable and thought to be influenced by inflammation and sex hormones (Chughtai et al., 2016). It has been found that DHT (5α-dihydrotestosterone) is necessary for the development of BPH (McNicholas and Mitchell, 2008).

### 4-3 Prostate cancer (PCa)

To be treated extensively in next chapter.

Prostate cancer is the malignant transformation of prostate gland (epithelium cells). Adenocarcinoma is the most frequent PCa type, which is an invasive carcinoma of the prostate. There are eight histological prostate adenocarcinoma variants: Atrophic, Pseudohyperplastic, Microcystic, Foamy gland, Mucinous, Signet ring-like cell, Pleomorphic giant cell and Sarcomatoid variant. They are multifocal, with an average of two to three separate tumors per gland which are macroscopically variable (Szymańska and Hainaut, 2019).

## 1- Epidemiology

Each year 1.6 million men are diagnosed with PCa, and 366,000 men die due to it (Pernar et al., 2018). Prostate cancer is the third most common cancer in men, and the most common cancer in men in Europe, North America, and some parts of Africa (Grönberg, 2003). It is the most frequently diagnosed cancer among men in over one-half (105 of 185) of the countries of the world (Bray et al., 2018).

Prostate cancer is the second most common cancer diagnosed in men in the United States with an incidence of 164.690 new cases detected in 2018, with approximately 29.430 deaths (McClure et al., 2018).

In France, 40.309 new cases estimated in 2000, prostate cancer by its frequency now ranks 2$^{nd}$ among all cancers and ranks 1$^{st}$ for men where it accounts for 25% of all new cases (Villers et al., 2004).

The highest incidence rates were reported in Australia /New Zealand (111.6 per 100,000) and Northern America (97.2 per 100,000), and in Western and Northern Europe. This is due to the use of (PSA) testing in standard clinical practice and due to the aging population. Incidence rates are also relatively high in certain less developed regions, such as the Caribbean (79.8 per 100,000), Southern Africa (61.8 per 100,000), and South America (60.1 per 100,000), but remain low in Asian populations (10.5 per 100,000) (Szymańska and Hainaut, 2019).

In Algeria, the cancer registry in 2009 places prostate cancer in 4$^{th}$ place, it is the first urologic cancer, and virtually the 1$^{st}$ cancer in the subject after the age of 50 years. In 2014, PCa represents 10% of cancers in men, 1645 new cases were recorded with an average incidence of 10.8 per 100,000 (Hamdi Cherif et al., 2014; 2015).

## 2- Pathophysiology

Almost all prostate cancers are adenocarcinomas which progress locally then became metastatic (Fig. 4) (McClure et al., 2018). About 4% of PCa cases exhibit transitional cell

morphology that develops from the urogenital lining of the prostatic urethra. Most PCa cases (about 70%) begin in the peripheral zone, up to 25% of PCa cases in the transitional zone and (15%-20%) in the central zone. Most cases are multifocal; different malignant clones are present in the same gland. These multicentric lesions are often present in different zones with different characteristics, so it is not possible to be sure that the characteristics of a carcinoma identified by biopsy represent the status of the gland until the prostate is removed and examined (Crawford, 2009; Selman, 2011).

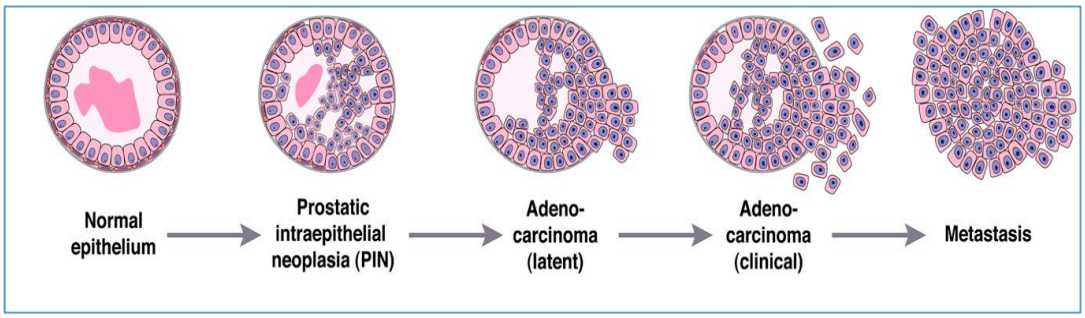


**Fig. 4:** Progression pathway for human prostate cancer (Shen and Abate-Shen, 2010)**.**

Other prostate tumors (<2%) include intraductal carcinoma (a variant of prostate cancer with more limited response to hormonal manipulation and radiation therapy), carcinosarcomas, squamous cell carcinoma, and urothelial carcinoma involving the prostatic urethra with or without stromal invasion (McClure et al., 2018).

## 3- Risk factors

Several risk factors associated with the development of prostate cancer have been described:

### 3-1 Age

Older age is associated with prostate cancer. Only 1 in 10.000 under age 40 will be diagnosed with prostate cancer, compared to 1 in 39 for ages 40–59, 1 in 14 for ages 60–69 (Moreira and Abern, 2018). The incidence rate of prostate cancer increases dramatically after 55 years of age (Pernar et al., 2018).

### 3-2 Family history and genetics

Prostate cancer risk increases according to the number of family members with prostate cancer (Moreira and Abern, 2018). Having a first-degree relative with prostate cancer incurs a twofold to threefold risk which increases with the number of relatives affected and earlier age at diagnosis (Giles, 2017). However, most PCa cases are sporadic at 80% of cases (Roehl et al., 2006).

### 3-3 Smoking

There is evidence, that smoking is associated with increased prostate cancer mortality (Giles, 2017). That's why current smokers had a 60% higher risk of prostate cancer mortality compared to men who never smoked. Also, former smokers who quit 10 or more years before diagnosis, have a similar PCa mortality risk and recurrence to those whom never smoked before. Several mechanisms were suggested to explain the association between smoking and PCa risk including tumor promotion through carcinogens contained in tobacco smoke, changes in testosterone levels, and epigenetic and nicotine-induced effects (Pernar et al., 2018). Overall, the association between smoking and the risk of developing prostate cancer is still unclear (Giles, 2017).

### 3-4 Alcohol

The consumption of low to moderate quantity of alcohol has no association with prostate cancer. However heavy drinking may increase the risk of developing PCa (Giles, 2017).

### 3-5 Obesity

Since 1980, worldwide obesity has doubled and became a public health issue. It is associated with an increased risk of developing prostate cancer by 1% to 5% (Pernar et al., 2018). Many mechanisms may promote the development and progression of prostate cancer, in obese men: such as increased levels of insulin-like growth factor 1 (IGF-1), sex hormones, and adipokines. Obesity can promote an inflammation, which can affect the development of prostate cancer (Sierra et al., 2016; McClure et al., 2018).

### 3-6 Dietary factors

There is a relationship between several foods and nutrients and the risk of developing prostate cancer (Sierra et al., 2016). Several studies have suggested that dietary fat, especially from red meat, is the major risk factor for the development of this disease (McClure et al., 2018). Other dietary elements may have an acute effect on the disease like lycopene and tomato-based products; notably, the associations between tomato products are stronger for risk of advanced or lethal prostate cancer, compared with overall risk, suggesting that tomato products may play a role in disease progression. Also, calcium, dairy products and vitamin D, coffee, fish and statin (Pernar et al., 2018).

### 3-7 Occupation

Several studies showed an inverse association between occupational activity and risk of advanced prostate cancer (Pernar et al., 2018), few of them have been firmly established.

# 4- Anatomopathology of prostate cancer

## 4-1 Precancerous lesion

Two putative premalignant lesions of the prostate have been identified.

- Prostatic intraepithelial neoplasia (PIN) is characterized by proliferation and anaplasia of cells lining ducts and acini.
- Atypical adenomatous hyperplasia (AAH) consists of a localized proliferation of small round glands without cytologic atypia (Putzi and Marzo, 2001).

PIN and AAH may be confused with well-differentiated carcinoma as: florid hyperplasia, basal cell hyperplasia, transitional metaplasia, seminal vesicular epithelium, and atypia due to inflammation, infarction, and radiation. These premalignant lesions appear to have a high predictive value for carcinoma, and their presence on prostatic biopsy warrants further search for concurrent invasive adenocarcinoma. Only high-grade PINs (HG-PIN) should be reported on histological reports of biopsy specimens (Bostwick, 1988).

## 4-2 Anatomopathological classification

### 4-2-1 Gleason Score

The Gleason score is a system used to grade and help evaluate the prognosis of men with prostate cancer using samples from a prostate biopsy. The predominant and secondary patterns of glands within the tumor are identified and each is assigned a grade of 1 to 5, with 1 being the least aggressive (most differentiated) and 5 being the most aggressive (least differentiated) (Fig. 5).

- Well-differentiated tumors: score 2 to 4.
- Moderately differentiated tumors: score 5 to 7.
- Tumors with little or no differentiation: score 8 to 10 (Salomon et al., 2010).

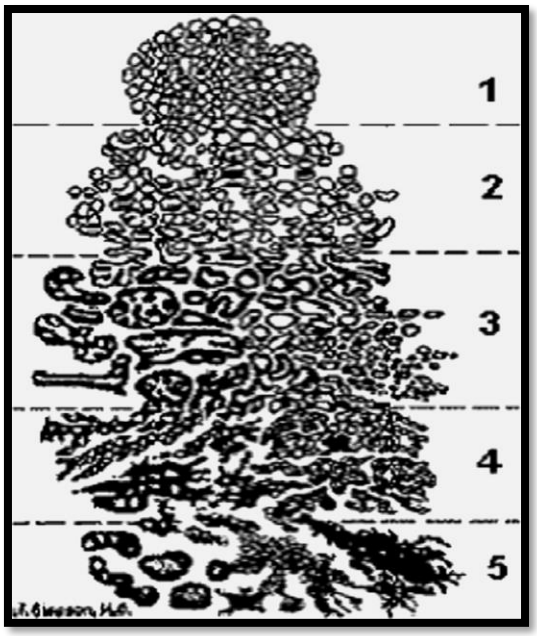


**Fig. 5:** Gleason score patterns; from 1 the least aggressive (most differentiated) to 5 the most aggressive (least differentiated) (Lee et al., 2011).

### 4-2-2 TNM classification

Before TNM classification, the Whitmore and Jewett classification has been long used. It is a classification of four stages (A, B, C and D) (Fournier et al., 2004). Then it was replaced in 1940-1950, by the French surgeon Pierre Denoix who staged the prostate cancer using T, N, and M classification system. Which classifies according to their local (T), regional (N) and distant (M) results of clinical, radiological and histological examinations (Appendix 1) (Salomon et al., 2010).

### 4-2-3 AIMCO's classification

- It classifies tumors according to their potential aggression at the time of diagnosis:
- **Low risk**: PSA <10 ng / ml and Gleason score ≤ 6 and T1c or T2a clinical stage.
- **Intermediate risk**: PSA between 10 and 20 ng / mL or Gleason score of 7 or T2b stage.
- **High risk**: PSA> 20 ng / mL or Gleason score ≥ 8 or T2c clinical stage.

***P.S***: CT3a is integrated into the high-risk group (Salomon et al., 2010).

## 5- Symptoms

Prostate cancer usually causes no signs or symptoms in the early stages of the disease (Giles, 2017). The signs and symptoms often appear when the tumor grows and cause changes

in urinary habits or other problems (Moreira and Abern, 2018). Other medical conditions can cause the same symptoms as prostate cancer can cause.

**In early stages:**

- lower urinary tract symptoms (LUTS)
- presence of blood in the sperm
- erectile dysfunction
- pain or stiffness in the bones of the hips, back or chest
- weakness or numbness in the legs or feet
- cough that does not go away or shortness of breath

**Late symptoms of PCa:**

- loss of weight
- asthenia
- hematuria
- sexual impotence
- pain, dysfunction and failure of other organs are related to the presence of metastases (Fournier et al., 2004; Moreira and Abern, 2018).

# 6- Detection of Prostate Cancer

## 6-1 Suspicion of the presence of cancer

The use of PSA in combination with DRE, as an aid to early diagnosis is widely used in clinical practice (Heidenreich et al., 2008). However, an abnormal DRE (asymmetric prostate) of the prostate or an elevated PSA blood test level suggest the presence of prostate cancer and it is an indication for prostate biopsy (McClure et al., 2018).

## 6-2 Prostate-Specific Antigen

Prostate-specific antigen, first described in 1981, is a glycoprotein secreted by prostatic epithelial cells. Its protease activity lyses clotted ejaculate to increase sperm motility. PSA enters the circulation through unknown mechanisms where it is present in low quantities (Szymańska and Hainaut, 2019). The half-life of PSA range between 2 to 3 days (Fournier et al., 2004). Unfortunately, PSA is organ-specific and not prostate cancer-specific, and this explains the overlap in PSA levels between benign pathologies (BPH), prostatitis and PCa (Descotes, 2019). PSA is normally secreted outside of the body in urine or semen. If PSA backs up into the body and is present at elevated levels in the blood, then an abnormal

condition is present within the prostate. This abnormality may be caused by trauma to the prostate, infection, benign enlargement, or prostate cancer (McClure et al., 2018).

### 6-3 Digital rectal examination

The digital rectal exam is a clinical examination of the prostate by palpating it with the finger through the rectum wall to assess the volume, consistency and regularity of the prostate. The latter is suspect when it is of hard consistency (nodule or stony lobe), deformed, asymmetrical or bulky (Fournier et al., 2004), its performance for initial detection of cancer is limited: because most patients detected with PCa during screening PSA program have normal DRE. However, palpation of irregularity or nodule during DRE still remains an indication for prostate biopsy regardless of the level of PSA (Descotes, 2019).

### 6-4 Biopsy

Prostatic biopsy is indicated in patients with elevated total PSA and/or abnormal DRE or family history of prostate cancer (Vakar-Lopez and True, 2019). It consists in taking Transrectal echo guided under local anesthesia to get prostate tissue samples to confirm diagnosis of the disease (McClure et al., 2018). Transrectal ultrasound (TRUS) is also used to evaluate prostate size and to ensure that biopsies have an adequate distribution of samples throughout the prostate gland. Today, transrectal ultrasound prostate biopsy remains the gold standard examination to confirm diagnosis and to classify the tumor according to Gleason classification (Descotes, 2019).

### 6-5 Other tests for prostate cancer detection

Adjunctive tests used to quantify the risk of prostate cancer with men who have indeterminate PSA levels or elevated levels with prior negative biopsies which have been applied, these include:

- **PCA-3,**

Prostate cancer gene 3 (PCA3) is a prostate-specific, non-coding mRNA biomarker which is detectable in urine sediments obtained during DRE and can be measured by real-time PCR (Szymańska and Hainaut, 2019).

- **4kScore**

4K score is a four-kallikrein panel including kallikrein-related peptidase 2, intact PSA, fPSA and tPSA (Descotes, 2019). It is a blood test that evaluates PSA forms and other proteins, and taking into account patient characteristics (age, prior biopsy history). 4kScore

results are reported as the percentage risk of the patient having larger or more aggressive prostate cancer (McClure et al., 2018).

- **Nomograms**

It is a pictorial representation of a complex mathematical formula that use biological and clinical variables, such as tumor grade and patient age, to graphically depict a statistical prognostic model that generates a probability of a clinical event, such as cancer recurrence or death (Balachandran et al., 2015) and to predict the risk of disease recurrence after definitive treatment. Multiple individual variables have been shown to be useful in this setting (Descotes, 2019).

PCa is both clinically and genetically heterogenous disease, which complicate its better understanding (Giri and Beebe-Dimmer, 2016). Genetic components that cause PCa are either inherited from parents or acquired during lifetime (Porkka and Visakorpi, 2004; Cussenot and Cancel-Tassin, 2015), which means that there are two forms of PCa: the familial and hereditary form, and the sporadic form (Bratt, 2002; Shen and Abate-Shen, 2010).

## 1- Familial and hereditary forms of PCa

It was estimated by several epidemiological studies that 5-10% of all prostate cancer incidence is caused by inheritance of predisposition genes with high penetrance (Demichelis and Stanford, 2015; Giri and Beebe-Dimmer, 2016). This inheritance is either autosomal dominant, recessive, X-linked or Y-linked in some cases (Bratt, 2002; Edwards and Eeles, 2004; Attard et al., 2016). Predisposition genes can be categorized into high-penetrance genes and low-penetrance genes (Elo and Visakorpi, 2001).

The heritability of PCa have been estimated to 58% (Demichelis and Stanford, 2015), which is the highest reported heritability of any major cancer (Rebbeck, 2017).

Men with one first-degree relative with PCa have a twofold higher risk to be affected by PCa then men with no family history, while men with two first-degree relatives or with one relative that was diagnosed with PCa before age of 55 years have a fivefold higher risk of developing prostate cancer compared with men with no family history (Gonzalgo and Isaacs, 2003; Salinas et al., 2014; Demichelis and Stanford, 2015). This risk increases with the number of affected relatives, and is inversely related to the age at diagnosis among affected relatives (Giri and Beebe-Dimmer, 2016).

Familial prostate cancer (FPC) is having at least one first degree relative with prostate cancer, its defined as families having either: two first-degree relatives diagnosed with PCa at any age, or one first-degree relative with two or more second-degree relatives diagnosed with PCa at any age (Wallis and Nam, 2015; Giri and Beebe-Dimmer, 2016).

Hereditary prostate cancer (HPC) characterize families with a particularly strong history of PCa, its defined as families having either: three successive generations of the same lineage diagnosed with PCa, three or more first‑degree relatives diagnosed with PCa, or two relatives diagnosed with PCa before age 55 years (early-onset disease) (Wallis and Nam, 2015; Giri and Beebe-Dimmer, 2016).

### 1-1 Predisposition genes

Many PCa predisposition genes have been identified such as *HPC1* (Hereditary Prostate Cancer 1) located at chromosome 1q24-25, *PCAP* (Prostate Cancer Predisposing) located at chromosome 1q42.2-q43, *HPCX* (Hereditary Prostate Cancer X-Linked) located at chromosome Xq27-28, *CAPB* (Cancer Prostate And Brain) located at chromosome 1q36, *HPC20* (Hereditary Prostate Cancer 20) located at chromosome 20q13, *MSR1* (Macrophage Scavenger Receptor 1) located at chromosome 8p22-23, *HOXB13* (Homeobox B13) located at chromosome 17q21-22 (Porkka and Visakorpi, 2004; Rubin and De Marzo, 2004; Rebbeck, 2017). Other loci have been identified in many chromosomal regions such as 16q23.2 region, 17p region also known as *ELAC2* (elaC Ribonuclease Z 2) or *HPC2* (Hereditary Prostate Cancer 2), 7q32-q34 and 19q12 regions (Visakorpi, 2003: Edwards and Eeles, 2004). Of this genes the most notable predisposition genes candidates in hereditary PCa are: *HOXB13* G84E variant, *BRCA2* (Breast Cancer 2) (Demichelis and Stanford, 2015; Giri and Beebe-Dimmer, 2016; Rebbeck, 2017), *HPC1/RNASEL* (Ribonuclease L) and *MSR1* (Rubin and De Marzo, 2004; Demichelis and Stanford, 2015).

## 2- Sporadic form of PCa

Sporadic forms of PCa are the most frequent, and they are defined as patients with no known family history of PCa (Roehl et al., 2006). Genetic aberrations in PCa results in inactivation of tumor suppressor genes and activation of oncogenes (Porkka and Visakorpi, 2004).

### 2-1 Cytogenetic alterations

There are many chromosomal alterations occur in PCa tumoral cells. The most common of this chromosomal alterations are: deletions at chromosomes 1q,3p, 5q, 6q, 8p, 9p, 10q, 12, 13q, 16q, 17p, 18q, 22 and Y and gains at 7p, 7q, 8q and Xq (Elo and Visakorpi, 2001; Visakorpi, 2003; Shen and Abate-Shen, 2010; Gandhi et al., 2018; Köseoğlu, 2018). Thus far, only a few target genes have been associated with these aberrations such as *NKX3‑1* (NK3 homeobox 1), *PTEN* (Phosphatase and tensin homolog), *MYC* (Myelocytomatosis), *BRCA2* and *RB1* (Retinoblastoma Transcriptional Corepressor 1) (Shen and Abate-Shen 2010; Wallis and Nam, 2015; Gandhi et al., 2018).

It has been demonstrated in many studies of PCa that loss of DNA material is 5 times more common than DNA gains and amplifications in primary stages of tumors, which means that in early prostate cancer, the inactivation of recessive tumor suppressor genes is more

important than amplification of oncogenes. In addition it appears that losses and gains are obvious in hormone-refractory tumors, which indicate that activation of oncogenes and inactivation of tumor suppressor genes, are involved in late-stage cancers (Elo and Visakorpi, 2001; Visakorpi, 2003).

### 2-2 Epigenetic alterations

Epigenetic alterations are very common in PCa and had an important role in prostate carcinogenesis initiation and progression. They are considered as one of the earliest somatic changes in PCa development which found even before any histological evidence of malignant transformation (Porkka and Visakorpi, 2004; Valdés-Mora and Clark, 2015). There are a lot of epigenetic mechanisms identified in PCa, they are either work independently or with each other to modify gene regulation and form the PCa 'epigenome' (Valdés-Mora and Clark, 2015). These processes includes primarily: DNA methylation, chromatin remodeling, post-translational histone modifications, the incorporation of histone variants and expression of non-coding RNAs (Fig. 6) (Gonzalgo and Isaacs, 2003; Wang et al., 2018).

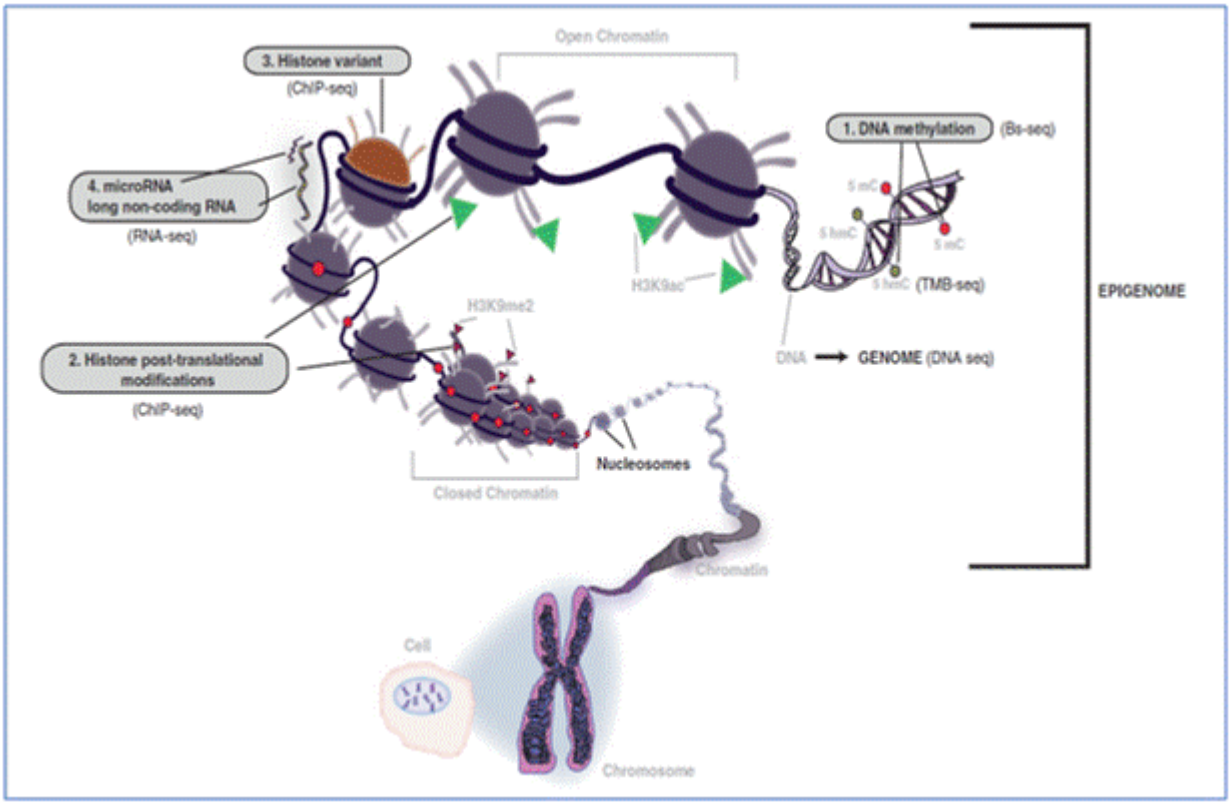


**Fig. 6:** Epigenetic mechanisms implicated in PCa (Valdés-Mora and Clark, 2015)**.**

### 2-2-1 Aberrant DNA methylation

In early stages of PCa initiation a severe alteration of the CpG DNA methylation landscape occurs which continues to change during cancer progression (Valdés-Mora and Clark, 2015). These alterations are characterized by a global DNA hypomethylation that lead to genome instability and oncogene activation (Gonzalgo and Isaacs, 2003; Shen and Abate-Shen, 2010), and local DNA hypermethylation of CpG island promoter regions of tumor suppressor genes which cause malignant transformation results in silencing of tumor suppressor genes (Netto et al., 2017; Wang et al., 2018).

Hypermethylation loci are considered as potential prostate cancer detection and prognosis biomarkers. Genomic DNA hypomethylation occurs in both early and late stages of PCa, which can be used as a potential biomarker for early detection and prognosis (Gonzalgo and Isaacs, 2003; Porkka and Visakorpi, 2004; Valdés-Mora and Clark, 2015).

### 2-2-2 Histone modifications

Histone epigenetic modifications (e.g., acetylation, methylation, phosphorylation, sumoylation and ubiquitylation) have been found in PCa, simultaneous with cancer progression that result in a considerable deregulation of gene expression of tumor cells leading to uncontrolled cell proliferation and invasion. H3K27-me3, a trimethylation of lysine residue 27 of histone H3 by EZH2 enzyme, is a key histone modification associated with prostate carcinogenesis by repression of tumor suppressor genes such as *DAB2IP* and *E-cadherin* (Shen and Abate-Shen, 2010; Valdés-Mora and Clark, 2015; Netto et al., 2017).

### 2-2-3 Non-coding microRNAs

Modifications of miRNA expression in tumor cells affect potentially oncogene and tumor suppressor gene regulation, this alteration is caused by both genetic and epigenetic mechanisms. These alterations in miRNAs expression has been widely related with local invasion and early stages of the PCa (Valdés-Mora and Clark, 2015).

### 2-2-4 Chromatin remodelers

Certain mutations in many epigenetic regulators such as ASXL1 (Additional Sex Combs Like 1), KMT2C (Lysine(K) Methyltransferase 2C) and KDM6A (Lysine Demethylase 6A) and in chromatin remodelers include ARID1A (AT-Rich Interaction Domain 1A), ARID4A (AT-Rich Interaction Domain 4A), ARID2 (AT-Rich Interaction Domain 2), and other members of the SWI/SNF nucleosome remodeling complex have been found in many PCa cases (Wang et al., 2018).

## 2-3 Genetic alterations

In this study, we talked about the most frequent genetic alterations in PCa, for a more completed list see appendix 2.

### 2-3-1 Gene fusion

The most common PCa genetic alterations are translocations which lead to gene fusions, these recurrent gene fusions have been identified by multiple studies that they manifests in 50% of primary PCa cases (Barbieri et al., 2012; Wallis and Nam, 2015; Gandhi et al., 2018) .

### 2-3-2 *AR*

The androgen receptors mediate the androgens activity and have a key role in both normal prostate developments and prostate carcinogenesis (Hatcher et al., 2009; Shen and Abate-Shen, 2010). This receptor is found mutated in about 10–30% of the hormone refractory and metastases PCa cases (Porkka and Visakorpi, 2004; Dong, 2006).

The AR is considered as the central molecular signaling pathway for the physiological function of the normal prostate gland (Shen and Abate-Shen, 2010; Gandhi et al., 2018). It has been found that the majority of primary and metastatic prostate cancers particularly the hormone refractory cancers contain alterations in *AR*, such as *AR* amplification and somatic mutations, which lead to castration resistant PCa (Dong, 2006; Shen and Abate-Shen, 2010; Netto et al., 2017; Wang et al., 2018). The somatic mutations result in the diminution of ligand-binding specificity and improper activation of AR by other substances such as estrogens and progestins, while genomic amplification of the *AR* gene maintain an active androgen signaling axis even with very low levels of androgen (Porkka and Visakorpi, 2003; Visakorpi, 2004; Dong, 2006; Wang et al., 2018).

### 2-3-3 *PTEN*

The deletion of chromosomal regions of the long arm of chromosome 10 (10q23) is very frequent in human PCa, which contain *PTEN* gene along others (Visakorpi, 2003; Porkka and Visakorpi, 2004). These deletions have been found in prostate carcinomas but highly detected especially in metastatic stage of PCa (reach up to 60% of metastatic prostate carcinomas) (Elo and Visakorpi, 2001; Dong, 2006).

*PTEN* gene is involved in PCa with recurrent somatic mutations occur mostly in aggressive prostate cancer, which are found also in many other human malignancies, in addition to the deletion of *PTEN* gene (Dong, 2006; Elo and Visakorpi, 2001). *PTEN* gene mutations or deletion lead to reduction or loss of expression of this gene which lead to

increased cell proliferation and reduced apoptosis (Porkka and Visakorpi, 2004; Dong, 2006; Shen and Abate-Shen, 2010). It has been shown that mutations of *PTEN* exist as well in cancer predisposition syndromes, such as Cowden's disease and the Bannayan-Zonana syndrome (Elo and Visakorpi, 2001; Dong, 2006).

### 2-3-4 *MYC*

It was demonstrated in numerous studies that there is an amplification of *MYC* gene in up to 50% of PCa tumors including cancer initiation and progression, localized tumors (40%), metastasis (90%) and even at the PIN stage (Dong, 2006; Shen and Abate-Shen, 2010; Wang et al., 2018). Mutated *MYC* in PCa tumors shows a varying level of copy number increases depending on PCa stage such as simple gain of an extra copy of 8q in *MYC* in case of located tumor, while regional amplification of *MYC* is more frequent in higher grade tumors, metastases and androgen-resistant tumors, which indicate that *MYC* gene is more commonly involved in prostate cancer progression (Dong, 2006; Gandhi et al., 2018).

### 2-3-5 *NKX3-1*

The loss of short arm of chromosome 8 (8p) is the most common deletion of PCa, which include the *NKX3-1* gene that is a frequently deleted gene in PCa (Gonzalgo and Isaacs, 2003; Shen and Abate-Shen, 2010; Wang et al., 2018).

The *NKX3-1* PSA-regulated homeobox gene is considered as a prostate specific tumor suppressor. It is expressed in high levels in adult prostate, while in prostate cancer cells its expression level is reduced (Gonzalgo and Isaacs, 2003; Dong, 2006; Shen and Abate-Shen, 2010; Wang et al., 2018). Loss of *NKX3-1* expression has been associated with hormone-refractory PCa and advanced stage of prostate tumor (Porkka and Visakorpi, 2004). The *NKX3-1* gene can be silenced through promoter hypermethylation which is an epigenetic down-regulation (Shen and Abate-Shen, 2010; Gandhi et al., 2018).

### 2-3-6 *TP53*

The tumor suppressor gene *TP53* located at chromosome 17p13 (Elo and Visakorpi, 2001), is the most frequently mutated gene in human cancer, it is commonly inactivated by point mutations leading to inactivation of tumor suppressor function while grant oncogenic characteristic to the mutated protein (Vassilev, 2004; Andrysik et al., 2017). The TP53 protein is a transcription factor which plays a central role in the regulation of cell cycle, senescence, apoptosis, and DNA repair, thus prevent tumor progression and lead to protection from cancer (Moll and Petrenko, 2003; Shangary and Wang, 2008; Tovar et al., 2013), they

are called “the guardian of the genome” (Brooks, 2013). *TP53* gene is tightly controlled by its negative regulator the *MDM2*. In unstressed cells, TP53 exist in a latent and unstable form at low levels, however in cells that sustain various types of stress and damage its level increase dramatically leading to growth arrest, senescence, or apoptosis of those damaged cells (Onel and Cordon-Cardo, 2004; Vassilev, 2004; Brooks, 2013).

Normal TP53 protein tend to degrade quickly in normal cells by mediation of MDM2 protein due to its very short half-life range between 5-30 min, but in mutated TP53 it was observed that their half-life prolonged because of abnormal stability of the mutated TP53 (Moll and Petrenko, 2003; Dong, 2006). This prolonged half-life of TP53 leads to its nuclear accumulation in tumor cells (nuclei), which allow its detection by Immunohistochemistry (IHC) (Elo and Visakorpi, 2001; Porkka and Visakorpi, 2004).

## 3- *MDM2* Gene family

The murine double minute 2 (*Mdm2*) gene was identified for the first time as the gene responsible for the spontaneous transformation of the non-transformed BALB/c 3T3 mouse cell line into the tumorigenic mouse cell line 3T3-DM. It was originally discovered as one of three expressed genes (*Mdm1, Mdm2,* and *Mdm3*) within an amplicon cloned from the derivated tumorigenic mouse cell line 3T3-DM (Freedman et al., 1999; Mendoza et al., 2014). The product of *Mdm2* gene is the one responsible for cells tumorigenic transformation ability when overexpressed and the only one of the *MDM* genes which has this transforming ability (Freedman et al., 1999; Iwakuma and Lozano, 2003)..

There is a second member of the *MDM2* gene family which is *MDM4* gene (also known as *MDMX*, *HDM4*, or *HDMX*), which is a paralog of *MDM2* forming both a small family called the *MDM2* gene family (Freedman et al., 1999; Mendoza et al., 2014).

MDM2 and MDM4 form a heterodimer in cells to strengthens the efficacy of MDM2’s inhibitory activities(Freedman et al., 1999; Iwakuma and Lozano, 2003; Mendoza et al., 2014).

### 3-1 *MDM2* Gene

The *MDM2* gene or as known in humans as the *HDM2* is located on chromosome 12q14.3-q15, encode a 491 amino acid protein, with an E3 ubiquitin ligase activity, which negatively regulates the TP53 tumor suppressor protein by polyubiquitylation of this latter, the polyubiquitylated TP53 then subsequently degraded by the 26S proteasome (Manfredi, 2010; Mendoza et al., 2014). The *MDM2* gene is about 25 kilobases (kb) in size (Freedman et al., 1999), consist of 12 exons (Fig.7) at least that have several different isoforms of MDM2

messenger RNA (mRNA), and can generate many different proteins in various cell lines because of the presence of multiple *MDM2* transcripts in most cell lines; at least seven unique transcripts have been described in both mouse and human cells (Freedman et al., 1999; Iwakuma and Lozano, 2003).

The human *MDM2* gene can also be classified as an oncogene based on its behavior in human cancers, where it was found overexpressed in a wide variety of different human tumors such as soft tissue sarcomas, osteosarcomas, acute myeloid leukaemias and breast carcinomas, due to one of three different mechanisms: gene amplification, increased transcription or enhanced translation (Levav-Cohen et al., 2005; Mendoza et al., 2014).

The *MDM*2 gene has two different promoters: the P1 promotor site found in upstream of exon 1 which regulates the constitutive expression of the full-length p90 *MDM2* mRNA. The P2 promotor site inside the first intron of which is very responsive to p53 which regulates the damage inducible expression of the alternatively spliced shorter protein p76 mRNA (Iwakuma and Lozano, 2003; Onel and Cordon-Cardo, 2004). The full-length p90 MDM2 oncoprotein inactivates the TP53 tumor suppressor protein that can bind to it, whereas the short p76 MDM2 protein cannot bind to TP53 because it lacks the first 49 amino acids of the p90 MDM2 protein (Chang et al., 2004).

MDM2 protein contains three domains (Fig. 7): the N-terminal region which interact with TP53 and inhibit its transcriptional activation function, the central region that includes an acidic region that mediates MDM2's interaction with the ribosomal protein L5 and its associated 5S ribosomal RNA (rRNA) and a C4 zinc finger domain, and the C-terminal RING finger domain which have the E3 ubiquitin ligase activity that mediate MDM2 autoubiquitination, p53 ubiquitination and provides binding sites for MDM4 (Moll and Petrenko, 2003; Manfredi, 2010; Zheng et al., 2015). In addition to the conserved nuclear localization sequence (NLS) and the conserved nuclear export signal (NES) motifs that mediate MDM2 capacity to shuttle between the nucleus and the cytoplasm (Freedman et al., 1999; Moll and Petrenko, 2003).

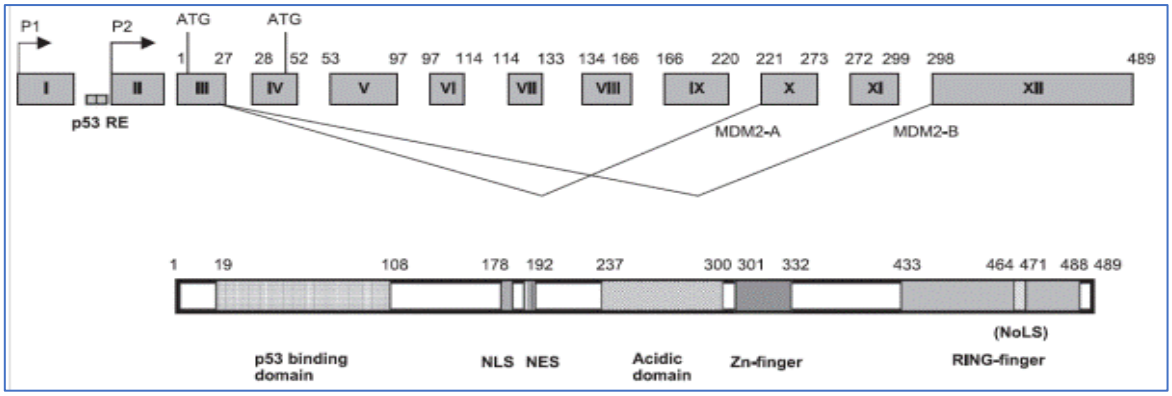


**Fig. 7:** Structure of *MDM2* gene and protein (Iwakuma and Lozano, 2003)**.**

The main cellular function of MDM2 is the physiological regulation of TP53 (Klein and Vassilev, 2004). MDM2 and TP53 regulate each other through an autoregulatory feedback loop intended for keeping low cellular levels of TP53 in the absence of cellular stress (Manfredi, 2010), where TP53 turnover is regulated by MDM2 and expression of MDM2 is under the transcriptional control of TP53 (Mendoza et al., 2014): When nuclear TP53 level is elevated, the transcription of the *MDM2* gene is activated, which increase the level of MDM2 protein, in the same time MDM2 binds to TP53 which blocks its N-terminal transactivation domain which then aim to degrade TP53 (Klein and Vassilev, 2004).

MDM2 can work independently of TP53, play a regulatory role in its interaction with other tumor-related genes that are important for cell-cycle control, and contributes to carcinogenesis through interaction with transcriptional factors of the E2F family, inhibition of the Rb growth regulatory function and inhibition of G0/G1-S-phase transition in normal cells (Li et al., 2006; Shangary and Wang, 2008).

### 3-2 MDM2 polymorphisms

Mutations in the MDM2 oncoprotein are very rare but the overexpression of *MDM2* gene has been found in wide variety of human tumors due to different mechanisms which are mentioned above (Levav-Cohen et al., 2005; Lalonde et al., 2012).

The *MDM2* gene is highly polymorphic, it has at least 4,765 single-nucleotide polymorphisms (SNPs) and 18 insertion polymorphisms, none of these polymorphisms cause amino acid changes, thus *MDM2* polymorphisms in a regulatory region, such as the promoter, may alter its transcriptional activities, and as a result degrade TP53 and cause carcinogenesis in humans (Li et al., 2006; Gansmo et al., 2016; Hua et al., 2017; Moazeni-Roodi et al., 2019).

The most studied *MDM2* SNPs, are SNP309T>G (rs2279744) and SNP285G>C (rs117039649), located in *MDM2* promoter P2, which were found to increase the binding affinity of the *MDM2* promoter 2 region to the transcription factor Sp1 (specificity protein 1), resulting in higher levels of MDM2 mRNA and protein, in addition to del1518 (rs3730485) polymorphism (Wang et al., 2016; Gansmo et al., 2017).

### 3-2-1 Del1518 (rs3730485) polymorphism

The del1518 (rs3730485) polymorphism of *MDM2* gene is a 40 base pairs insertion/deletion polymorphism located in position −1208 to −1169 in the promoter P1 with a putative TATA motif (Fig. 8) (Lalonde et al., 2012; Gansmo et al., 2017; Hua et al., 2017), which have three variants: I (Ins), D (Del) and I/D (Indel) (Hashemi et al., 2017). The del variant of this polymorphism has been associated with decreased MDM2 expression, and have almost complete linkage disequilibrium with the MDM2 promoter P2 polymorphism SNP309T>G (rs2279744) (Gansmo et al., 2016; 2017).

The 40bp indel polymorphism was found to increase cancer risk of various tumor types, such as increased risk of overall cancer and gastrointestinal cancer (Moazeni-Roodi et al., 2019), increased risk of hepatocellular carcinoma, colon cancer and uterine leiomyoma (Gansmo et al., 2017), increased risk of breast cancer (Hashemi et al., 2014) and increased risk in PCa (Hashemi et al., 2017). But the findings of several studies for impact of 40bp indel polymorphism on cancer risk were inconsistent and controversial (Moazeni-Roodi et al., 2019).

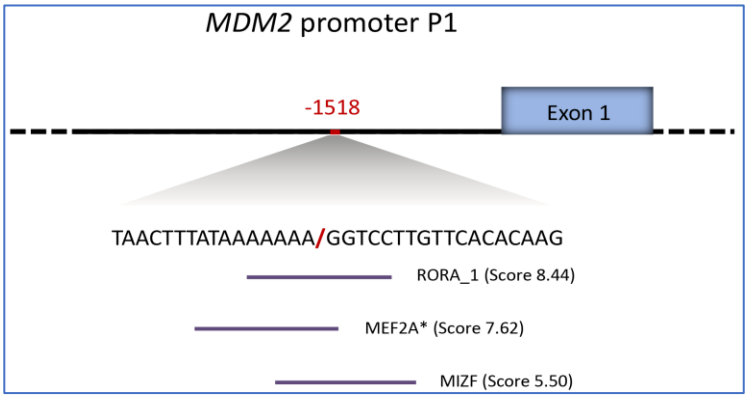


**Fig. 8:** *MDM2* promoter P1 del1518 break-point sequence context (Gansmo et al., 2016)**.**

# 1- Diagnosis of prostate cancer

## 1-1 Transrectal prostate ultrasound

Transrectal ultrasound guided systemic biopsies is the recommended diagnostic method in most cases with the suspicion of PCa on an abnormal DRE and/or elevated serum PSA levels (Heidenreich et al., 2008). It allows a uniform spatial separation and sampling of prostate regions, visualization of the prostate gland to measure its volume and to distinguish a localized cancer from an extracapsular cancer (Moreira and Abern, 2018).

## 1-2 Magnetic resonance imaging (MRI)

Magnetic resonance imaging (MRI), an imaging modality that uses a magnetic field instead of rays. MRI is used to evaluate and localize prostate tumors and determine their size (McClure et al., 2018). MRI can identify suspicious lesions in the prostate that can be targeted by biopsies or focal treatments as well as aid in local staging by identifying signs of extraprostatic extension (Moreira and Abern, 2018).

## 1-3 Abdominal-pelvic scan

Also named sonography, is a test which depend on X-rays to visualize and precise the targeted area, the extracapsular extension of PCa and pulmonary metastases, cerebral, bone, etc (Fournier et al., 2004).

## 1-4 Bone scintigraphy

Bone scintigraphy is a highly sensitive nuclear medicine imaging technique that uses a radiotracer to evaluate if the bones are affected with malignant diseases (Van den Wyngaert et al., 2016). It is indicated for Gleason grade 4 and /or PSA greater than 10 ng/ml and/or bone pain. This diagnosis technique detects bone metastases in asymptomatic patients (Attard et al., 2016).

# 2- Treatment of prostate cancer

PCa can be treated by many therapeutic methods. The choice of treatments depends on: the histological type, stage, grade, the characteristics of the prostate (volume, impact on the bladder functions), age, medical and surgical history (Moreira and Abern, 2018).

## 2-1 Treatment of localized prostate cancer

Men with localized prostate cancer are treated with active surveillance, radical prostatectomy, radiotherapy, or focal therapies (D'Amico, 2011).

### 2-1-1 Active Surveillance (AS)

Active surveillance has emerged as a therapeutic alternative to men whom have PCa with low risk of disease progression (Heidenreich et al., 2008). It is based on repeated PSA, exams, and prostate biopsies in the hope of delaying definitive treatment. It helps affected men with limited chance of benefiting from local treatment to avoid the side effects of treatment (McClure et al., 2018).

### 2-1-2 Radical Prostatectomy (RP)

RP involves the surgical removal of the entire prostate and seminal vesicles. It is appropriate for any patient whose cancer clinically appears as a localized PCa. The goal of radical prostatectomy is to eradicate the disease, while preserving continence and potency whenever possible (Szymańska and Hainaut, 2019). The most common risks and side effects associated with RP are: infections, damage to nearby structures, urinary incontinence, and erectile dysfunction (Moreira and Abern, 2018).

### 2-1-3 Radiotherapy

Prostate cancer can be treated with radiation therapy. It involves the delivery of high-energy radiation that creates lesions in the DNA to treat the disease. For external radiotherapy, at least (72 Gy) are recommended for the management of low-risk PCa (Heidenreich et al., 2008). The effectiveness of radiation therapy is limited by the relative insensitivity of prostate cancer to radiotherapy. It is prescribed for patients who cannot undergo surgery (McClure et al., 2018).

### 2-1-3 Focal therapies

The rationale for focal therapies of the prostate is to treat the tumor while sparing the normal prostate. Although, there are several means of delivering energy to kill the tumor, the most used technique is cryotherapy. Freezing of the prostate is accomplished by using a multiprobe cryosurgical device. Multiple probes are placed percutaneously under transrectal ultrasound guidance, and the temperature is lowered to -25c°to -50°C (Moreira and Abern, 2018).

## 2-2 Treatment of metastatic prostate cancer

Men with metastatic PCa are usually treated with hormonotherapy, chemotherapy or immunotherapy.

### 2-2-1 Androgen Deprivation Therapy (ADT)

Androgens are required for normal function and growth of the prostate. They also stimulate growth of PCa cells. Lowering androgen levels or stopping them from getting into

prostate cancer cells. Several types of ADT can be used to treat prostate cancer (Moreira and Abern, 2018) :

- Bilateral orchiectomy (surgical castration)
- Luteinizing hormone-releasing hormone (LHRH)
- *CYP17* inhibitors
- Antiandrogens
- Estrogens (Szymańska and Hainaut, 2019).

### 2-2-2 Chemotherapy

The cytotoxic chemotherapy is usually used in cases of metastatic disease, especially castration-resistant prostate cancer (CRPC). The most commonly prescribed drugs are: docetaxel, cabazitaxel, mitoxantrone, and estramustine that target cell division mechanisms (McClure et al., 2018).

### 2-2-3 Immunotherapy

Many immunomodulatory agents are being investigated. For now, only Sipuleucel-T is approved for the treatment of metastatic prostate cancer (Attard et al., 2016). Sipuleucel-T is a vaccine against PCa cells, after a patient's white blood cells have been trained to target a ligand which is specific to PCa. Treating men with Sipuleucel-T has been shown in a multicenter, randomized control trial to have a 22% reduction in mortality (McClure et al., 2018).

The present study is a case-control study, performed at the department of urology, EHS -Daksi Constantine (for blood samples and patients data collection), and carried out at the laboratory of biology and molecular genetics of the CHU Ibn Badis Constantine (for DNA extraction), and at the laboratory of molecular biology of the nature and life science faculty (SNV Faculty) at the University of Brothers Mentouri Constantine 1 (for PCR). Our study was performed between March 2019 and April 2019

# 1- Subjects recruitment

## 1-1 Patients recruitment

We enrolled a population of 30 male patients diagnosed with PCa.

## 1-2 Controls recruitment

We enrolled a population of 30 age-matched presumably healthy men from the biology and molecular genetic laboratory DNA bank.

Before collecting blood samples from both patients and controls, these subjects should respond to our inclusion and exclusion criteria.

- **Inclusion criteria**

**Patients**: male subjects diagnosed with prostate cancer.

**Controls**: male subjects presumably healthy.

- **Exclusion criteria**

Patients who received a blood transfusion.

Patients diagnosed with BPH or other prostate conditions.

Controls with a family history of prostate cancer.

In addition to blood sample, every participant in our study is submitted to a survey accompanied with a written informed consent (Appendix 2 and 3), for the purpose of identify implication of genetic risk factors such as family history, and environmental risk factors such as life style and smoking. For the gathering of patient's clinical information's, we performed an analysis of every patient medical file.

# 2- Methods

## 2-1 Blood samples collection

The collection of blood samples was performed under sterile conditions and collected in EDTA-containing tubes (Ethylene Diamine Tetraacetic Acid) which is an inhibitor of the DNase enzymes and nucleases and preserve molecular integrity of DNA. To extract a good

amount of DNA, the blood sample of each patient was made in two 5ml tubes, stored at +4° C for up to 10 days.

### 2-2 DNA extraction

The extraction of the DNA consists of the isolation of the pure DNA from the blood leukocytes of each individual. The ideal extraction method should fit the following criteria: it should be sensitive, consistent, quick, and easy to use also the quality and quantity of genomic DNA extracted from blood samples is a key feature most facilities consider when choosing a protocol. During this study, we used the NaCl extraction method (the salting out method).

#### 2-2-1 NaCl extraction method

The DNA of each subject was extracted from peripheral blood leukocytes collected in an EDTA-containing tube, following the salting out method. The leucocytes are separated from the total blood by hypotonic lysis and then treated with sodium sulphate detergent (SDS) and proteinase K, which leads to the releasing of the nuclear DNA into the medium. The DNA ball is formed in the supernatant by precipitation with ethanol. The DNA is solubilized in the aqueous phase. For the detailed NaCl extraction protocol look in appendix 4.

### 2-3 *MDM2* promoter p1 del1518 polymorphism genotyping

The genotyping of 40-bp ins/del polymorphism (del1518) of *MDM2* was performed by using PCR (Polymerase Chain Reaction). After the realization of PCR, the results are verified by Agarose gel electrophoresis, and then observed under UV light.

#### 2-3-1 Reaction medium (mixture) preparation

For this step, the reagents used in the PCR must be diluted first according to the following formula:

$$C1 \times V1 = C2 \times V2$$

Knowing that:

C1: initial concentration of each reagent (mentioned on the tube).
V1: initial volume required for dilution (unknown).
C2: final concentration (mentioned in the protocol used).
V2: final volume (depending on the number of tubes).

So:

$$\mathbf{V1 = C2 \times V2 / C1}$$

### 2-3-2 PCR

It's a method used widely in molecular biology. Allows to make many copies of a specific DNA segment. Using PCR, copies of DNA sequences are exponentially amplified to generate thousands to millions of more copies of that particular DNA segment. Each cycle represents a succession of three reactions: denaturation of the DNA strands, hybridization of the primers and elongation. The preparation of PCR mixture was done as below (Table 1).

**Table 1:** Mixture preparation

| PCR mixture reagents | For 1 sample (µL) |
|---|---|
| **dNTP** | 1.6 |
| **F primer** | 1 |
| **R primer** | 1 |
| **Buffer 10X** | 1 |
| **Taq polymerase 5U** | 0.08 |
| **Distilled water** | 4.02 |
| **$MgCl_2$** | 0.3 |
| **DNA** | 1 |
| **Total** | 10 |

The used *MDM2* gene primers sequences are:

**Forward primer** (F oligo): 5'- GACCACTATGTTTAAGGAAG 3'
**Reverse primer** (R oligo): 5'- TGACTCACCTACTTTCCCAC 3'

After the preparation of the PCR mixture, we took 9 µl of this mixture with 1 µl DNA for each reaction. Then we ran PCR cycling using thermocycler and the following cycling conditions (Table 2).

**Table 2**: PCR cycles programming

| N° of cycles | Steps | T°C | Duration |
|---|---|---|---|
| **1** | Initial denaturation | 95 | 4 min |
| **30** | Denaturation | 95 | 30 sec |
| | Hybridization | 56 | 30 sec |
| | Elongation | 72 | 30 sec |
| **1** | Final elongation | 72 | 4 min |

### 2-3-3 PCR control

The PCR products were controlled onto 2% agarose gel using electrophoresis. Agarose gel was prepared using the following ingredients (Table 3). Then after agarose gel preparation, we put it in the electrophoresis system and ran the migration.

**Table 3**: Agarose gel preparation

| Compositions of agarose gel | Quantities |
|---|---|
| **Agarose** | 2 g |
| **TBE (Tris/Borate/EDTA) 1X** | 100 µl |
| **EtBr (Ethidium Bromide)** | 10 µl |

After migration, the amplification products were (Fig. 9).

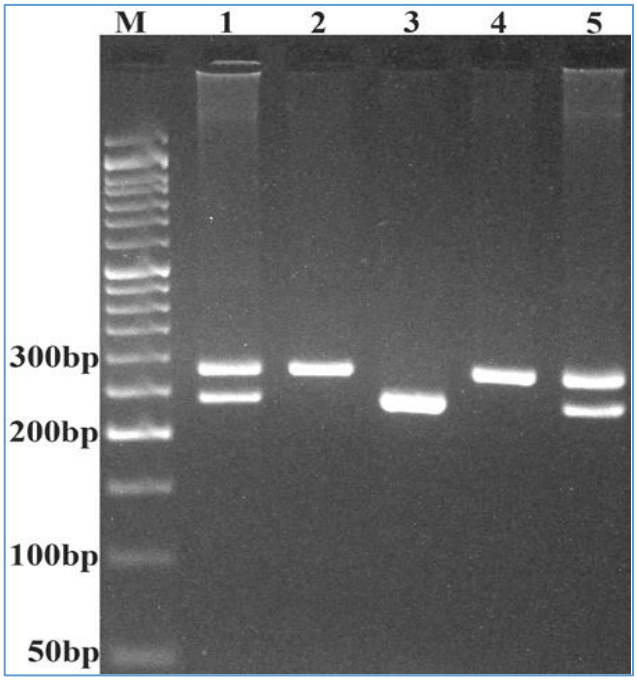


**Fig. 9:** Electrophoresis pattern of the PCR product for the *MDM2* 40-bp I/D polymorphism. M: 50 bp DNA marker; lanes 1 and 5: I/D; lanes 2 and 4: I/I; lane 3: D/D. I: insertion, D: deletion (Hashemi et al., 2017).

### 2-4 Statistical Analysis

We used in this statistical analysis multiple tests and methods to determine existence of any significative association between prostate cancer and different factors such as advanced age, smoking, family history, *MDM2* del1518 polymorphism (rs3730485), etc.). We used Microsoft excel and Epi info 6.0 version to process subjects information and calculate statistical data.

### 2-4-1 Mean and standard deviation

Sample mean is computed from a collection (the sample) of data on one or more random variables, it is an estimator of the population mean where the term population refers to the set from which the sample was taken. It is calculated as the formula below:

$$\bar{X} = \sum ni\ xi / N - 1$$

Where:

$\bar{X}$**:** mean

xi: variable

ni: size value

N: total size of sample

Standard deviation is a measure that is used to quantify the amount of variation or dispersion of a set of data values. In case of samples we use sample standard deviation, which compute an estimate of the population's standard deviation, we can calculate it using the formula below:

$$\sigma = \sqrt{\sum} (xi - \bar{X})^2 / N - 1$$

Where: σ: standard deviation

The quantitative variables are represented as mean ± standard deviation.

### 2-4-2 Odds ratio

An odds ratio (OR) is a measure of association between an exposure and an outcome. Odds ratios are most commonly used in case-control studies, however they can also be used in cross-sectional and cohort study designs as well (with some modifications and/or assumptions). Odds ratios are used to compare the relative odds of the occurrence of the outcome of interest (e.g. disease or disorder), given exposure to the variable of interest (e.g. health characteristic, aspect of medical history). The odds ratio can also be used to determine whether a particular exposure is a risk factor for a particular outcome, and to compare the magnitude of various risk factors for that outcome. We calculated odds ratios (OR) by using a two-by-two contingency table (Table 4).

**Table 4:** Contingency table

| | Patients | Control | Total |
|---|---|---|---|
| **Exposed** | a | b | a+b |
| **Unexposed** | c | d | c+d |
| **Total** | a+c | b+d | a+b+c+d |

Where:

a = Number of exposed patients

b = Number of exposed controls

c = Number of unexposed patients

d = Number of unexposed controls

odd ratio is calcuted by the formula below:

$$OR = a/c \,/\, b/d = a*d / b*c$$

If:

OR=1: Exposure does not affect odds of outcome

OR>1: Exposure associated with higher odds of outcome

OR<1: Exposure associated with lower odds of outcome

Calculating 95% confidence intervals with the formula below:

Upper 95% CI = e ^ [1n (OR) + 1.96 √(1/ a + 1/ b + 1/ c + 1/ d)]

Lower 95% CI = e ^ [1n (OR) - 1.96 √(1/ a + 1/ b + 1/ c + 1/ d)]

### 2-4-3 P-value

The P-value is defined as the probability under the assumption of no effect or no difference (null hypothesis), of obtaining a result equal to or more extreme than what was actually observed. The P stands for probability and measures how likely it is that any observed difference between groups is due to chance. Being a probability, P can take any value between 0 and 1. Values close to 0 indicate that the observed difference is unlikely to be due to chance, whereas a P-value close to 1 suggests no difference between the groups other than due to chance.

Five different genetic models were constructed to determine pooled ORs in accordance with the assumed genetic effect of the D allele including: allele model (D versus I), co-dominant model (DD versus II), heterozygous model (ID versus II), dominant model (DD + ID versus II) and recessive model (ID + II versus DD).

$P<0.05$ was considered to indicate a statistically significant difference.

In our study, we performed a study analysis of environmental, genetic and biologic criterias in our sample of 30 pateints such as age, smoking, occupation, family history, total PSA rate, and gleason score and their association with PCa, in order to better understand the disease's etiology.

# 1- Case study analysis

## 1-1 Age

Our sample, is subdivided according to age groups of 5 years (Table 5):

**Table 5**: Distribution of patients according to their age

| Age | n | % |
|---|---|---|
| [50-54] | 1 | 3.33 |
| [55-59] | 0 | 0 |
| [60-64] | 2 | 6.67 |
| [65-69] | 9 | 30 |
| [70-74] | 11 | 36.67 |
| [75-79] | 5 | 16.67 |
| [80-84] | 1 | 3.33 |
| [85-89] | 1 | 3.33 |
| Total | 30 | 100 |

In our study, the mean age of our sample is 73.28 ± 6.34 years with extreme values of 53 and 86 years, the disease was more frequent in [70-74] years old age group with a rate of 36.67% (11 cases) followed by [65-69] years old age group with a rate of 30% (9 cases), on the age group [75-79] years old with a rate of 16.67% (5 cases) is found in third place. In younger age [50-54] and older age [85-89], only 1 case was found with a rate of 3.33%. We didn't find any patients in the group age [55-59].

Our results show that prostate cancer occurrence begins at the age of 50 and increase dramatically after 60 years which are close to those of Pernar et al. (2018) that explained the noticeable increase of prostate cancer incidence rate after 55 years old. In addition, Malik et al. (2018) have found that both PCa incidence and mortality rates increase with age, specifically above the age of 60 years, and considered increased age as a strong risk factor for PCa. Moreira and Abern (2018) also show that older age is associated with prostate cancer.

As for Hamdi Cherif et al. (2015) they have indicated that the median age of diagnosis of patients is 71 years in the Algerian population, which are almost identical to our results.

### 1-2 Smoking

The comparison between smokers and non-smokers (Fig. 10) reveals that the majority of patients with PCa are smokers, with a high frequency (smokers and former smokers) of 73.33% (22 cases), while the frequency of non-smokers represents 26.67% (8 cases).

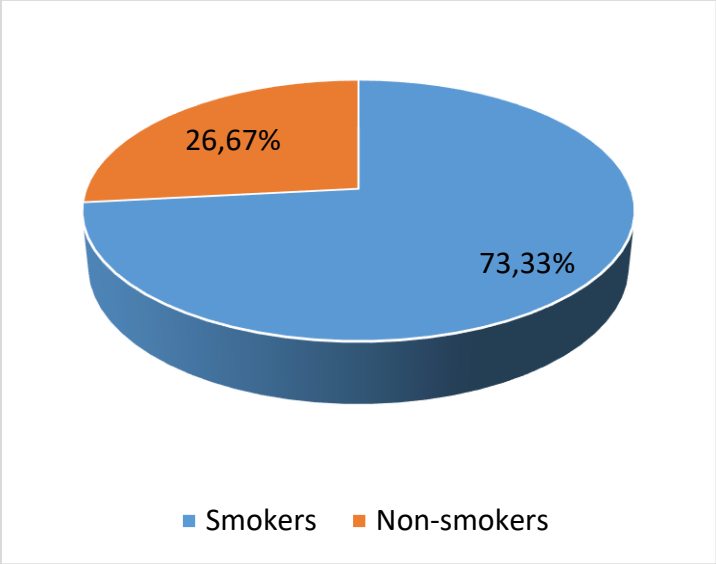


**Fig. 10:** Distribution of smoking and non-smoking patients

The obtained results from the comparison are similar to those of Huncharek et al. (2010) which showed an association of smoking with prostate cancer incidence and mortality. Pernar et al. (2018) showed also that smokers had 60% higher risk of PCa occurrence than men who never smoked and those who quit it 10 years or more before diagnosis. Moreover Kenfield et al. (2011) found that smokers have an increased risk of dying from PCa and may be diagnosed with PCa at more advanced age than non-smokers, hence cigarettes increase PCa mortality. Malik et al. (2018) revealed a statistically significant association between numbers of cigarettes smoked per day and advanced stage of prostate cancer. However, in spite of all this, Giles (2017) indicate that the association between PCa and smoking is still unclear.

### 1-3 Family history

We performed a distribution of patients according to family history (Table 6).

**Table 6:** Distribution of patients according to the presence of family history.

| | n | % |
|---|---|---|
| **None** | 25 | 83.33 |
| **1st degree** | 4 | 13.33 |
| **2nd degree** | 1 | 3.34 |
| **Total** | 30 | 100 |

Of the 30 patients recruited, 83.33% (25 cases) have no family history, 13.33% (4 cases) have at least one 1st degree relative with PCa diagnosed. While only one patient has 2nd degree relative diagnosed with PCa with a rate of 3.34%. Of the 4 cases that have first degree relative with PCa, one patient have one 1st degree family member and one have 2nd degree family member, his father and his uncle, that were affected with PCa, which suggest that they may have the familial form of prostate cancer in their paternel side of the family, and that other non affected family member have two-fold higher risk of developing PCa. The majority of patients with family history (75% of cases) have their father affected with PCa (3 cases).

Our findings shows that the sporadic form of PCa is the most common among prostate cancer patients. This results are similar to those of Abdel-Rahman (2019), Bratt et al. (2016), Giri and Beebe-Dimmer (2016) and Randazzo et al. (2016) which found that 5-15% of PCa cases were familial and hereditary cases, and that the sporadic form of the PCa is majoritary in PCa incidence. While, Randazzo et al. (2016) remarked that 72.5% of patients with family history have their father diagnosed with PCa which are in accordance with our results.

### 1-4 Occupation

A distribution of patients by profession was also performed (Table 7).

**Table 7:** Distribution of patients according to their occupation

| Profession | n | % |
|---|---|---|
| **Administration employee** | 7 | 23.33 |
| **Baker** | 2 | 6.67 |
| **Builder** | 1 | 3.33 |
| **Bus driver** | 1 | 3.33 |
| **Chef cooker** | 1 | 3.33 |
| **Company manager** | 1 | 3.33 |
| **Farmer** | 1 | 3.33 |
| **Firefighter** | 1 | 3.33 |
| **Mechanician** | 2 | 6.67 |
| **National army administrator** | 1 | 3.33 |
| **Security guard** | 3 | 10 |
| **Teacher** | 3 | 10 |
| **Trader** | 3 | 10 |
| **Waiter** | 1 | 3.33 |
| **Undefined** | 2 | 6.67 |
| **Total** | 30 | 100 |

In 30 patients, we found that administration employees are the most affected with PCa among our population (sample) with a rate of 23.33% (7 cases) followed by teachers, traders, and security guards with a rate of 10% (3 cases) for each. Afterward mechanicals and bakers come in 3$^{rd}$ place with a rate of 6.67% (2 cases) for each one. Builder, firefighter, waiter, company manager, chef cooker, bus driver, farmer and national army administrator comes last with a rate of 3.33% (1 case) for each. These results highly suggest that white collar occupations such as administration workers are implicated in PCa occurrence.

These findings are similar to those of Sauvé et al. (2016) and Sritharan et al. (2017) which found that administration related jobs have higher risk rate of PCa. Blanc-Lapierre et al. (2017) have similar findings for white collar and administrative related jobs, and have found that these jobs are related to highly-continued stress (chronic), long working hour and the very demanding nature of this jobs which may explain the association between these occupations and the high risk of PCa (both high and low grade of PCa). Stress induce

hormones such as testosterone, cortisol and some hormones implicated in neuroendocrine pathway which contribute to PCa development (Blanc-Lapierre et al., 2017). However, Giles (2017) shows that large literature exists on occupational associations with PCa, but very little has been firmly established. In addition, Pernar et al. (2018) stated that the mechanism through which occupation may alter prostate cancer risk is yet unclear but may act through changes in sex hormone levels.

## 1-5 Biological and clinical criteria

### 1-5-1 Total PSA rate

**Table 8:** Distribution of patients according to their PSA values.

| **Total PSA rate** | **n** | **%** |
|---|---|---|
| **PSA ≤ 10** | 5 | 20.83 |
| **10 < PSA < 100** | 12 | 50 |
| **PSA ≥ 100** | 7 | 29.17 |
| **Total** | 24 | 100 |

P.S: 6 patients don't have PSA value.

In our study population, 50% of our population (12 patients) have PSA value which varies between (10 < PSA < 100), 29.17% of patients (7 patients) have a very high level of PSA (PSA ≥ 100ng / ml), and only 20.83% of patients (5 patients) have total PSA levels below 10 ng / ml (Table 8). The results indicate that the vast majority of our sample (79.17%) have high total PSA levels (total PSA > 10). These results are identical to those of Descotes et al. (2019) showing that high PSA value is clearly associated with an increased risk of prostate cancer. Mcclure et al. (2018) also publish that the vast majority of men with elevated PSA levels (>30 ng/mL) have prostate cancer. Nevertheless, our findings are different from those of Yu-Rong Lin et al. (2014) who found that PSA was not the ideal predictor of PCa for men with a PSA of (2.5–10.ng/ ml) or (10.1–20 ng /ml) in Chinese men. In addition, if there are elevated levels of PSA in blood, then an abnormal condition is present within the prostate. This abnormality may be caused by trauma to the prostate, infection, benign enlargement, or prostate cancer (McClure et al., 2018).

### 1-5-2 Gleason score

To evaluate the aggressiveness of prostate cancer cells, the Gleason score has been reported in anatomopathology reports.

**Table 9:** Distribution of patients according to their Gleason score

| **Gleason score** | n | % |
|---|---|---|
| **2 ≤ SG ≤ 5** | 0 | 0 |
| **6 ≤ SG ≤ 7** | 8 | 42.11 |
| **8 ≤ SG ≤ 10** | 11 | 57.89 |
| **Total** | 19 | 100 |

P.S: 11 patients without Gleason score

In our sample, 57.89% of patients (11 patients) have undifferentiated and aggressive adenocarcinomas (8 ≤ SG ≤ 10), 42.11% of patients (8 patients) have moderately differentiated adenocarcinomas with intermediate aggressiveness (6 ≤ SG ≤ 7), while well-differentiated and mildly aggressive adenocarcinomas (2 ≤ SG ≤ 5) are not diagnosed in any patient of our sample.

Our results are similar to those of Epstein et al. (2016) who noted that Gleason scores 2 to 5 has virtually disappeared from current clinical practice. He demonstrated that from 1996-2000 to 2005, reported Gleason scores 2 to 4 decreased from 2.7% to 0% and reported Gleason score 5 decreased from 12.2% to 0.3%. Our results suggest that, the more Gleason score is high the more PSA is high and thus the more the tumour is aggressive according to MCclure et al. (2018) who showed that high-risk prostate cancer is associated with Gleason score 8–10.

## 2- Case-control study of the genotypic and allelic profiles of *MDM2* promoter p1 del1518 polymorphism (rs3730485)

Our molecular study aims to search for possible association between *MDM2* promoter p1 del1518 polymorphism (rs3730485) and prostate cancer susceptibility.

The patients and controls electrophoresis profile of *MDM2* amplification products by PCR has showed two bands: the ins band by the size of 287 bp and the del band by the size of 247 bp, and different combination of this bands in patients and controls according to their

genotypes: the heterozygote genotype ins/del which have two heterogeneous ins and del bands together, the homozygote genotypes del/del and ins/ins which have only one band: the ins or del band (Fig. 11).

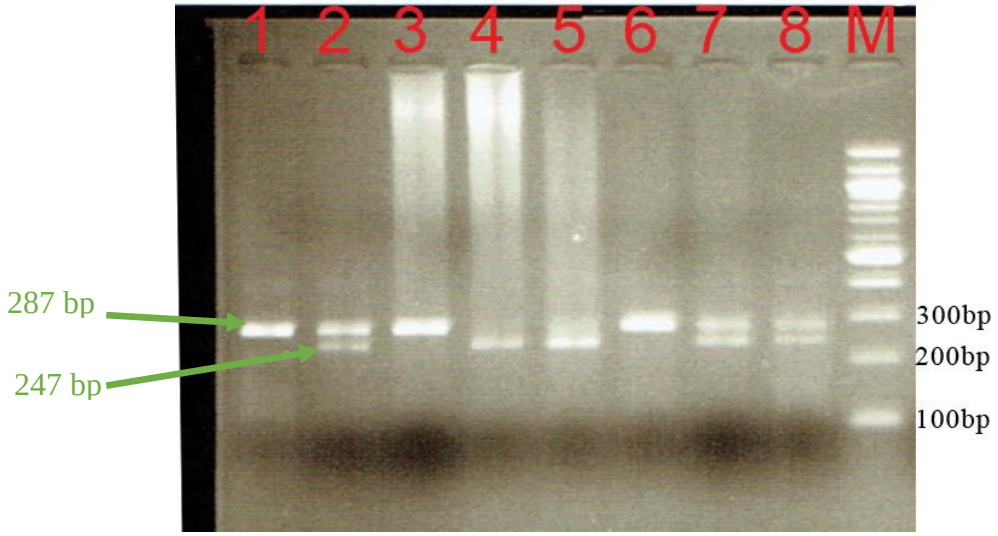


**Fig. 11:** Electrophoresis profile of the PCR products of *MDM2* del1518 polymorphism. M: 100 bp marker; 1-8: subjects.

The genotype and allele frequencies of *MDM2* ins/del polymorphism in prostate cancer patients and healthy subjects are shown in table 10.

**Table 10**: Genotypic and allelic frequencies 40-bp ins/del polymorphism of *MDM2* in prostate cancer patients and control subject.

| ***MDM2* 40-bp ins/del** | **Prostate cancer n (%)** | **Control n (%)** | **OR (95% CI)** | ***P* value** |
|---|---|---|---|---|
| **Co-dominant** | | | | |
| ins/ins | 8 (36.36) | 10 (37.04) | | |
| ins/del | 9 (40.91) | 13 (48.15) | 0.87 (0.20-3.67) | 0.92 |
| del/del | 5 (22.73) | 4 (14.81) | 2.50 (0.44-15.2) | 0.41 |
| **Dominant** | | | | |
| ins/ins | 8 (36.36) | 10 (37.04) | | |
| ins/del + del/del | 14 (63.64) | 17 (62.96) | 1.03 (0.27-3.22) | 0.80 |
| **Recessive** | | | | |
| ins/ins + ins/del | 17 (77.27) | 23 (85.19) | | |
| del/del | 5 (22.73) | 4 (14.81) | 0.59 (0.11-3.09) | 0.73 |
| **Alleles** | | | | |
| ins | 25 (56.82) | 33 (61.11) | | |
| del | 19 (43.18) | 21 (38.89) | 1.19 (0.49-2.91) | 0.82 |

Distribution of genotypic frequencies of *MDM2* del1518 polymorphism shows that the heterozygous genotype ins/del is the most frequent in both patients and controls, with a genotypic frequency of 40.91% for patients and 48.15% for controls. Next, the homozygote genotype ins/ins is the second in both populations, with a genotypic frequency of 36.36% in patients and 37.04% in controls. Lastly, the homozygote genotype del/del is found with a genotypic frequency of 22.73% in patients and 14.81% in controls.

Distribution of allelic frequencies of *MDM2* del1518 polymorphism indicate that the ins allele is found in the majority of patients and control altogether, with allelic frequency of 56.82% and 61.11%, respectively. While, del allele is found in less than half in both populations with 43.18% for patients and 38.89% for controls.

The odds ratio and p-value values show no significant results which strongly suggest that there is no association between *MDM2* del1518 polymorphism and prostate cancer susceptibility in any genetic model (Table 10).

Our results are in agreement with several studies: Moazeni-Roodi et al. (2019) showed that there is no significant association between indel variant and PCa in Asian and Caucasian population. Furthermore, Gansmo et al. (2016) have found that *MDM2* del1518 polymorphism and prostate cancer occurrence are not associated in Caucasians, African Americans and Chinese. The same with Hua et al. (2017) that failed to prove a relation between *MDM2* del1518 polymorphism and prostate cancer risk. However, Hashemi et al. (2017) established an association between *MDM2* del1518 polymorphism and susceptibility in Iranian population.

It appears that *MDM2* gene is polymorphic, and the *MDM2* promoter P1 del1518 polymorphism is implicated in *MDM2* transcriptional activity alteration. The overexpression of *MDM2* gene lead to the degradation of *TP53* which may cause tumorigenesis.

Although we didn't find an association between *MDM2* promoter P1 del1518 polymorphism and prostate cancer susceptibility. We suggest that *MDM2* del1518 polymorphism is conserved in our population. On the other hand, our sample is relatively small, so our results aren't conclusive; we propose to raise sample size so the results will may be more conclusive and have significant outcome in the Algerian population.

Prostate cancer is a major burden for all men around the world and a public health issue especially in older men (>50 years). In Algeria, PCa is the most widespread cancer in men after 50 years, which mean that understanding its etiology, pathology, epidemiology, and associated risk factors and genetic is a necessity to a better diagnostic, treatment and management of this disease, and even to help prevent it in the future. The PCa as a disease is remarkably heterogenic and multifactorial which make it a complex disease.

Our case-control study enrolled two populations: a diseased population of 30 patients and a presumptively healthy population of 30 controls, allowed us to identify risk factors involved in prostate cancer appearance, such as advanced age (>60 years), smoking, family history and occupation impact. Thereby, our study enabled us to conclude that prostate cancer sporadic form is most prevalent of all PCa cases which occur after the age of 60 years. Moreover, our results show that the majority of prostatic adenocarcinomas are aggressive (Gleason score between 8 and 10, PSA>10 ng/ml) and are limited (localized) to the prostate only. This is due to the fact that PCa spread slowly over the years, and discovered generally in late stage of the disease, which make it a silent killer if not diagnosed in early stages.

Our research work also intent to explore possible correlation between the *MDM2* promoter P1 del1518 polymorphism (rs3730485) and prostate cancer. Our molecular study of del1518 polymorphism in 22 patients diagnosed with PCa and 27 controls, concluded that this latter do not seem to be involved in the manifestation of prostate cancer. Nonetheless, our sample size is small, which did not allow us to obtain conclusive results.

**Perspective**

1- Increasing the sample size in order to draw consistent conclusions about the association of *MDM2* del1518 polymorphism with prostate cancer.
2- Trying other polymorphisms of *MDM2* like SNP309, or other genes implicated in PCa such as *GSTP1, TMPRSS2: ERG* gene fusion and *TP53*.

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**Appendix 1:** TNM Classification (Sobin et al., 2009).

**Primitive tumor (T)**

- Tx Tumor not assessable.
- T0 No detectable tumor.
- T1a Fortuitous Histological Finding Tumor Occupying 5% or Less of Tissue resected.
- T1b Incidental Histological Finding Tumor Occupying More than 5% of Resected Tissue.
- T1c Tumor discovered by biopsy puncture during elevation of PSA.
- T2a Tumor limited to half a lobe.
- T2b Tumor interesting more than half of a lobe and limited to a lobe.
- T2c Tumor interesting both lobes.
- T3a Extra unilateral or bilateral capsular extension.
- T3b Tumor invading the seminal vesicle (s).
- T4 Tumor fixed or invasive of structures other than seminal vesicles: cervix bladder, external sphincter of the bladder, rectum, anus and / or wall elevating muscles Pelvic.

**Regional Ganglions (N)**

- Nx Unscreened pelvic ganglia.
- N0 Lack of pelvic lymph node involvement.
- N1 Regional lymph node metastasis.

**Remote metastasis (M)**

- Mx Non evaluable metastases.
- M0 No distant metastasis.
- M1 Remote metastasis.
- M1a Extra pelvic ganglion metastases.
- M1b Bone metastases.
- M1c Metastases to other sites.

**Appendix 2:** Common genetic aberrations in prostate cancers and their biological functions (Wang et al., 2018).

| Gene | Genomic alterations | Locus | Altered frequency (The Cancer Genome Atlas Research Network 2015) | Biological function in prostate cancer | References |
|---|---|---|---|---|---|
| APC | Deletion | 5q22.2 | 5.0% | Antagonist of the Wnt signaling pathway; also involved in other processes, including cell migration and adhesion, transcriptional activation, and apoptosis | Grasso et al. 2012 |
| AR | Amplification/ mutations/ splicing variants | Xq12 | 1.2% | A steroid hormone-activated transcription factor, which remains important in development; amplification and mutations of AR contribute to the progression of prostate cancer and the failure of ADT by allowing constitutive activation of the AR pathway | Taplin et al. 1995; Visakorpi et al. 1995 |
| ATM | Deletion/ mutation | 11q22.3 | 7.0% | One of the master controllers of the cell cycle checkpoint signaling pathways that are required for cell response to DNA damage and for genome stability | Pritchard et al. 2016; Fraser et al. 2017 |
| BRCA1<br>BRCA2 | Deletion/ mutation | 17q21.31<br>13q13.1 | 1.2%<br>3.0% | Play key roles in transcription, DNA repair of double-stranded breaks, and recombination. | Mateo et al. 2015<br>Robinson et al. 2015 |
| CHD1 | Deletion | 5q21.1 | 7.0% | Involved in transcription-related chromatin remodeling but also required to maintain a specific chromatin configuration across the genome; CHD1 cooperation with H3K4me3 regulates NF-κB pathway gene transcription | Barbieri et al. 2012; Burkhardt et al. 2013; Zhao et al. 2017 |
| ERF | Deletion/ mutation | 19q13.2 | 1.5% | Transcriptional repressor that binds to E26 transformation-specific 2 (ETS2) promoter; ERG competes with ERF to bind DNA at consensus ETS sites | Bose et al. 2017; Huang et al. 2017 |
| ERG<br>ETS2<br>ETVs | Fusion/deletion<br>Deletion<br>Fusion/deletion | 21q22.2<br>21q22.2<br>NA | 46.0%<br>14.0%<br>29.0% | ETS activation enhances tumorigenesis through broad mechanisms, including lineage specification, genome instability, epigenetic alterations, and metabolism remodeling | Tomlins et al. 2005<br>Grasso et al. 2012<br>Sizemore et al. 2017 |
| EZH2 | Mutation | 7q36.1 | 0.6% | Acts a coactivator for critical transcription factors, including AR | Xu et al. 2012b |
| FOXA1 | Mutation | 14q21.1 | 6.0% | Required for epithelial cell differentiation in murine prostate and promotes cell cycle progression in CRPC | Zhang et al. 2011a; Barbieri et al. 2012 |
| IDH1 | Mutation | 2q34 | 1.2% | IDH1 mutant subtype shows strongly elevated levels of genome-wide DNA hypermethylation | The Cancer Genome Atlas Research Network 2015 |
| KMT2A (MLL1)<br>KMT2C (MLL3)<br>KMT2D (MLL2) | Mutation/deletion | 11q23.3<br>7q36.1<br>12q13.12 | 2.4%<br>5.0%<br>4.0% | Process histone methylation and involved in transcriptional coactivation | Malik et al. 2015<br>Robinson et al. 2015<br>Beltran et al. 2016b |

*Continued*

| Gene | Genomic alterations | Locus | Altered frequency (The Cancer Genome Atlas Research Network 2015) | Biological function in prostate cancer | References |
|---|---|---|---|---|---|
| KDM1A (lysine-specific demethylase 1 [LSD1]) | Mutation/deletion | 1p36.12 | 1.5% | Process histone demethylation and involved in transcription, acting as coactivators or corepressors, depending on the context | Sehrawat et al. 2018 |
| KDM3A (JMJD1A) | | 2p11.2 | 1.8% | | Fan et al. 2018 |
| KDM6A (UTX) | | Xp11.3 | 4.0% | | |
| MYC | Amplification | 8q24.21 | 8.0% | Contributes to prostate cancer by directly activating the transcription of protumorigenic factors involved in cell growth and proliferation | Jenkins et al. 1997; Ellwood-Yen et al. 2003 |
| MYCN | Amplification | 2p24.3 | 0.6% | Overexpressed or amplified in ~40% of NEPCs; a driver of NEPC initiation | Beltran et al. 2011; Dardenne et al. 2016; Lee et al. 2016b |
| NCOR1 | Deletion/ mutation | 17p11.2 | 3.0% | AR corepressors | Hodgson et al. 2005 |
| NCOR2 | | 12q24.31 | 3.0% | | Taylor et al. 2010 |
| NKX3-1 | Deletion | 8p21.2 | 17.0% | A PSA-regulated homeobox gene; a tumor suppressor controlling tumorigenesis, cell proliferation, and invasion activities in prostate cancer | He et al. 1997; Bhatia-Gaur et al. 1999 |
| PTEN | Deletion/ mutation | 10q23.31 | 17.0% | Suppresses the PI3K–AKT–mTOR pathway to regulate cell survival, proliferation, and energy metabolism | Wang et al. 2003; Barbieri et al. 2012; Grasso et al. 2012 |
| RB1 | Deletion/ mutation | 13q14.2 | 0.9% | A negative regulator of the cell cycle; stabilizes constitutive heterochromatin to maintain the overall chromatin structure | Beltran et al. 2016; Ku et al. 2017 |
| SETD2 | Deletion | 3p21.31 | 3.0% | Histone methyltransferase that trimethylates H3K36 and activates transcription | |
| SETDB1 | Amplification | 1q21.3 | 1.8% | Histone methyltransferase that trimethylates H3K9 and represses transcription | |
| SMAD4 | Deletion/ mutation | 18q21.2 | 3.0% | Tumor suppressor; acts as a downstream effector of the TGFβ pathway, regulates gene transcription, inhibits epithelial cell proliferation, and remodels the TME | Ding et al. 2011; Wang et al. 2016a |
| SMARCA1 | Deletion/ mutation | Xq26.1 | 2.1% | Components of the SWI/SNF complex, which has been shown to drive prostate tumorigenesis | |
| SMARCB1 | | 22q11.23 | 1.2% | | |
| SPOP | Mutation | 17q21.33 | 12.0% | Component of a BTB–CUL3–RBX1 E3 ubiquitin–protein ligase complex; SPOP mutants cause stabilization of oncogenic substrates such as JNK, NCOA3, DEK, and BET family proteins | Barbieri et al. 2012; Theurillat et al. 2014; Blattner et al. 2017 |
| TP53 | Deletion/ mutation | 17p13.1 | 8.0% | Responds to diverse cellular stresses to regulate expression of genes involved in cell cycle arrest, apoptosis, senescence, DNA repair, or changes in metabolism | Barbieri et al. 2012; Beltran et al. 2016b; Mu et al. 2017 |

**Appendix 3:** The survey

| | | |
|---|---|---|
| Date: / / | Department: | |
| **Identity** | | |
| Last name: | First name: | Age: |
| Phone number: | Address: | Profession: |
| Origin: | Weight: | Children number: |
| **Lifestyle and risk factors** | | |
| **Smoking:** | yes | no |
| Cigarettes number: | Packets number: | Duration: |
| | | Former smoker since: |
| **Obesity:** | yes | no |
| **Stress:** | Domestic problems: | Occupational problems: |
| **PCa family history:** | yes | no |
| **Clinical information** | | |
| **Related conditions** | | |
| **PCa diagnosis age:** | | |
| **Tumor:** | localized | non localized |
| **PSA:** | ng/ ml | |
| **DRE:** | | |
| **Gleason Score:** | | |
| **TNM Classification:** | | |
| **Scintigraphy:** | | |
| **Prostatectomy:** | no | Yes in: |
| **Current therapeutics intake:** | Under chemotherapy | Under hormonotherapy |

**Appendix 4:** Informed consent form for participation in a study

I, the undersigned, Mr., ……. Certify that I have received the information note concerning the study above.

It has been made clear to me that I am completely free to accept or refuse to participate in this research.

I certify that I understood the objective and the modalities of this study. I give my consent to participate in this study.

On the genetic tests performed from the blood that was taken from me on ……

Patient signature

In the absence of reading and writing autonomy of Mr. ……, the third party identified below, attests to have personally and faithfully informed the patient, about the information note and this consent form, and thus agrees to sign for this consent form.

*Third party*

Mr/ Mrs ……

Signature

Investigator signature

**Appendix 5:** The NaCl extraction method

## 1. Hemolysis of blood and preparation of leukocyte pellet

The freshly collected blood in the EDTA tubes is vigorously mixed with a hypotonic solution to burst the red blood cells.

The lysis is carried out at + 4 ° C. for 10 to 20 min. Then, the lysate is centrifuged for 10 minutes at 3900 r/m. Once the supernatant is removed, after two washes, a white pellet, consisting essentially of leucocytes, is obtained. The steps were as follows:

1. Put the 10 ml of blood in a 50 ml Falcon tube and make up the volume to 25 ml with Tris EDTA (TE) 20: 5.
2. Leave it for 10 min in the freezer -18 ° C.
3. Centrifuge for 10 min at 3900 g.
4. Remove the supernatant by carefully pouring into a container without detaching the leucocyte pellet contained at the bottom of the wall of the tube.
5. Add the TE 20: 5 to the pellet (make up to 25 ml).
6. Leave for 10 min in the freezer at -18 ° C.
7. Centrifuge under the same conditions as above.
8. Gently pour the supernatant and keep the leucocyte pellet form.

(If we want to stop at this stage, we put the resulting pellet in a 15 ml conical tube with 10: 1 TE for storage at -20 ° C).

## 2. Leukocyte lysis, digestion of the nucleoprotein complex and release of the DNA for the release of the DNA

The dissolution of the leucocyte membranes and the digestion of the proteins associated with this DNA proceed as follows:

1. transfuse the leukocyte pellet in a 15 ml Falcon tube.
2. Add 3 ml of lysis buffer (400 mM NaCl, 2 mM EDTA, and 10 mM Tris, pH 8.2) by dilating the pellet with a sterile pastel.
3. Add 200 µl of 10% SDS (Sodium Dodecyl Sulfate) anionic detergent for leukocyte lysis, nuclease inhibition, protein denaturation and activation of proteinase K.
4. add 100 µl of proteinase K at 10% mg / ml in order to digest all the proteins including the nucleoproteins to release the nuclear DNA.

5. Put the tubes under agitation (wheel) at 37 ° C overnight.
6. The next day, put the tubes in the freezer at -18 ° C to cool their contents for 10 min.
7. add 1 ml of 4M NaCl, and shake vigorously by hand.
8. Leave for 5 min in the freezer at -18 ° C (protein precipitation).
9. Centrifuge for 15 min at 2500 g.
10. transfer the supernatant into a 15 ml falcon tube, add 2 times its volume of absolute ethanol previously cooled (approximately 8 ml) and shake by inverting the tube several times: the DNA ball is formed.
11. Leave for 30 minutes at -20 ° C if the ball is not formed.
12. Wash the ball of DNA twice in 70% ethanol to remove the salts.
13. Gently recover the ball in Eppendorf tubes which must remain open for about 1 hour for the drying the DNA.

**Solubilization:**

1. add between 300 to 1000 µl of TE 10:1 depending on the size of the DNA ball and the desired concentration.
2. Leave overnight on a rotator shaker at 37 ° C., then at + 4 ° C. until complete dissolution during 1 to 2 days.

## Abstract

Prostate cancer is the malignant transformation of prostate gland. It is the most common solid tumor diagnosed among men in the world. Our study was conducted on a sample of 60 people; where 30 patients were diagnosed with prostate cancer and 30 presumed healthy volunteers. The aim of our study is to investigate the possible relationship between the Del1518 polymorphism (rs3730485) of the *MDM*2 gene and prostate cancer using the PCR technique for genotyping.

Our study showed that the mean age of our sample is 73.28 ± 6.34 years. In addition, the frequency of smoking patients (73.33%) is higher than that of non-smokers (26.67%). Also, prostate cancer is sporadic in 83.33% of cases. In terms of occupational distribution, we found that administrative workers are the most affected by prostate cancer, with a rate of 23.33%. Moreover, our results indicated that the vast majority of the population (79.17%) has a high total PSA level (total PSA> 10). In addition, 57.89% (11 patients) had undifferentiated and aggressive adenocarcinoma ($8 \leq SG \leq 10$) according to the Gleason score.
According to the statistical analysis of the genotypic and allelic frequencies of the Del1518 polymorphism (rs3730485) in patients and controls and the calculation of OR and p-value, we can shift the absence of association between the Del1518 polymorphism (rs3730485) of the *MDM*2 gene with prostate cancer, ($p>0.05$).

In conclusion, the Del1518 (rs3730485) polymorphism of *MDM2* gene is not a risk factor for prostate carcinoma in East Algerian population. However, these results remain inconclusive and preliminary and cannot be generalized to our entire population.

**Key words:** Prostate cancer, Age, PSA, Adenocarcinoma, Del1518 (rs3730485) polymorphism, PCR, *MDM2*.

**Résumé**

Le cancer de la prostate est la transformation maligne de la glande prostatique. C'est la tumeur solide la plus répandue parmi les hommes diagnostiqués dans le monde. Notre étude a été réalisée sur un échantillon de 60 personnes ; où 30 patients ont été diagnostiqués avec un cancer de la prostate et 30 témoins présumés en bonne santé. Le but de notre étude est de rechercher d'éventuelle relation entre le polymorphisme Del1518 (rs3730485) du gène *MDM2* et le cancer de la prostate en utilisant la technique de PCR pour le génotypage.

Notre étude a montré que l'âge moyen de notre échantillon est de 73,28 ± 6,34 ans. En outre, la fréquence des patients fumeurs (73,33%) est largement supérieure à celle des non-fumeurs (26,67%). De plus, le cancer de la prostate est sporadique dans 83,33% des cas. Pour ce qui est de la répartition professionnelle, nous avons constaté que les employés administratifs sont les plus touchés par le cancer de la prostate, avec un taux de 23,33%. Nos résultats ont indiqué que la grande majorité de la population (79,17%) a un taux de PSA total élevé (PSA total> 10). 57,89% (11 patients) ont un adénocarcinome non différencié et agressif ($8 \leq SG \leq 10$) selon le score Gleason.

Selon l'analyse statistique des fréquences génotypiques et alléliques du polymorphisme Del1518 (rs3730485) chez les patients et les témoins et le calcul de l'OR et de la *p-value*, nous pouvons déclarer l'absence d'association entre le polymorphisme Del1518 (rs3730485) du gène *MDM2* avec le cancer de la prostate, ($p>0,05$).

Le polymorphisme étudié ne peut être considéré comme un facteur de risque pour la population Est Algérienne. Ces résultats restent préliminaires et ne peuvent êtres généralisés à l'ensemble de notre population.

**Mots clés :** Cancer de la prostate, Age, PSA, Adénocarcinome, polymorphisme Del1518 (rs3730485), PCR, *MDM*2.

سرطان البروستات هو تحول خبيث في غدة البروستات. هو الورم الصلب الأكثر شيوعا بين الرجال في العالم. تم إجراء دراستنا على عينة من 60 شخصًا. حيث تم تشخيص 30 مريضا بسرطان البروستاتا و30 شخص سليم أخذ كشاهد. الغرض من دراستنا هو البحث عن علاقة بين سرطان البروستات وتعدد الأشكال الجيني (rs3730485) Del1518 باستخدام تقنية ال PCR للقيام بالتنميط الجيني.

تشير دراستنا أن متوسط العمر لعيناتنا هو 73،28 ± 6،34 سنة. بالإضافة إلى ذلك، تشير المقارنة بين المدخنين (73،33٪) وغير المدخنين (26،67٪) إلى وجود علاقة قوية بين التدخين وسرطان البروستات. الشكل الفردي لهذا المرض هو الأكثر شيوعا في (83،33 ٪) من الحالات. بالنسبة للتوزيع المهني، وجدنا أن موظفي الإدارة هم الأكثر تضررا من المرض من بين عينتنا بمعدل 23،33 ٪. علاوة على ذلك، تشير نتائجنا إلى أن أغلبية المصابون بالمرض (79،17٪) لديهم مستويات عالية من ال PSA الكلي (PSA> 10). من جهة أخرى (57،89٪) (11 مريض) لديهم سرطان غدي غير متمايز وعدواني ($10 \geq SG \geq 8$).

التحليل الإحصائي للترددات الوراثية والأليلية لتعدد الأشكال الجيني (rs3730485) Del1518 ممثلة بنسبة الاحتمالات وقيمة P يشير إلى عدم وجود علاقة، بين تعدد الأشكال الجيني (rs3730485) Del1518 وسرطان البروستات.

في الختام، تعدد الأشكال الجيني (rs3730485) Del1518 للمورثة *MDM2* ليس عامل خطورة لسرطان البروستات عند سكان الشرق الجزائري. لكن تبقى نتائجنا أولية ولا يمكن تعميمها على كل المجتمع الجزائري.

**الكلمات المفتاحية:** سرطان البروستات، السن، تعدد الأشكال الجيني (rs3730485) Del1518، المورثة *MDM2*، PCR، PSA