Hormone related cancers, namely breast, endometrium, prostate, testis, ovary and thyroid share a unique mechanism of carcinogenesis referred to as “cell proliferation mechanism”. Hormones, both endogenous and exogenous drive cell proliferation and thus increase the chance of accumulation of random genetic errors. The key distinction between cell proliferation and chemical carcinogenesis model is that no specific initiator is required for the former. Equally important is that the hormonal stimulus to cell proliferation continues all along the progression pathway (145). Further, the interruption of hormone stimulus through anti-hormone therapy will slow the progression until hormonal independence occurs.

Steroid hormones, particularly androgens play an important role in regulating prostate growth. Testosterone, the male androgen in circulation and dihydrotestosterone (DHT), the principal androgen in the prostate tissue are the most important androgens in an adult male. Testosterones, as well as DHT have shown to induce adenocarcinoma of the prostate (146) and anti-androgen therapy has reported to regress the cancer in animal models (147). However, epidemiological studies on the role of androgens in prostate cancer have produced conflicting results. Although Gann et al 1996 (35) reported a significant association between serum levels of testosterone and prostate cancer, several studies have found a suggestive but statistically non-significant association between prostate cancer and serum levels of testosterone and DHT (148,149). The most important limitation is that serum levels of androgens do not reflect the levels in the tissue; hence it is difficult to interpret results from serological studies. It is also unclear whether cumulative exposure to androgens over a lifetime or at certain points in life is more relevant in prostate carcinogenesis.

Since androgens play an important role in the regulation of the prostate, genes involved in the biosynthesis and metabolism of androgens have been under intensive study in prostate cancer.
3.1 Regulation of Androgen metabolism

3.1.1 Hypothalamic-Pituitary-Gonadal Regulation of androgen production:

Androgen is secreted by the testes and adrenals and metabolized in the prostate and skin. The testes produce more than 95% of testosterone and adrenals less than 5% (150). Androgen production is regulated by the hypothalamic–pituitary–gonadal axis (Fig:3.1). Luteinising hormone-releasing hormone (LHRH) secreted by the hypothalamus induces the anterior pituitary to release luteinising hormone (LH), which in turn stimulates the leydig cells of the testis to produce testosterone. Testosterone is taken up from circulation by the prostate epithelium, where it is converted to the most active androgen, 5α-dihydrotestosterone (DHT), by steroid 5α-reductase (SRD5A2). In a second pathway, corticotrophin-releasing hormone (CRH) from the hypothalamus stimulates the release of adrenocorticotrophic hormone (ACTH) from the pituitary. This in turn causes the release of adrenal androgens: androstenedione and dehydroepiandrosterone (DHEA). The adrenal precursor DHEA is converted into testosterone and then DHT either in the plasma or in the prostate itself.

![Fig 3.1 Hypothalamic-Pituitary-Gonadal Regulation of the androgen production](image)
3.1.2 Enzymes regulating androgen metabolism

Androgen metabolism takes place in the mitochondria and endoplasmic reticulum of the testes and adrenal cortex, where different cytochrome P-450 (CYP) isoforms are involved. As a first step in the biosynthesis of testosterone, cholesterol enters the mitochondria with the assistance of steroidogenic acute regulatory protein (StAR) for its conversion to pregnenolone by the cholesterol side chain cleavage enzyme, CYP11A. In the endoplasmic reticulum, pregnenolone is converted to DHEA by the 17 alpha hydroxylase activity of CYP17 (151). Pregnenolone is also converted to progesterone by HSD3B1 and progesterone in turn is converted to androstenedione by the 17, 20-lyase activity of CYP17. Androstenedione is the important precursor which gives rise to testosterone, estrone and estradiol. Fig 3.2 illustrates the testosterone metabolism and highlights the various enzymes involved in the regulation.

Fig 3.2 Illustration of androgen metabolism in the testes and prostate highlighting the key enzymes regulating the metabolic pathway.
Testosterone is released into circulation and in the blood roughly 44% of testosterone is bound with high affinity to sex hormone-binding globulin (SHBG), 54% with low affinity to albumin and only 1–2% of testosterone exists in a free bioavailable state. CYP1B1 catalyzes the hydroxylation and thereby breakdown of testosterone in circulation, while CYP19 mediates the conversion of testosterone to estradiol. In the cells of the prostate, testosterone is converted to dihydrotestosterone (DHT), by steroid 5α reductase activity of SRD5A2.

### 3.1.3 Androgenic action within the prostate

Both testosterone and DHT exert their androgenic effects in the prostate through the androgen receptor (AR) (Fig3.3.). The AR is associated with heat shock proteins in an inactive state. Androgen binding induces dissociation of AR from heat shock proteins, followed by hyperphosphorylation, conformational changes and dimerization of AR.

![Fig 3.3 Androgenic action within the prostate](image)
The DHT-AR complex translocates to the cell nucleus where it binds to specific DNA sequences termed androgen responsive elements (ARE) located in the promoter of androgen-responsive genes. In conjunction with co-activators and other transcription factors, AR upregulates or down-regulates the transcription of several genes. In vitro studies have shown that certain AR co-activators such as ARA54, ARA55, ARA70, ARA160, p160 and cortisol binding proteins enhance AR transcriptional activity (152). AR transactivation induces the expression of PSA and cyclin-dependent kinases 2 and 4 (153) as well as induces down-regulation of the cell cycle inhibitor p16. The overall effect of androgens in cells expressing AR is an increase of cyclin-dependent kinase activity and stimulation of cells to S phase of the cell cycle, thereby inducing cellular proliferation. The excess DHT in the prostate is metabolized to $\beta$ androstenedione and $\alpha$ androstenedione glucuronide (AAG), which is released from the prostate (Fig 3.2).

### 3.2 Polymorphisms in androgen metabolizing enzymes and prostate cancer

Although there is evidence that hormone metabolism can be environmentally influenced through diet and physical activity, the control of hormonal patterns is largely genetically regulated. Hence it is essential to characterize genetic variations that contribute to carcinogenesis in hormone responsive tissue and identify candidate loci in genes responsible for interindivid ual differences in steroid hormone levels. Polymorphisms in any of the genes encoding the enzymes involved in the androgen metabolism may affect the metabolic rate thus attributing to the susceptibility to prostate cancer. Racial differences in genetic polymorphisms in the metabolism of testosterone and other androgens may partly account for the ethnic differences in prostate cancer risk. A detailed list of the genes in AR metabolism and their polymorphisms have been listed in Table 1.3. The present study focused on the reported polymorphisms in CYP17, SRD5A2, AR and PSA genes.
3.3 Cytochrome P450c17α gene polymorphism in prostate cancer

3.3.1 CYP17 Gene

CYP17 gene mapped to 10q24.3, consists of 8 exons (Fig 3.4). It encodes the enzyme cytochromeP450c17α which functions at key branch points in the steroid hormone biosynthesis (151). CYP17 mediates both steroid 17α-hydroxylase activity, which converts pregnenolone to dehydroepiandrosterone and 17, 20-lyase activity, which generates androstenedione from progesterone (Fig 3.2).

3.3.2 CYP17 gene polymorphism

Three common polymorphisms have been reported in CYP17 gene namely, T to C transition in the 5’untranslated region (5’UTR), G 1951 A transition in the promoter sequence (154) and C5471A transversion in intron 6 (155). The one in the 5’ UTR has been extensively investigated while the information on the functional effects of the other two polymorphisms is lacking.

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Fig: 3.4 Diagrammatic representation of chromosomal location and exon-intron organization of CYP17 gene. Exons are numbered; introns are represented by white box. The position of the SNP analyzed in the present study is indicated.
5’UTR polymorphism

The 5’UTR of CYP17 gene has a T to C transition 34 bp upstream from the initiation of translation and 27 bp downstream from the transcription start site (156) (Fig 3.4). In line with the literature, the common allele (-34 T) is referred to as A1 and the variant allele (-34 C) as A2. This variation is reported to create an additional Sp1-type (CCACC box) promoter site. Since the number of promoter elements correlate with promoter activity, it has been postulated that the variation might result in increased transcription and thus higher androgen levels (78). However, in an in-vitro assay, Kristensen et al 1999 (157) did not observe Sp-1 binding at this polymorphic site. A positive association first reported by Carey et al 1994 (156) between the A2 allele of CYP17 and hyperandrogenic diseases such as polycystic ovarian syndrome and male baldness suggested CYP17 5’UTR as a candidate loci for study in relation to hormone related cancers. Molecular epidemiological studies have presented contradictory results concerning the role of CYP17 in prostate cancer.

3.3.3 Studies reporting association of CYP17 A1/A2 and A2/A2 genotypes with prostate cancer

Two studies, one in North Carolinian Caucasians comprised of 108 patients and 176 controls (84) and another in Caucasians in United States comprised of 590 patients and 782 controls reported a borderline significant association between A1/A2 genotype and prostate cancer (158). Gsur et al 2000 (159) assessed 63 patients and 126 controls in Austria and reported an increased risk in men with A2/A2 genotype. In a study on 105 patients and 210 controls from Japan, Yamada et al 2001 (160) observed a significant association between CYP17 A2/A2 and prostate cancer. Likewise, Kittles et al 2001 (161) in their study on 71 patients and 111 control African Americans reported a significant association of CYP17 A2/A2 genotype and prostate cancer. In a study on 100 patients and controls in North India, significant association between A2/A2 and prostate cancer risk has been reported (162).
3.3.4 Studies reporting association of CYP17 A1/A1 genotype and prostate cancer

Wadelius et al 1999 (163) assessed 178 patients and 160 controls from Sweden and reported a significant elevation in risk for the A1/A1 genotype. Similarly, among 252 prostate cancer patients and 131 controls from Japan, Habuchi et al 2000 (164) observed A1/A1 genotype to exhibit increased risk for prostate cancer. Further, a study on Italians comprised of 384 patients and 360 controls also revealed a significant association between A1/A1 and A1/A2 genotypes and prostate cancer risk (165).

3.3.5 Studies reporting no association between CYP17 and prostate cancer

Allen et al 2001 (166) studied 621 British men and reported no significant association between the A2 allele and endogenous androgen levels. In order to determine the role of this polymorphism in familial prostate cancer, Chang et al 2001 (167) studied 159 families, each of which had at least 3 first-degree relatives with prostate cancer, 249 sporadic prostate cancer patients and 211 controls. Their study did not provide evidence for over transmission of either alleles of the CYP17 to affected individuals in familial prostate cancer. A study comprised of 226 sporadic prostate cancer patients and 156 controls from France, Latil et al 2001 (80) observed no significant relation between CYP17 and prostate cancer. Likewise, in a Brazilian population of 92 prostate cancer patients and 200 controls, Dos Santos et al 2002 (168) found no association between CYP17 polymorphism and prostate cancer. In yet another family-based case-control study in Caucasians and African Americans comprised of 440 patients and 480 controls from 411 families, Cicek et al 2004 (169) reported A2 as the common allele in African-American patients, however the association was not significant. Further, a study on 93 patients and 121 controls in Taiwan did not find a significant association of the CYP17 polymorphism and prostate cancer (170). The association of CYP17 polymorphism with prostate cancer in various populations are shown in Table 3.1.
Androgen metabolizing gene polymorphisms in prostate cancer

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