CHAPTER 1

Review of Literature
Cancer

Second only to cardiovascular diseases, cancer represents leading causes of deaths. Cancer is the most dreadful disease that causes huge mortality in human beings around the world. The disease being most dreadful itself, the palliative period of the same is associated with several mental, physical and other complications as well as lifestyle compromises. This disease affects almost every kind of organs of the body.

WHO estimates suggest that the global burden of cancer has more than doubled during the past 30 years? In 2010, it is estimated that there were over 12 million new cases of cancer diagnosed, 7 million deaths from cancer and 25 million persons alive with cancer. The continued growth and ageing of the world’s population will greatly affect the cancer burden. By 2030, it could be expected that there could be 27 million incident cases of cancer, 17 million cancer deaths annually and 75 million persons alive with cancer within five years of diagnosis. The most commonly diagnosed cancers were found to be lung, breast, colorectal and prostate cancer whereas the most common cause of cancer deaths were suggested to be the cancers of lung, prostate and liver. In nutshell, this data suggested prostate cancer to be the second leading cause of deaths and fourth most common among all types of cancer worldwide [20].

Prostate cancer

A total of 1,479,350 new cancer cases and 562,340 deaths from cancer were projected to occur in the United States in 2009. Approximately 192,280 American men may have been diagnosed with prostate cancer and almost 27, 360 may have died of the disease in the 2009 [21]. Prostate cancer remains the most commonly diagnosed malignancy and second leading cause of cancer death in men older than age of 40 years in the United States. The widespread use of serum prostate specific antigen (PSA) and digital rectal examination (DRE) for prostate cancer screening has resulted in earlier disease detection in the last decade. Treatment options, including nerve sparing radical prostatectomy, external beam radiation and brachytherapy,
have been increasingly used to manage localized disease. Despite these efforts, prostate cancer continues to be a significant health problem in most western countries. Recent scientific discoveries have led to better understanding of the molecular and genetic events associated with prostate carcinogenesis.

The prostate is part of a man's reproductive system. It's an organ located in front of the rectum and under the bladder. The prostate surrounds the urethra, the tube through which urine flows as shown in the Fig 1. Prostate growths can be benign (not cancer) or malignant (cancer). In mammals, we have two steroid hormones, testosterone and dihydrotestosterone. Both hormones bind to a steroid hormone receptor, called as androgen, and activate genes containing androgen-responsive DNA sequences. In the past, studies show testosterone as the major androgenic hormone and postulated that dihydrotestosterone was an inactive metabolite of testosterone. The dihydrotestosterone is a potent androgen having physiological roles different from those of testosterone. It can be predicted by two observations first, androgen target tissues contained a steroid 5 alpha reductase enzyme activity which result in conversion of testosterone to dihydrotestosterone [22], and second, the product of this enzyme accumulated in the nuclei responsive cells, such as those of the rat ventral prostate [23, 24]. In further study of an inborn error of male phenotypic sexual differentiation, now termed steroid 5-alpha reductase 2 deficiency, provided genetic proof of the critical role of dihydrotestosterone [25, 26]. Males with this genetic disease have a biochemical defect in the synthesis of dihydrotestosterone in the embryo, which in turn leads to a developmental defect in the formation of the external genitalia and the prostate [25, 26]. They exhibit a striking phenotype in which the internal genitalia (epididymis, seminal vesicles and vas deferens) are normal, but the external genitalia resemble those of the female. In addition, these subjects appear to have less baldness and acne. The facts that dihydrotestosterone mediates growth of the prostate and that individuals who lack five a -reductase failed to develop a prostate led to the development of therapeutic inhibitors of the enzyme. These drugs are
used in the treatment of endocrine disorders whose underlying etiology requires dihydrotestosterone action.

Figure 1: Prostate cancer natural history

Steroid 5 alpha reductase (SRD5A2)

SRD5A2 plays an important role in male developmental biology, physiology, and pharmacology.

Figure 2: Anatomy of human male reproductive tract. Men have a prostate at the base of the bladder

The first androgenic hormone isolated and characterized was androsterone, a $5\alpha$-reduced 19-carbon steroid that was isolated by Butenant in 1931 from 25,000 liters of urine from adult men. This steroid was a potent androgen in bioassay systems and was assumed to be the male hormone
until Ernst Laquer and his colleagues demonstrated in 1935 that the androgen secreted by the testis is in fact testosterone, a 19-carbon steroid with a 4, 5 double bond [27]. The Steroid 5α-reductase enzyme was initially characterized in the 1950s in rat liver slices based on its ability to convert deoxycorticosterone to five α-reduced metabolites [28, 29]. In further studies by Tomkins and others showed that the enzyme was present in the particulate fraction, utilized reduced pyridine nucleotide as a cofactor, and was capable of metabolizing a variety of steroid substrates [30, 31] but it was not clear in these early studies whether a single enzyme or multiple enzymes are responsible for the 5α-reduction of steroids.

It is studied that DHT, the 5α-reduced precursor of androsterone formation, is a more potent androgen than testosterone in bioassays involving the prostate [32] and that the administration of radiolabeled testosterone to rats resulted in a time-dependent accumulation of dihydrotestosterone in the nuclei of ventral prostate cells [23, 24]. Finally, dihydrotestosterone was shown to bind preferentially to specific nuclear (androgen) receptor proteins [33, 34]. This study indicated that the 5α-reduction of testosterone is a crucial step in androgen action and focused attention on 5α-reductase. To discover the evidence for the key role of five α-reductase was subsequently obtained from two evidences. First, developmental studies showed that the activity of 5α-reductase in mammalian embryo was highest in the primordia of the prostate and external genitalia prior to their virilization, but very low in wolffian duct structures [35, 36] suggesting that the reaction is crucial for formation of the normal male phenotype during embryogenesis. Second, genetic studies showed that a rare disorder of male sexual differentiation, originally termed pseudovaginal perineoscrotal hypospadias [37] was caused by mutations in 5α-reductase [25, 26]. The analysis of enzyme activity in skin slices [25], and of the urinary and serum steroids in these subjects [26], showed a generalized defect in the conversion of testosterone to dihydrotestosterone as shown in Fig 3.
Testes

Figure 3: Androgen receptor signaling pathway in healthy prostate. In androgen responsive target cells testosterone (T) is converted to DHT by 5αR enzymes. DHT binds to AR and causes dissociation of heat shock proteins (Hsp), allowing translocation of DHT-AR complex into nucleus, where it binds to AREs. Recruitment of co activator proteins (Co) enables transcriptional activation of target genes.

This disease was subsequently referred to as steroid 5α-reductase deficiency. To study the phenotype of 5α-reductase deficiency cells of affected individuals were cultured [38]. Like, fibroblasts grown from genital skin possessed a 5α-reductase activity with an acidic pH optimum that was absent in the genetic disease [39].

Table 1 # Characteristics of Human 5α-reductase Type 1 and Type 2

<table>
<thead>
<tr>
<th>Gene structure</th>
<th>SRD5A1</th>
<th>SRD5A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exons / Introns</td>
<td>5 / 4</td>
<td>5 / 4</td>
</tr>
<tr>
<td>Chromosome location</td>
<td>5p15</td>
<td>2p23</td>
</tr>
<tr>
<td>Size</td>
<td>259 Amino acids, MW = 29,462</td>
<td>259 Amino acids, MW = 28,398</td>
</tr>
<tr>
<td>PH optima</td>
<td>Neutral to Basic</td>
<td>Acidic to Basic</td>
</tr>
<tr>
<td>Tissue distribution</td>
<td>Liver, nongenital skin, prostate, Brain, Ovary, Seminal vesicle, genital skin, breast, Hair, Fetal placenta, testis</td>
<td>Prostate, epididymis, seminal vesicle, genital skin, breast, Hair, Fetal placenta, testis</td>
</tr>
<tr>
<td>Prostate level</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Cell distribution in prostate</td>
<td>Epithelial cells</td>
<td>Stromal &amp; Basal epithelial cells</td>
</tr>
<tr>
<td>Level in BPH</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Level in primary PCa</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Level in metastatic PCa</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>Gene in 5αRD deficiency</td>
<td>Normal</td>
<td>Mutated</td>
</tr>
<tr>
<td>Finasteride inhibition</td>
<td>IC50 = 360nM</td>
<td>IC 50 = 69 nM</td>
</tr>
<tr>
<td>Dutasteride inhibition</td>
<td>IC50 = 6nM</td>
<td>IC 50 = 7 nM</td>
</tr>
</tbody>
</table>
Steroid-5-alpha-reductase type 2 and prostate cancer

Analyses of the 5α-reductase activity in the ethnic groups demonstrate that 5α-reductase activity in Asian males is lower than African-Americans and American Whites [40, 41], and is increased in Asian males who live in North American compared to Asians in Asia [41]. These data correlate with the progressive increase in clinical prostate cancer incidence in immigrants who have moved from Asia to United States, in the second-generation Asian-American males when compared to the first-generation immigrants [42, 43]. These data suggest that increased 5α-reductase activity may relate to the pathogenesis of prostate cancer. Studies in various ethnic groups and in patients with prostate cancer suggest that allelic variants in 5α-reductase-2 gene may be associated with prostate cancer risk and prostate cancer progression. [44] reported that a missense polymorphism, V89L, which has a substitution of leucine for valine at amino acid position [45] of the enzyme, was associated with lower 5α-reductase activity. The prevalence of this variant in African-American, Asian, and Latino men parallels the frequency of prostate cancer in these races.

Another 5α-reductase-2 gene polymorphism, A49T, which changes an alanine to a threonine residue at amino acid 49 with an increase in 5α-reductase activity has found to be associated with prostate cancer risk in African-American, Latino and Hispanic men, and be most common in African-American and Latino men with advanced prostate cancer [44]. Recently, Jaffe et al., 2000 has found that the presence of the A49T variant is associated with a greater frequency of extracapsular disease, a higher pathological tumor-lymph node-metastasis and a poorer prognosis, while V89L genotypes has no association with any of the characteristics studied. However, such association between 5α-reductase-2 gene polymorphism and prostate cancer has not been demonstrated in other studies [46, 47]. Further supporting evidences come from the clinical studies of male pseudohermaphrodites with an inherited DHT deficiency due to 5α-reductase-2 gene mutations from the Dominican kindred, the largest pedigree in the world. As stated above, these patients have an underdeveloped prostate and undetectable plasma PSA levels. After followed for many years, neither BPH nor prostate cancer has been observed in these patients.
Figure 4: Schematic of the hypothalamic-pituitary-testicular-adrenal axis. ACTH = adrenocorticotropic hormone; DHT = dihydrotestosterone; LH = luteinizing hormone; LHRH = luteinizing hormone releasing hormone. Steroid 5-alpha-reductase (EC 1.3.99.5) catalyzes the conversion of testosterone into the more potent androgen, dihydrotestosterone (DHT). The two isoforms of the enzyme are: SRD5A1 and SRD5A2.

Studies of specific 5α-reductase inhibitors provide additional evidence. Administration of a 5α-reductase inhibitor prevents the development of spontaneous prostate cancer in animals [48] and in humans [49]. In a large randomized, placebo controlled chemoprevention trial [45, 49], prophylactic use of finasteride, in males over 50 years, decreases the incidence of prostate cancer by 25% compared to those in the placebo group. However, the tumor malignancy is increased in the finasteride-treated group, a dilemma in using finasteride for the prevention of prostate cancer. Although both type 1 and 2 5α-reductases are expressed in prostate tumor cells [50] and the administration of 5α-reductase inhibitor alone or in combination with androgen antagonist inhibits prostate tumor cell growth in culture [45, 51] the efficacy of finasteride in the treatment of prostate cancer is disappointed [52]. Since the 5α-reductase-1 activity is 3 to 4 times greater in malignant than in benign prostate tissues, while the 5α-reductase-2 activity is similar in these 2 diseases [53], whether inhibition of both isozymes by combination of specific
type 1 and type 2 inhibitor or using dual inhibitor is more effective in the prevention and therapy of prostate cancer is an interesting strategy and remains to be determined. A high dietary fat intake is a major risk factor of prostate cancer [54, 55]. However, how dietary fat stimulates prostate growth and prostate cancer development is unclear. It has been recently demonstrated that a high dietary fat intake increased prostate 5α-reductase-2 gene expression and circulating DHT levels in the rat without significant alteration in prostate and hepatic 5α-reductase-1 gene expression and plasma testosterone concentration, suggesting that alteration in prostate 5α-reductase activity after a high-fat diet may be a potential mechanism for dietary fat stimulation of prostate growth and pathogenesis. Numerous studies have supported the concept that genistein, a phytoestrogen, has beneficial effects on the prevention and treatment of prostate cancer [56, 57], acting via multiple mechanisms such as inhibiting 5α-reductase activity [58], and tyrosine kinase activity [57]. Recently, we have also observed that genistein completely blocked the dietary fat induced increases in prostate 5α-reductase-2 gene expression and plasma DHT levels, as well as inhibited DHT actions via estrogen receptors, [59]. These data provide further evidence to support the above concept.

Figure 5: Type 1 and 2, 5αR immunostaining in BPH, PIN, primary prostate cancer, recurrent cancer and metastases.
SRD5A2 and Benign Prostatic Hyperplasia

BPH is responsible for considerable morbidity due to urethral obstruction. Histological evidence of BPH is found in 50% of males by the age of 50 and 90% of males by the age of 80 [60]. The development of BPH is exclusively dependent on androgens, and BPH does not occur in men castrated prior to puberty [60]. Although testosterone is the major androgen from the testes, DHT is known to be the major intracellular androgen to mediate androgen actions in the prostate cells [23, 24, 50]. Androgen withdrawal by castration leads to atrophy of prostate gland due to prostatic cell apoptosis [61]. Nearly 87% had rapid atrophy of their enlarged prostate and almost 58% had relief of symptoms. Administration of either DHT or testosterone to castrated dogs results in an increase in intra-prostatic DHT, and in BPH [62]. However, the concomitant administration of testosterone with a 5a-reductase inhibitor results in a decreased DHT formation and a prevention of BPH [63, 64].

Administration of finasteride, a specific 5a-reductase-2 inhibitor causes a selective decrease in both circulating and intraprostatic DHT, a significant decrease in prostate size due to prostatic cell apoptosis [65, 66], and an improvement of clinical symptoms in BPH. In human prostates examined after finasteride administration, the atrophy of glandular tissue in the prostate proceeds from the distal to the proximal acinar ducts [67] found that finasteride triggers a similar reduction in morphometric and volumetric measures in the transition and peripheral zones of the prostate. However, Montironi et al., 1996 noted that shrinkage is predominantly in the transition zone. It is known that BPH originates in the transition zone, while prostate cancer originates in the peripheral zone of the prostate. Finasteride has been shown to reduce blood flow in both the ventral and dorsal lobes of the rat prostate, which may be mediated by decreasing vascular-derived endothelial growth factor gene expression. The specific 5a-reductase-2 inhibitor, finasteride has now been used for the treatment of BPH. This novel therapeutic strategy evolved from the clinical observation that adult male pseudohermaphrodites with 5a-reductase-2 deficiency and a deficiency in DHT production have rudimentary prostate [26, 68]. Recently, specific 5a-
reductase-1 inhibitor and dual 5α-reductase-1 and 2 inhibitor have been developed. Pre-clinical and preliminary clinical studies have shown that inhibition of both 5α-reductase isozymes resulted in greater and more consistent suppression of circulating DHT than that observed with the selective inhibitor of 5α-reductase-2, finasteride [69].

**Steroid-5-alpha-reductase type 2**

Steroid 5α-reductase isozymes are microsomal NADPH-dependent proteins that reduce the double bond steroids at the 4–5 position of a variety of C19 and C21 including testosterone. Although testosterone is the primary androgen synthesized and secreted from the testes, it functions as a prohormone in certain tissues such as prostate where it converts to DHT, a more potent androgen, by 5α-reductase isozymes. Although testosterone and DHT produce some distinct biological responses, they bind to the same intracellular androgen receptor, which is a member of the nuclear steroid/thyroid hormone receptor superfamily, to regulate target gene expression [13]. The molecular mechanism for the differential testosterone and DHT action is unclear though differences in receptor binding [14] and DNA interaction [70] between testosterone and DHT. In the early 60’s, it was theoretically said that multiple 5α-reductase isozymes existed. Two different pH optima for 5α-reductase activity in genital and nongenital skin were detected in the 70’s [39, 71]. The major peak of 5α-reductase activity with a narrow, acidic pH optimum of 5.5 was found to be low in the genital skin of male pseudohermaphroditism with 5α-reductase deficiency. Another broader peak of activity which had a neutral to alkaline pH (pH 7–9), and was present in both genital and nongenital skin, was found to be normal in the genital skin of male pseudohermaphroditism with 5α-reductase deficiency. Kinetic analysis of 5α-reductase activity in the epithelium and stroma of the prostate also suggested different 5α-reductase activities [22, 72]. Studies of specific 5α-reductase inhibitors further indicated that multiple 5α-reductase isozymes were present in human prostate tissues [73].

In the early 90’s, two genes encoding two 5α-reductase isozymes were eventually identified, and named steroid 5α-reductase type 1 (gene symbol:
SRD5A1) and steroid 5α-reductase type 2 (gene symbol: SRD5A2) [74, 75].

Both human 5α-reductase-2 and 5α-reductase-1 genes have five exons and four introns, and encode a highly hydrophobic 254 and 259 amino acid protein with a molecular weight of approximately 28.4 and 29.5 kilo daltons, respectively. 5α-reductase-2 is mapped to the short arm of chromosome 2p23, and 5α-reductase-1 to chromosome 5p15. There are 50% homologies in amino acid compositions between human type-1 and type-2 isozymes. The type-2 isozyme has a much higher affinity than type-1 isozyme for substrates such as testosterone. The type-2 isozyme is sensitive to finasteride, a 5α-reductase-2 inhibitor, while the type-1 has a lower sensitivity. The apparent Km (3–10 μM) for the NADPH cofactor is similar for both isozymes. The type-2 isozyme has an acidic pH optimum in the enzymatic assays, while the type-1 has a broad alkaline pH optimum [17, 75]. However, studies with transfected Chinese hamster ovary cells suggest that the type-2 isozyme may actually have a neutral pH optimum in its native state, and that the acidic optimum described may actually be an artifact of cell lysis [76]. Additionally, the affinity of the type-2 isozyme for steroid substrates is higher at a neutral pH than an acidic pH (pH 5.0), suggesting that this isozyme acts at neutral pH in the cell [76, 77].

The functional domains of 5α-reductase-2 have been deduced from in vitroc mutagenesis transfection analysis of natural mutations of 5α-reductase-2 gene in cultured mammalian cells [75, 78, 79] and mutagenesis analysis of 5α-reductase-1 isozyme. Mutations affecting NADPH binding map to the last half of type-2 isozyme, suggesting that the carboxyl-terminal of the isozyme appears to be a cofactor-binding domain even though consensus adenine dinucleotide-binding sequences are not identified. In contrast, 5α-reductase-2 mutations that affect substrate (testosterone) binding appear to be located at both ends of the protein. However, the amino acid determinants of the substrate binding domain appear to be mainly located at the amino terminal of the protein [75]. At birth, 5α-reductase-1 is detected in the liver and non-genital skin, and is present throughout life. Its expression in embryonic tissues, however, is quite low. In adulthood, it is expressed in non-genital skin,
liver and certain brain regions; whereas its presence in the prostate, genital skin, epididymis, seminal vesicle, testis, adrenal and kidney is low.

The function of 5α-reductase-1 in human physiology remains to be defined. 5α-reductase-2 is expressed in external genital tissues early in gestation [76]. In adulthood, its expression in prostate, genital skin, epididymis, seminal vesicle and liver is relatively high, while it is quite low in other tissues. This isozyme also appears to be expressed in the ovary and hair follicles [80, 81]. In the human prostate, both 5α-reductase isozymes are present in epithelial cells and stromal cells, while 5α-reductase-2 is the predominant isozyme expressed in the stromal cells [19, 75, 76]. Both isozymes are expressed in BPH and prostate cancer tissues, and in prostate tumor cells [50, 82]. It is the mutations in the 5α-reductase-2 gene that are responsible for male pseudohermaphroditism due to 5α-reductase deficiency [17, 50]. SRD5A2 is also named alternatively like: 3-oxo-5α-steroid 4-dehydrogenase; steroid 5α-reductase; 3-oxosteroid delta4-dehydrogenase; steroid delta4-5α-reductase; 3-oxo-5α-steroid delta4-dehydrogenase; 3-oxo-5α-steroid delta4-reductase; 4-ene-3-keto steroid 5α-reductase; 3-keto-delta4-steroid-5α-reductase; 5α-reductase; testosterone 5α-reductase; 4-ene-3-keto-steroid-5α-oxidoreductase; 5α-reductase; testosterone 5α-reductase; 4-ene-3-keto-steroid-5α-oxidoreductase; 3-keto-delta4-steroid-5α-reductase; 5α-reductase; testosterone 5α-reductase; 4-ene-3-keto-steroid-5α-oxidoreductase; 5α-reductase.

SRD5A2 is a put under the class of enzyme called oxidoreductases, which act on the CH-CH group of donors with other acceptor. The primary structures of the human 5α-reductase isozymes were determined from their respective cDNAs. They are hydrophobic proteins composed of 254 amino acids with predicted molecular weights of 28393 Da. There are no consensus sequences for N-linked glycosylation (Asn-X-Ser/Thr) or for O-linked glycosylation (Ser/Thr/Pro-rich regions). An average of 37% of the residues have side chains commonly found buried in the hydrophobic interior of globular proteins (Cys, Ile, Leu, Met, Phe, Val). These hydrophobic amino acids are distributed throughout the enzyme, and do not give rise to clear-cut transmembrane regions in hydropathy plots. This structural feature suggests that the 5α-reductase isozymes are intrinsic membrane proteins deeply embedded in the lipid
bilayer, and it explains the need for detergent to solubilize the enzyme in earlier purification attempts. The hydrophobic amino acid content may also underlie the aberrant electrophoretic mobilities in sodium dodecyl sulfate-containing polyacrylamide gels that have been reported for the five α-reductase isozymes. In these gels, the isozymes migrate with molecular weights of 21 KD -27 KD instead of the predicted 28 KD- 29 KD [74, 76, 83]. In vitro translation studies indicate that the 5 α-reductase isozymes do not have cleavable signal sequences [74]. Database comparisons have so far revealed five proteins with extended sequence identities with the 5 alpha-reductases. These include a protein of unknown function encoded by a partial cDNA isolated from the nematode Caenorhabditis elegans [84], the Epstein Barr virus terminal protein possibly involved in maintaining the virus’s latent infection cycle [85], a rat protein of unknown function referred to as SC2 [86], a tobacco chloroplast NADH ubiquinone oxidoreductase chain 5 homolog [87], and a portion of the reverse transcriptase (pol) gene of the Cas-Br-E murine leukemia virus [88]. The significance of these homologies is unclear. In the cases of the C. elegans, Epstein Bare virus, and SC2 proteins, the identities may reflect a simple conservation of a hydrophobic transmembrane region. However, the fact that each of these proteins shares the same carboxyl-terminal end with the 5α-reductases implies a functional conservation.

Several groups have succeeded in expressing the human cDNAs in the baculovirus system. Infected insect cells express a large amount of immune reactive 5α-reductase isozyme and may thus be appropriate hosts for subsequent purification attempts. Reaching this goal may be facilitated by the use of affinity chromatography inhibitor column [78]. To study the subcellular Localization of the enzyme work have been conducted like, in pulse-chase experiments with transfected CHO cell lines, both human 5α-reductase isozymes have long half-lives (20-30 hours), which are not altered by the presence of micromolar concentrations of two 4-azasteroid inhibitors.

One of these 4-azasteroids (finasteride) is thought to bind irreversibly to the human 5α-reductase. These results suggest that the enzyme is not regulated at the level of protein degradation, at least not in cultured hamster
fibroblasts. In agreement with this hypothesis, no alterations in steady-state protein level or enzyme activity could be detected in synchronized CHO cells at various phases of the cell cycle [76]. No evidence been found for posttranslational modification of the isozymes (e.g. phosphorylation, fatty acylation, isoprenylation). Indirect fluorescent immunocytochemistry indicated that both human 5 alpha reductase isozymes reside in the endoplasmic reticulum [76], presumably embedded in the lipid bilayer of this organelle. The subcellular localization of 5α-reductase was previously shown to differ depending on the tissue source of the enzyme. Thus, enzyme activity sedimented with the nuclear fraction of human [72] and rat [71, 89] prostate cells, but with endoplasmic reticulum fraction of liver cells [39]. These results have been confirmed by immunohistochemical studies in both the human (type isozyme) and rat prostate (type 1 isozyme), in which the subcellular distribution of the antigen is perinuclear, and in rat and human liver (type 1 and type 2 isozymes, respectively), in which a reticular distribution is detected [19, 76, 78].

The different subcellular localization of 5α-reductase in liver and prostate may reflect a difference in the proliferation of the endoplasmic reticulum, since this organelle is continuous with and extends outwards from the nuclear membrane. Alternatively, the difference may have a regulatory mechanism, as the cells of the prostate are androgen dependent whereas those of the liver are not. A perinuclear localization of the enzyme might facilitate subsequent binding of product by nuclear androgen receptors.

The prostate is a ductal-acinar gland whose growth and development initiates in fetal life and completes at sexual maturity. Development of prostate begins when prostatic buds emerge from the urogenital sinus that derived from endoderm at 10th week in human fetuses, on day 17 in embryonic mice and on day 19 in embryonic rats. The urogenital sinus also forms the bulbourethral glands, and the prostatic and membranous portion of the urethra. Normal prostate development requires many coordinated cellular processes and involves multiple genes and hormonal actions [90]. DHT plays an essential role in the prostate development and growth. Studies in rabbit, rat [89], and human [35] fetuses have shown that 5α-reductase activity is present
in the urogenital sinus and external genital enlarge prior to prostate and external genital differentiation.

However, 5α-reductase activity is not present in the Wolffian duct, at the time of epididymal, vas deferens, and seminal vesicle differentiation. Thus, testosterone and its 5α-reduced metabolite DHT have selective roles in male sexual differentiation during embryogenesis. Testosterone mediates Wolffian ductal differentiation, while DHT mediates male external genital and prostate differentiation. This hypothesis is confirmed by the study of 5α-reductase-2 deficiency syndrome. Patients with 5α-reductase-2 deficiency syndrome have decreased circulating and prostatic DHT concentration due to attenuated 5α-reductase activity. In the affected male adults, the prostate is no palpable on rectal examination [26, 91] and is found to be rudimentary on transrectal ultrasound and MRI visualization [92]. Prostatic volumes are approximately 1/10th of age-matched normal controls. Histological analysis of prostate biopsy from these patients reveals fibrous connective tissue, smooth muscle, and no identifiable epithelial tissue, suggesting atrophic epithelium or lack of epithelial differentiation [93]. Plasma PSA is low or undetectable in these patients. Administration of DHT results in enlargement of the prostate [68, 94] and an elevation of plasma PSA levels. These findings provide clinical evidence that prostate differentiation and growth as well as circulating PSA level is mediated largely by DHT. However, the mere presence of a prostate in these individuals supports the notion that other growth factors are also involved in its organogenesis. Further supportive evidence is provided by animal studies using 5α-reductase-2 inhibitors and gene knockout. Administration of a 5α-reductase-2 inhibitor, finasteride, in rats [95, 96] and monkeys [97] impairs male sexual differentiation and prostate development. The prostate in mice with genetic disruption of either 5α-reductase-2 or 5α-reductase-2 plus 5α-reductase-1 gene is small, but it is puzzling that these knockout animals have normal genitalia in male offspring [98].

The clinical syndrome of 5α-reductase deficiency was first described in large Dominican kindred [26], and in two siblings from Dallas [25]. Subsequently large cohorts in New Guinea [99] and Turkey were described [79, 93, 100] as well as many other cases worldwide [13]. Most affected 46XY
subjects with 5α-reductase-2 deficiency have ambiguous external genitalia with a clitoral-like phallus, severely bifid scrotum, pseudovaginal perineoscrotal hypospadias and a rudimentary prostate [26, 93]. More masculinized subjects have been described; they either lack a separate vaginal opening [101], or have a blind vaginal pouch which opens into the urethra [102], penile hypospadias [103] or even a penile urethra [104]. Wolffian duct differentiation in affected males is normal with seminal vesicles, vasa deferentia, epididymides and ejaculatory ducts; no Mullerian structures are present. Cryptorchidism is frequently described though it is not invariably present with testes usually found in the inguinal canal or scrotum and occasionally located in the abdomen. In humans, with the onset of puberty, the affected males have increased muscle mass and deepening of the voice [26]. The genitalia enlarge with growth of the phallus as well as rotation and hyperpigmentation of the scrotum. The inguinal testes have been observed in some subjects to descend into the scrotum at puberty [13, 105]. Libido is intact and affected men are capable of erections [101]. Although most subjects studied are generally oligo- or azoospermic due to undescended testes, normal sperm concentrations have been reported in subjects with descended testes [106, 107]. Affected men from the Dominican kindred [107] and from Sweden [108, 109] have been reported to father children. These findings suggest that pubertal events, including male sexual function and spermatogenesis, appear to be primarily testosterone mediated. The other possibility is that the amount of DHT derived from 5α-reductase-1 action is enough for spermatogenesis. The facial and body hair is decreased, and male pattern baldness has never been observed in genetic males with this condition [26, 100]. Sebum production is normal in 5α-reductase-2 deficient subjects although it is an androgen-dependent process [92].

The biochemical features of this syndrome have been well defined over the years [13, 68]. These include: (a) high normal to elevated levels of plasma testosterone; (b) low normal to decreased levels of plasma DHT; (c) an increased testosterone to DHT ratio at baseline and/or following hCG stimulation; (d) decreased conversion of testosterone to dihydrotestosterone in vivo; (e) normal metabolic clearance rates of testosterone and DHT; (f)
decreased production of urinary 5α-reduced androgen metabolites with increased 5β/5α urinary metabolite ratios; (g) decreased plasma and urinary 3α-androstanediol glucuronide, a major metabolite of DHT; (h) a global defect in steroid 5α-reduction as demonstrated by decreased urinary 5α-reduced metabolites of both C21 steroids and C19 steroids other than testosterone, i.e. cortisol, corticosterone, 11β-hydroxy-androstenedione and androstenedione; increased plasma levels of LH and an increased LH pulse amplitude with a normal LH frequency; and plasma FSH levels may be elevated.