Introduction

Prostate cancer, one of the most common forms of cancer in males, is a complex, heterogeneous disease, exhibiting a spectrum of clinico-pathologic presentations. It is the fifth most common cancer in the world and the second most common cancer among men with substantial differences in prevalence in different ethnic groups (1). Despite the high incidence and mortality rates, its etiology is poorly understood.

No single environmental or lifestyle factor has been consistently associated with the risk of prostate cancer. Despite the absence of strong exogenous risk factors, endogenous genetic factors may partly explain the variation in risk between ethnic groups. A better understanding of the role of various gene-gene and gene-environment interactions will enable to establish the genesis of cancer and identify high-risk individuals.

Genetic risk factors include both high penetrance and low penetrance genes; the former contributes to only 5-10% of prostate cancer risk whereas the latter in combination with environmental risk factors may attribute to a greater proportion of prostate cancer risk (2). Thus the association of prostate cancer with the genetic status can result from two kinds of mechanisms. First, a genetic predisposition associated with high risk can explain hereditary prostate cancer in cases of an inherited mutation involving the known hereditary predisposing loci. Secondly, the genetic mechanism associated with prostate cancer risk may result from genetic susceptibility, via individual or ethnic polymorphisms. Identification of susceptibility genes is important especially in cancers like the prostate, which are known to be associated with lifestyle and ethnicity.

The introductory chapter describes the epidemiology and heterogeneity of prostate cancer. The various risk factors have been elaborated with emphasis on the genetic factors. Genetic variations reported in association with prostate cancer have been reviewed.
1.1 Prostate gland -Structure and Functions

Prostate, an exocrine male sex gland, is present beneath the urinary bladder surrounding the urethra. The growth and function of the prostate are regulated by androgens. It has four distinct anatomical zones that have a clinical correlation (3). The peripheral zone represents 70% of the prostatic volume, where 60-70% of the cancers originate. The central zone comprises 25% of the prostate, where inflammatory processes like prostatitis arise. The transitional zone represents only 5% of the prostatic volume where benign prostatic hyperplasia (BPH) originates, and the anterior zone is fibromuscular with no glandular structures (Fig1.1). BPH is only a benign condition of the prostate and is not a premalignant lesion. The prostate nourishes and protects the sperms with an alkaline fluid and controls rate of urine flow from bladder into the urethra.

1.2 Epidemiology of prostate cancer

Incidence of prostate cancer has been increasing steadily in almost all countries and the average increase in the age-adjusted incidence worldwide, between 1985 and 2002, was 1.1% annually. Worldwide, more than 650,000 men are diagnosed with prostate cancer every year, accounting for a tenth of all new male cancers. Age standardised incidence rate of prostate cancer world wide was 25.3 per 100,000 in 2002 (1). Incidence of prostate cancer varies widely and the rates differ by as much as 90-fold between populations. The highest incidence was recorded in North America (119.9 per 100,000) especially in African-Americans, followed by New Zealand /Australians (79.9 per 100,000), Western European (61.6 per 100,000) while the
lowest rates were in Eastern Asia (3.8 per 100,000) (Fig 1.2). In India, the age-standardised incidence rate was 4.6 per 100,000 in 2002 (1). Among all cancers, prostate cancer exhibits the largest differences between incidence and mortality. Fig 1.2 reveals the worldwide incidence and mortality rates of prostate cancer.

Fig 1.2: Worldwide incidence and mortality rates of prostate cancer
Although men in the United States, Western Europe and Australia have the highest incidence rates, their mortality-incidence ratios are lesser than that in the developing
countries. This ratio is affected by the reporting of incidence and mortality, degree of screening, treatment and survival. Mortality is affected by survival, and survival rates are significantly better in high-risk countries. Estimated survival rates in various developed and developing countries are depicted in Fig 1.3. Aggressive screening and improved treatment have increased the survival rates in developed countries (4).

![Fig 1.3 Estimated age adjusted survival % in developed and developing countries](image)

Migrants from low-risk to high-risk countries show a marked increase in prostate cancer incidence. The incidence among Asians who migrate to North America has been reported to increase, although their rates are still lower than their American counterparts (5). The Chinese and Japanese communities residing in North America exhibit higher incidence rates than their Asian counterparts (6). The fact that incidence rates increase significantly in groups who immigrate to North America indicates that diet and life-style play a major role in the etiology of the disease. The United States Surveillance, Epidemiology and End Results (SEER) Program (1997-2001) reported age adjusted incidence rate (per 100,000) of 271 in African Americans
,167 for White Americans and 100 for Asian Americans (7) thus revealing the interethnic variations in incidence. The differences in ethnic-specific risk may be mediated via population differences in genes involved in various pathways associated with the cancer. Thus, the epidemiology of prostate cancer suggests that it is a complex, multifactorial disease involving both genetic and environmental factors.

**Incidence of prostate cancer in India**

In India, Mumbai and Delhi have reported highest incidence rates for prostate cancer. In Chennai, prostate cancer is the fourth most common cancer among men (8). The age adjusted incidence per 100,000 reported in important centers from North India were 7.9 in Mumbai, 5.7 in Delhi and 2.6 in Kolkata. In South India, the incidence rates were 6.1 in Trivandrum, 4.7 in Bangalore and 3.6 in Chennai (9). While the incidence rates may be low in India, studies have reported that 84% of patients in India present with advanced stages and high-grade prostatic intraepithelial neoplasia (10,11), which suggest that early screening for prostate cancer is limited in the Indian population.

The cancer registry of our institute, SRMC &RI while reporting the relative proportion of cancers by specific site reported the highest incidence for prostate cancer (14.67%) in males, followed by the Brain (11.3%), stomach (9%) and others (12). The high incidence of prostate cancer recorded from our institute further stressed the need to identify the genetic variations associated with prostate cancer risk.

**1.3 Heterogeneity of Prostate Cancer**

Clinically detected prostate cancer displays a variety of phenotypic features and malignant potential. Prostate cancer may be an indolent, latent disease without clinical symptoms during the lifetime of an elderly patient, or it may take an aggressive clinical course metastasizing into seminal vesicles, bladder, rectum, lymphnodes, bone and other organs.
It is proposed that the normal prostatic epithelium gives rise to Prostatic Inflammatory Atrophy (PIA) as a result of chronic inflammation. In 1999, De Marzo et al (3) proposed PIA as the precursor to prostatic intraepithelial neoplasia (PIN) and prostate cancer. Inflammatory cells elaborate numerous microbicidal oxidants that might cause cellular or genomic damage in the prostate (13,14). Epithelial cells in the lesions of PIA show molecular signs of stress and DNA damage indicated by high levels of glutathione S transferase P1 (GSTP1) and cyclooxygenase-2 (COX-2). The PIA upon numerous other genomic alterations gives rise to low grade and subsequently High-Grade Prostatic Intraepithelial Neoplasia (HGPIN). Several lines of evidence implicate HGPIN as a preneoplastic lesion (15). The model proposed to explain this carcinogenesis mechanism is shown in Fig 1.4.

Prostate carcinoma is frequently multifocal and several distinct foci of carcinoma and PIN can be found in one prostate varying in the degree of cellular dysplasia, tissue disorganization and genetic alterations. Chromosomal aberrations, gene mutations, variations, aberrant expression of cell cycle regulatory proteins like p16 and p21 (16); signal transduction proteins of Epidermal Growth Factor Receptor (EGFR) family like Her2/Neu (17), cell adhesion molecules like E-cadherin, chondroitin sulphate (18, 19) and angiogenesis factors like VEGF and VEGFR (20) have been variedly reported in cancer progression. Unlike other neoplasms, mutations in oncogenes like bcl2 and c-myc or tumor suppressor genes like p53 and Rb are uncommon in primary prostate cancer and have been observed only in certain advanced stages and androgen independent prostate cancer (21, 22).

Exploring and understanding the molecular mechanisms associated with the development and progression of the disease has been difficult due to this marked heterogeneity and multifocality of the disease. Multistep carcinogenesis is therefore, a widely accepted model for cancer progression, which suggests that latent prostate cancer, as well as putative precursor lesions, such as prostatic intraepithelial neoplasia (PIN), have multiple genetic alterations in several regulatory pathways like cell proliferation, apoptosis, androgen metabolism, carcinogen metabolism, metastasis and so on; leading to the development and progression of prostate cancer. This extreme
variability in the clinical course complicates the diagnosis and therapy of prostate cancer.

1.4 Diagnosis and treatment of prostate cancer

**Digital rectal examination (DRE)** was the principal method of prostate cancer detection and staging. Although DRE allows the clinician to directly evaluate the prostate it only allows the detection of palpable abnormalities, representing usually locally advanced cancers. Currently, the diagnosis is based on elevated level of serum **prostate specific antigen (PSA)**, secreted by epithelial cells of the prostate. Diagnosis is suspected if serum PSA is above 4 ng/ml. However, PSA levels may be increased not only in cancer but also in other prostatic disorders like BPH. Hence PSA level alone does not confirm prostate cancer. Thus diagnosis is suspected if the serum PSA is above the normal, or a suspicious DRE. At present, the diagnosis is confirmed only by **histopathological examination** of the prostate biopsy. As a practical consequence of its heterogeneity, histological grading has been replaced by Gleason grading, which considers the degree of tissue disorganization in two main components of the carcinoma (23).

There are several treatment options for prostate cancer, which need to be adapted to the individual patient. It is curable only in its localized stage for which **radical prostatectomy** or **radiation therapy** are the standard therapies and these are recommended only for patients with a life expectancy of 10-15 years. However, 20-40% of the cancers are diagnosed only in clinically advanced stages, when curative treatment is not possible. For these patients, **hormonal therapy**, which prevents androgen production or androgen action, is usually the effective treatment. Hormone therapy involves either **surgical castration** (orchidectomy) or administration of **Leutinizing Hormone Release Hormone (LHRH) analogues** and **anti-androgens**. About 70-90% of prostate cancers initially respond to hormone therapy and often hormone refractory prostate cancer arises (24). Thus effective therapy for prostate cancer remains elusive.
Given this background, expectations are high for the molecular biology of prostate cancer, which is expected to reveal insights into the mechanism of carcinogenesis and aid in identification of diagnostic and prognostic indicators. Moreover, it is essential to understand the potential risk as well as protective factors associated with prostate cancer so as to identify targets for treatment.

1.5 Risk factors for prostate cancer

Epidemiological studies have identified a number of risk factors. Most investigators suggest that prostate cancer results from interplay between genetic factors, endogenous hormones and environmental influences (25, 26). A better understanding of how these factors interact to cause prostate cancer will facilitate appropriate public health strategies to minimize high-risk factors and maintain protective factors that keep prostate cancer at bay. Despite the substantial morbidity from prostate cancer worldwide, age, ethnicity and family history are the only established risk factors. Evidence on diet, especially animal fat intake, is promising but inconclusive (27).

Data on other risk factors, such as circulating levels of hormones, physical activity, body size, smoking, drinking, sexual behaviour and occupational exposures are conflicting (28). A complicating factor in dissecting the risk factors for prostate cancer is that an individual’s metabolism and response to dietary factors, level of endogenous hormones, changes in hormonal factors as a result of diet may all be influenced by genetic factors as well. The various risk factors as well as preventive factors for prostate cancer are represented in Fig 1.5. Some of these important factors are described in brief.

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![Fig 1.5 Illustration of risk and protective factors in prostate cancer]

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“*A study on genetic polymorphisms associated with prostate cancer risk in South Indian men*”
1.5.1 Age

Incidence of prostate cancer increases with age, more so than any other cancer (29). It is a common malignancy among men in the age group of 50 to 70 years. It is diagnosed in very few people (<0·1%) younger than 50 years of age. The mean age of patients with this disorder is 72–74 years, and about 85% of patients are diagnosed after 65 years. In part, the higher rate of diagnosis in older men may be due to the slow-growing nature of prostate tumors. The tumors may well be present in younger men, but symptoms don't become apparent until many years later. However in familial prostate cancer, the age of onset is usually younger than 55 years.

1.5.2 Race

The number of males diagnosed each year with prostate cancer is not similar among different populations. In the United States the disease is 66% more common and twice fatal among African Americans than the Caucasians. In contrast, Blacks in Africa have lowest rates of prostate cancer worldwide (7). Differences in prostate cancer risk by race may reflect three factors: differences in exposure, such as diet (exogenous factors); differences in detection (reflecting exogenous factors) and genetic differences (endogenous factors).

1.5.3 Family History

Men with two or more affected first-degree relative have a 5- to 11-fold increased risk of developing prostate cancer (30). The risk of cancer further increases with increase in number of relatives with the history of cancer, especially if the relatives have cancer at a younger age (31). Family history is also a risk factor for early onset of prostate cancer (32).

1.5.4 Hormones

Hormones in particular, testosterone and its metabolite dihydrotestosterone (DHT) are crucial for the development of the prostate gland. The difference in incidence between
African-Americans and Caucasians has been suggested to be due to higher serum testosterone levels in African-Americans (33). There is a characteristic age-related decrease in the ratio of androgens to estrogens in men, which may be a contributing factor in prostate cancer (34). Prospective studies propose that prostate cancer risk may be increased by high serum concentrations of bioavailable testosterone (35), A-diol-G (marker of 5 alpha reductase activity) and DHT. Increased levels of IGF1 has also been proposed as a risk factor for prostate cancer (36) but conflicting results have been presented. In addition, low Serum Hormone Binding Globulin (SHBG) has also been suggested to increase risk of prostate cancer (35).

1.5.5 Diet

Dietary intake of fat, meat and dairy products have been positively associated with prostate cancer in several epidemiological studies. Increased total fat intake and consumption of red meat have been associated with increased risk of prostate cancer (37,38). Several studies have found direct association between dairy intake and prostate cancer (37, 39). The high incidence rate of prostate cancer in the Western countries and low incidence in Asian countries could be due to the high intake of animal fat by the former than the latter. It has been suggested that dietary patterns alter the production of sex hormones which inturn affect the risk of cancer.

1.5.6 Dietary Factors with protective effect

**Phytoestrogens:** Phytoestrogens are naturally-occurring plant compounds, consisting of flavones, isoflavones and lignans, which display oestrogen-like activity. Some phyto-oestrogens inhibit 5 alpha reductase activity, thereby decreasing the concentration of dihydrotestosterone. Genistein and daizein are the main flavones in the diet (40). *In vitro* at physiological concentration genistein can induce a G1 cell-cycle arrest and decrease PSA mRNA expression. Isoflavones have also been shown to inhibit prostate cancer-cell growth, angiogenesis and metastasis. **Soyabeans:** have high content of phytoestrogens, especially flavonoids, which have a prophylactic effect on prostate cancer (41,42). Among the differences between eastern and western diets is the greater intake of soyabean by the former than the latter.
**Vitamin D:** Low serum vitamin D has been associated with high incidence of prostate cancer in African-Americans; leading to the hypothesis that vitamin D has an antitumour effect (43). Vitamin D has been suggested to reduce the risk of prostate cancer and 1, 25 dihydroxyvitamin D3 (1,25 D) a vitamin D metabolite inhibits prostate cancer growth. *In vitro* addition of calcitriol to prostate cancer cell lines induces cell-cycle arrest in G1/G0 and promotes the differentiation of prostate-cancer cell lines. Furthermore, *in vivo* studies have shown tumor growth delay in animal models and a reduction of metastasis in mice administered with vitamin D (44). The translation of these results into clinical trials has been limited by the side effects of vitamin D, which dangerously increases serum calcium concentrations.

**Lycopene:** Frequent intake of tomato-based product has been reported to be associated with a reduced risk of prostate cancer. Tomatoes contain lycopene, a carotenoid and potent antioxidant. Giovannucci et al 2002 (45) reported a lowered risk of 16% for prostate cancer in men who consumed large amounts of lycopene than those who consumed small amounts of lycopene.

**Selenium and Vitamin E:** Of the micronutrients that have been investigated in prostate cancer, selenium and vitamin E are the most promising. Selenium inhibits tumorigenesis; 5 years of follow-up of patients with a history of prostate cancer revealed that the prostate cancer incidence was 66% lower in the selenium group than the placebo group (46). Vitamin E (Alpha Tocopherol) has antioxidant property which inhibits oxidative DNA damage. Results from a Finnish alpha tocopherol-beta carotene cancer trial revealed a 40% decrease in incidence and mortality of prostate cancer in men administered alpha tocopherol (47). The low incidence of prostate cancer in Asia might be due to high intake of dietary phyto-oestrogens and lycopene.
1.6 Genetics of Prostate Cancer

Genetic risk factors include both rare genes that confer a high risk of developing disease to more common genes that confer a low to moderate risk. Prostate cancer can be epidemiologically divided into hereditary and sporadic forms, but it has not been possible to distinguish these two groups at the molecular level. Although some tumors have inherited gene mutations, all tumors acquire genetic alterations as they progress. Association of candidate genetic markers to this multifactorial malignancy has been more difficult than other cancers such as breast, ovary and colon (48) due to the following reasons:

- Prostate cancer is diagnosed at a late age, often making it impossible to obtain DNA samples from living affected men for more than one generation.
- The lack of distinguishing features between the hereditary and sporadic forms of the disease is another problem. No overall significant difference has been found between the two groups in terms of clinical stage, Gleason score or pre-operative PSA value (49).
- The genetic heterogeneity of this complex disease with multiple susceptibility genes, frequently showing moderate or low penetrance.

An understanding of the genetic basis for the development and progression of prostate cancer may have important implications in diagnosis and prognosis.

1.6.1 Genetic alterations in prostate cancer

Genetic alterations reported in prostate cancer include somatic mutations, gene deletions, amplifications, chromosomal rearrangements and changes in DNA methylation. The most commonly reported chromosomal abnormalities are gains at 7p, 7q, 8q and Xq, and losses at 8p, 10q, 13q and 16 (50). A striking heterogeneity in chromosomal abnormalities has been seen in different cases, in different lesions in the same case, and in different areas within the same lesion. Most commonly described areas of chromosomal loss and gain in prostate cancer have been tabulated (Table 1.1).
Table 1.1: Commonly reported chromosomal abnormalities in prostate cancer

<table>
<thead>
<tr>
<th>Chromosome locus</th>
<th>Putative genes</th>
<th>Normal Gene function</th>
<th>Gene status in prostate cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>7p</td>
<td>EGFR</td>
<td>Growth factor</td>
<td>Amplified (51)</td>
</tr>
<tr>
<td>8p</td>
<td>MSR</td>
<td>Macrophage scavenger receptor</td>
<td>Deleted (52)</td>
</tr>
<tr>
<td></td>
<td>NKX3-1</td>
<td>Tumor suppressor gene</td>
<td>Deleted (53)</td>
</tr>
<tr>
<td>8q</td>
<td>c-myc</td>
<td>Transcriptional activator</td>
<td>Amplified (54)</td>
</tr>
<tr>
<td>10q</td>
<td>PTEN</td>
<td>Tumor suppressor gene</td>
<td>Mutated (55)</td>
</tr>
<tr>
<td>13q</td>
<td>Rb</td>
<td>Tumor suppressor gene</td>
<td>Deleted (56)</td>
</tr>
<tr>
<td>16q</td>
<td>E-CAD</td>
<td>Adhesion molecule</td>
<td>Deleted (57)</td>
</tr>
<tr>
<td>Xq</td>
<td>AR</td>
<td>Androgen receptor</td>
<td>Amplified (58)</td>
</tr>
</tbody>
</table>

1.6.2 Hereditary susceptibility genes

Although few chromosomal regions have been linked to prostate cancer susceptibility, replication has proved difficult in independent study populations. While data from studies are most consistent with an X-Linked inheritance (59, 60), three independent segregation analysis (31, 49, 61) support an autosomal dominant mode of inheritance. The genome wide screen of families with prostate cancer have identified the following chromosomal regions with putative linkage:

- Chromosome 1 is of particular interest, with three proposed loci of susceptibility. HPC1 at 1q24-25 (62, 49), PCAP, at 1q42.2-q43 (63) and CAPB at 1p36 (64). However, these loci have not been reported consistently.
- HPCX at Xq27-28 (65,66)
- A prostate cancer susceptibility loci at 20q13 has been reported particularly in families having less than five affected members (67)
The failure to identify high penetrance genes in hereditary prostate cancer may result from the fact that multiple genes with a small to moderate effect are involved in hereditary carcinogenesis. While possible inherited prostate cancer susceptibility genes such as the ELAC2, RNASEL, MSR1, NSB1 and CHEK2 have been identified in some families (Table 1.2), the proportion of hereditary prostate cancer cases attributable to germline mutations in these loci is small.

**Table 1.2: Inherited prostate cancer susceptibility genes**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chromosome loci</th>
<th>Putative function</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPC2/ELAC2</td>
<td>17p</td>
<td>Metal dependent hydrolase</td>
<td>Ser217Leu, Ala541Thr(68)</td>
</tr>
<tr>
<td>RNASEL</td>
<td>1q24-q25</td>
<td>Encodes a ribonuclease that degrades viral and cellular RNA.</td>
<td>Deleted (69)</td>
</tr>
<tr>
<td>MSR1</td>
<td>8p</td>
<td>Encodes macrophage scavenger receptor for cellular uptake of molecules</td>
<td>Deleted (52)</td>
</tr>
<tr>
<td>NBSI</td>
<td>5p</td>
<td>Encodes nibrin, a protein involved in repair of DNA double strand breaks</td>
<td>Deleted (70)</td>
</tr>
<tr>
<td>CHEK2</td>
<td>22q</td>
<td>Regulator of p53 in DNA damage signalling pathway</td>
<td>Deleted (71)</td>
</tr>
</tbody>
</table>

Although candidate genes for hereditary prostate cancer susceptibility are been identified, only 5 to 10 percent of prostate cancer cases in the population may arise from major susceptibility genes (2). Other genetic factors, in combination with possible environmental risk factors, may have greater public health importance. Genetic polymorphisms associated with prostate cancer risk are much more common in the population than the high-penetrance cancer susceptibility genes.
1.6.3. Genetic polymorphisms in prostate cancer

It is apparent that most of the population-attributable prostate cancer susceptibility is related not to the rare deleterious gene defects but to polymorphic variations in the DNA sequence (72). With sequencing of the human genome, it is evident that 99.9% of the DNA is identical in every human genome (73,74). The 0.1% difference is responsible for the inter-individual variation and the unique phenotype of each individual. Such minor genetic variations, seen as single base changes in the genome are referred to as single nucleotide polymorphisms (SNPs). The common genetic polymorphisms have small relative risks, yet large population attributable risk because of their high frequencies.

The search for genetic markers of disease susceptibility often utilizes the candidate gene approach, where a gene is targeted based on the properties and metabolic pathways of its protein product (75). When these genes are polymorphic and the variants are distributed differently across populations, interest in them increases since variation in the DNA sequence could alter protein function and result in variations in disease risk. The role of genetic polymorphisms that govern interindividual differences in metabolism and cancer risk is receiving widespread attention.

One form of defense against cancer involves a series of genes whose role is to metabolize and excrete potentially toxic compounds and repair subtle mistakes in the DNA. It is this caretaker role of carcinogenesis that is likely to vary strongly between individuals because of population variability in polymorphic genes that regulate these processes. The current concept of carcinogenesis suggests that cancer genes can be classified as either caretaker or gatekeeper genes (76). This concept of caretaker and gatekeeper genes acknowledges their respective roles in maintenance of genomic integrity and cellular proliferation, respectively. Some examples of the caretaker genes are those that are involved in carcinogen activation, detoxification and DNA repair; whereas gatekeeper genes are those involved in cell cycle control and DNA replication.
Dysfunctional caretaker genes increase the probability of mutations in gatekeeper genes, initiating and promoting the molecular pathogenesis of cancer. Under this polygenic model, each allele confers a small genotypic risk, which combine to confer a range of susceptibilities. Genetic predisposition alone may not be responsible for causing cancer but a combination of susceptibility genes and exposures including environmental factors could contribute to the development of sporadic cancers (77). Multiple gene-environment interactions in carcinogenesis is illustrated in Fig 1.6.

Clinically important genetic risk factors that result in differences in individual susceptibility to prostate cancer probably include genes directly involved in androgen biosynthesis, metabolism and regulation. Other likely genes include those involved in carcinogen metabolism, DNA repair, cell cycle regulators and apoptotic regulators. In addition, polymorphisms in genes encoding non-androgenic hormones like the vitamin D receptor, estrogen receptor, Insulin Growth Factor 1 (IGF1); cell adhesion and angiogenesis genes like E cadherin and Vascular Endothelial Growth Factor Receptor (VEGFR) have also been reported in prostate cancer.
1.6.3.a Genetic polymorphisms associated with androgen metabolism

Androgens play a very important role in the growth and regulation of the prostate gland. Polymorphisms have been reported in genes involved in the biosynthesis, activation and degradation of androgens. In the prostate, approximately 90% of testosterone is converted to dihydrotestosterone (DHT) by the enzyme 5α reductase type 2 (SRD5A2). DHT, the active intracellular androgen in prostate cells, activates the androgen receptor (AR), which mediates transcription of androgen responsive genes that regulate prostate cell proliferation. Androgen metabolism has been described in detail in chapter III.

Polymorphisms in various androgen-metabolizing genes like CYP17, CYP3A4, SRD5A2, HSD3B2, AR and PSA have been reported in prostate cancer with distinct ethnic differences. The various candidate androgen metabolizing genes, their function and the effect of their polymorphisms in prostate cancer have been tabulated (Table 1.3).
Table 1.3 Candidate genetic polymorphisms of androgen metabolism in prostate cancer

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Reported Polymorphisms</th>
<th>Effect of variants</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP17</td>
<td>Mediates 17α hydroxylase and 17,20 lyase activity in steroid biochemical pathway</td>
<td>-34 T to C in 5’ UTR</td>
<td>Increased transcription rate (78)</td>
</tr>
<tr>
<td>CYP1B1</td>
<td>Catalyses testosterone hydroxylation</td>
<td>G 355 T and C 142 G</td>
<td>Amino acid substitution (79)</td>
</tr>
<tr>
<td>CYP19</td>
<td>Encodes aromatase that catalyses conversion of androstenedione to estrone and testosterone to estradiol</td>
<td>Long (TTTA) n in intron 4, C to T substitution in exon 7</td>
<td>Affects aromatase activity(80), Arg264Cys amino acid substitution (81)</td>
</tr>
<tr>
<td>CYP3A4</td>
<td>Oxidative metabolism of testosterone</td>
<td>A392G (CYP3A4*1B)</td>
<td>Unknown effect (82, 83)</td>
</tr>
<tr>
<td>SRD5A2</td>
<td>Encodes the enzyme 5α-reductase which converts testosterone to DHT</td>
<td>G 1008 C, G 888 A, TA repeats in 3’UTR</td>
<td>Val89Leu substitution, decreases enzyme activity (84), Ala49Thr substitution, increases enzyme activity (85), Increase in TA repeats may decrease SRD5A2 activity (86, 87)</td>
</tr>
<tr>
<td>AR</td>
<td>Transactivation of genes regulating prostate cell growth and proliferation</td>
<td>Exon1 CAG, GGN repeats, R 726 L</td>
<td>Short repeats enhance transactivation (88), Alters AR transactivation (89)</td>
</tr>
<tr>
<td>HSD3B2</td>
<td>Inactivation of DHT</td>
<td>N 367 T, C 7519 G</td>
<td>Alters enzyme activity (90), Unknown effect (91)</td>
</tr>
<tr>
<td>PSA</td>
<td>Secreted by prostate by AR transactivation</td>
<td>–158 A to G</td>
<td>Differential binding to AR (92)</td>
</tr>
</tbody>
</table>
1.6.3 b Genetic polymorphisms associated with carcinogen metabolism

When carcinogens encounter biologic systems, they are altered by metabolic processes. This is an initial facet of the gene-environment interaction. Xenobiotics generally require activation to electrophilic reactive forms to produce DNA adducts, this being mainly catalyzed by phase I enzymes of the Cytochrome P450 family. In contrast, phase II enzymes, such as the Glutathione S transferase family, conjugate the reactive metabolic intermediates to water-soluble forms, which are then easily excreted. It can therefore be assumed that individuals with increased metabolic activity and decreased detoxifying activity are at higher risk of prostate cancer.

Molecular epidemiology studies in prostate cancer have examined association of phase I and phase II genes, such as CYP1A1, CYP1B1, CYP2D6, GSTM1, GSTT1, GSTP1, NAT1 and NAT2. The various candidate carcinogen metabolizing genes, their function, the effect of their polymorphisms have been tabulated (Table 1.4). The carcinogen metabolism has been described in detail in chapter IV.
Table 1.4 Candidate genetic polymorphisms of carcinogen metabolizing genes in prostate cancer

<table>
<thead>
<tr>
<th>Candidate genes</th>
<th>Function</th>
<th>Polymorphisms</th>
<th>Effect of Variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP1A1</td>
<td>Encodes aryl hydrocarbon hydroxylase</td>
<td>Exon 7 Ile 462 Val</td>
<td>Increase in enzyme activity (93,94,95)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3’UTR T to C transition</td>
<td></td>
</tr>
<tr>
<td>CYP1B1</td>
<td>Bioactivation of estrogens and carcinogens</td>
<td>Codon 119 G to T transition</td>
<td>Ala 119 Ser substitution (96) Hyperactivation of CYP1B1(97)</td>
</tr>
<tr>
<td>CYP2D6</td>
<td>Encodes debrisoquine hydroxylase which metabolizes drugs</td>
<td>G to A transition in exon 3</td>
<td>Enzyme with poor metabolism (98,99)</td>
</tr>
<tr>
<td>GSTM1, GSTT1</td>
<td>Detoxification of activated carcinogen intermediates</td>
<td>Gene deletion</td>
<td>Loss of enzyme activity (100)</td>
</tr>
<tr>
<td>GSTP1</td>
<td>Detoxification of activated carcinogen intermediates</td>
<td>A313 G in exon 5</td>
<td>Ile105Val substitution, alteration of enzyme activity (101)</td>
</tr>
<tr>
<td>NAT1</td>
<td>Encodes N acetyl transferase, catalyzes acetylation of aromatic and heterocyclic amine carcinogens</td>
<td>NAT1*10: T1088A, C1095A</td>
<td>Rapid acetylation (102)</td>
</tr>
<tr>
<td>NAT2</td>
<td>Encodes N acetyl transferase, catalyzes acetylation of aromatic and heterocyclic amine carcinogens</td>
<td>NAT2 mutant alleles M1, M2, M3</td>
<td>Slow acetylation (103)</td>
</tr>
</tbody>
</table>
1.6.3. Genetic polymorphisms associated with DNA repair pathway

Once a procarcinogen is metabolically activated to an ultimate carcinogenic form, it can bind covalently to cellular macromolecules, including DNA. The DNA damages are generally fixed by multiple DNA repair pathways like base excision, nucleotide excision, mismatch repair and double-strand break repair. Difference in the rates and fidelity of DNA repair influences the extent of carcinogen adduct formation and consequently the extent of genetic damage. Cells with unrepaired DNA damage either undergo apoptosis or unregulated growth to malignancy. A defect or reduced efficiency of DNA repair therefore plays a pivotal role in the development of cancer. Genes involved in DNA repair pathway reported in prostate cancer are XRCC1, XPD, XRCC3 and MGMT (Table 1.5)

### Table 1.5 Candidate genetic polymorphisms of DNA repair genes in prostate cancer

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Polymorphism</th>
<th>Effect of variant</th>
</tr>
</thead>
<tbody>
<tr>
<td>XRCC1</td>
<td>Base excision repair</td>
<td>Arg194Trp</td>
<td>Decreases repair activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arg280 His</td>
<td>(104)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arg399Gln</td>
<td></td>
</tr>
<tr>
<td>XPD 1</td>
<td>Nucleotide excision repair</td>
<td>Asp312Asn</td>
<td>Decreases repair activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Lys751Gln</td>
<td>(104)</td>
</tr>
<tr>
<td>OGG1</td>
<td>Base excision repair</td>
<td>A 7143 G</td>
<td>Decreases repair activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A 11657 G</td>
<td>(105)</td>
</tr>
<tr>
<td>XRCC3</td>
<td>Homologous recombination repair</td>
<td>Thr241Met</td>
<td>Decreases repair activity</td>
</tr>
<tr>
<td>MGMT</td>
<td>Repairs damage caused by alkylating agents</td>
<td>A to G:Ile143Val</td>
<td>Decreases repair activity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C to T: Leu84Phe</td>
<td></td>
</tr>
</tbody>
</table>
Other Gene Polymorphisms associated with prostate cancer

1.6.3.d p53 polymorphism

p53, the tumor suppressor gene contributes to the maintenance of genomic stability by controlling cell cycle and facilitating DNA repair in response to DNA damage (107). One of the regions of p53 that is believed to induce apoptosis is the proline-rich domain in which an Arg to Pro substitution at codon 72 has been reported (108). In vitro studies have indicated that p53 Arg/Arg genotype induces apoptosis with faster kinetics and suppresses transformation more efficiently than the Pro/Pro genotype (109). Very few studies have investigated the effect of p53 codon 72 polymorphism in prostate cancer and contrasting findings have been reported (110-112).

1.6.3.e Estrogen receptor gene polymorphisms

The prostate expresses both the estrogen receptors (ER); ER α and ER β at low levels. Unlike the androgen receptor, only a few studies have evaluated polymorphisms in the ER genes and prostate cancer. In the ER α gene, GGGA repeat polymorphism (113) and T to C in intron 1 (114) have been reported to be associated with prostate cancer. In the ER β gene, a T to C transition in the promoter region has been reported to increase the risk of prostate cancer (115).

1.6.3.f. Vitamin D receptor gene polymorphism

Physiological concentrations of vitamin D promotes the differentiation and growth arrest of prostate cancer cells in vitro (116). The precise mechanism through which vitamin D mediates this effect is unknown, although it is probably through its effect on cell growth proteins. Allelic differences in the vitamin D receptor (VDR) gene results in variation in VDR activity (117). VDR alleles have been significantly associated with prostate cancer and this association was stronger in advanced stages (118). Some of the polymorphisms of VDR associated with prostate cancer are Taq I
polymorphism in exon 9 (119), Poly A repeats in the 3’ UTR (118) and BsmI, ApaI polymorphisms in intron 8 (120).

1.6.3.g Insulin gene polymorphism

Insulin is hypothesized as a risk factor for prostate cancer because of its structural and regulatory relations with the IGF system. There are consistent reports on the risk of prostate cancer with high serum levels of IGF-I (36,121,122). The +1127 T/C polymorphism in the 3’ UTR of INS gene (Insulin gene) has been demonstrated to play a crucial role in regulating insulin production and reported to increase the risk of prostate cancer in Blacks and Caucasians (123).

1.6.3 h. Cell Cycle control gene polymorphisms

CDKN1A (p21) and CDKN1B (p27) function as important cell cycle regulators. The 3'-UTR of CDKN1A gene has a C to T transition and the genotypes CT and TT were associated with an increased risk of advanced prostate carcinoma (124). Further, a significant association has been observed between a codon 109 polymorphism of CDKN1B (p27) and prostate cancer (124,125).

1.6.3 i. E Cadherin Polymorphism

E-Cadherin (CDH-1) is a cell adhesion molecule which maintains cellular integrity and communication. C/T 3'-UTR polymorphism has been reported in CDH-1 gene and the CC genotype was reported to exhibit a higher risk for prostate cancer than other genotypes (126).

1.6.3 j Vascular endothelial growth factor (VEGF) gene polymorphism

Vascular endothelial growth factor (VEGF) is a potent inducer of endothelial cell growth and its levels are elevated in several tumor types including the prostate (127). C to T transition at 460 nucleotides upstream of the VEGF gene has been studied and
the homozygous TT genotype has been associated with an increased risk of prostate cancer (128).

1.6.4 Genetic alterations in Androgen Independent prostate cancer

The initial observation by Charles Huggins (129) over 30 years ago that orchidectomy induces regression of prostate cancer, led to the androgen ablation therapy. Androgen ablation slows tumor growth; eventually most men fail to respond to this therapy and die of recurrent androgen independent prostate cancer (AIPC). While the factors that determine the response to treatment and relapse are unclear, there is accumulating evidence that the response to androgen ablation results from apoptosis in androgen-dependent cells (130,131). Subsequently, patients suffer recurrence because of the androgen-independent cells as well as apoptosis-resistant clones (132).

Genetic alterations are crucial factors not only for tumor progression but also for the development of AIPC. Like other cancers, it is possible that prostate tumors initially select the genetic changes that increase the likelihood of subsequent mutations. An increase in the mutation rate would increase the likelihood of a cell developing ensuing mutations (‘multiple hits’), which allow the prostate cancer cells to grow independent of androgen (133,134).

There is evidence that receptors drive the proliferation of androgen-independent prostate cancer cells even in the absence of androgens (135). Somatic alterations in AR have been detected in AIPC (136,137). AR amplification accompanied by over expression may promote the growth of androgen-independent cancer cells by increasing the sensitivity of cancer cells to low levels of circulating androgens (138). In many AR mutations, the ligand-specificity of the receptor is altered, permitting activation by non-androgens or anti-androgens. Mutations and altered expression of apoptotic proteins have also been reported in the apoptosis resistant clone of androgen independent cells.
1.7 Apoptotic regulatory proteins in prostate cancer

The tumor-suppressor gene p53 and the protooncogene bcl-2 are important regulators of cell growth and apoptosis. Mutations in p53, which are most prevalent in many malignancies, have been reported especially in advanced grade of prostate cancer (21,139). Accumulation of p53 protein may occur due to p53 gene mutation, DNA damage, hypoxia and redox stress (140). The expression of bcl-2 in prostate cancer has also been correlated with tumor progression and androgen independence, implying the significance of bcl-2 in conferring apoptosis resistant phenotype following androgen deprivation (141,142). Evaluation of both p53 and bcl2 status would aid in better understanding of prostate cancer progression and prognosis.

From the above literature it is evident that genetic polymorphisms have a significant role in the individual susceptibility to cancer. These polymorphisms could explain the variation in incidence according to geographic or ethnic status. An improved understanding of the interplay of endogenous physiology, xenobiotic exposures and genetic variability at multiple loci may help to identify men who are at an increased risk for prostate cancer.

The contribution of genetic polymorphisms to the risk of prostate cancer is dependent on the population of study, as well as environmental and dietary factors that influence the population. Hence, each population has to evaluate its genetic profile for cancer risk that will help in understanding the geographic and racial differences in cancer incidence and mortality. Study on prostate cancer genetic polymorphism is very limited in the Indian population, especially in South Indians. Since India is known for its unique population structure, having about 5000 endogamous populations, one would expect the South Indian men to possess distinct genetic variations attributing to the risk of prostate cancer. Identification of susceptibility markers is essential to determine the genetically predisposed individuals.
Since androgens play an important role in regulating the growth and proliferation of the prostate cells and androgen metabolism being the pathway primarily targeted for therapy, the study was proposed to identify the genetic variations associated with androgen metabolism. Moreover, with aging there is increased oxidative stress and genotoxicity in the prostatic cells. Hence three other related pathways essential for the caretaker activity of the cells namely, carcinogen metabolism, DNA repair and apoptosis were selected. Dysfunctional carcinogen metabolism might lead to DNA damage, which can be rectified by DNA repair mechanism. However, an inefficient DNA repair might lead to the damage in the DNA to persist. The cells with damaged DNA either undergo apoptosis or there might be rapid proliferation of the damaged cells, which result in cancer. Hence it was felt essential to identify the genetic variations associated with a few important genes in carcinogen metabolism, DNA repair and apoptosis. An illustration of the role of androgen metabolism, carcinogen metabolism, DNA repair and apoptosis in a cell, highlighting the genes proposed in the study is represented in Fig 1.7.

Furthermore understanding the pathways that lead to AIPC is essential towards developing therapy for this lethal form of prostate cancer. Hence it was considered important to determine the genetic variations in androgen metabolism, carcinogen metabolism, DNA repair and apoptosis predominant in patients with AIPC so as to identify certain prognostic markers.
Fig1.7: An illustration of the role of androgen metabolism, carcinogen metabolism, DNA repair and apoptosis in a cell, highlighting the genes proposed in the study.

“A study on genetic polymorphisms associated with prostate cancer risk in South Indian men”
1.8. Aim and Objectives

The aim of the study is to determine the association between genetic polymorphisms and prostate cancer risk in South Indian men.

The specific objectives are:

1. To determine the risk attributed by polymorphism in genes regulating
   a. Androgen metabolism, with specific reference to
      - Cytochrome P450c17α (CYP17) gene
      - Steroid 5α reductase type II (SRD5A2) gene
      - Androgen receptor (AR) gene
      - Prostate specific antigen (PSA) gene
   b. Carcinogen metabolism, with specific reference to
      - Cytochrome P4501A1 (CYP1A1) gene
      - Glutathione-S transferase µ (GSTM1) gene
      - Glutathione-S transferase pi (GSTP1) gene
      - Glutathione-S transferase θ (GSTT1) gene
   c. DNA repair pathway, with specific reference to
      - X-ray repair cross-complementary group 1 (XRCC1) gene
      - Xeroderma pigmentosum group D (XPD) gene

2. To determine the risk attributed by a polymorphism in the apoptotic regulatory gene p53, as well as to assess the expression of the apoptotic regulatory proteins p53 and bcl2 in prostate cancer patients and correlate their expression with the genotype and tumor aggressiveness.

3. To perform a stratified analysis of the genotypes with clinical characteristics of the patients like age, tumor grade and the PSA levels at diagnosis.

4. To establish the gene-gene interactions of the various pathways towards the risk of prostate cancer.