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# THE ROLE OF THE PROSTATE IN ANDROGEN METABOLISM

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*“Given free will but within certain limitations*

*I cannot will myself to limitless mutations”*

(Wyatt, Kramer)



## ABSTRACT

The androgenic steroid hormones are essential for the physiological development of the prostate gland and are also implicated in pathological processes such as benign prostatic hyperplasia and prostate cancer. The systemic effect of endocrine activity in the prostate gland has so far been considered of minor importance. The overall aim of this thesis is to further investigate the role of the prostate in androgen bio-activation and inactivation and relate these processes to prostate cancer susceptibility.

Conjugation by UDP-glucuronosyl transferases (UGTs) is considered a major inactivation route for androgens in the prostate. Of these UGT2B15 and UGT2B17 are expressed in the prostate. Functional polymorphisms have been identified in both of these enzymes and were associated with intraprostatic androgen glucuronide levels (Paper IV). In paper I we investigated the UGT2B17 deletion polymorphism and found a 2.2-fold increased risk for prostate cancer for individuals with the deletion allele. In order to evaluate these findings we repeated the genotype association in a considerably larger population-based case-control study (paper II). In contrast to our previous results no association between the UGT2B17 deletion polymorphism and prostate cancer rate was found.

The dihydrotestosterone (DHT) metabolite 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol (3 $\beta$ Adiol) has been proposed as an intraprostatic ligand of the estrogen receptor  $\beta$  (ER $\beta$ ) promoting anti-proliferative activity. For the first time significant 3 $\beta$ Adiol levels were detected in human prostate tissue (paper IV). The cytochrome P450 7B1 (CYP7B1) enzyme is a putative regulator of intraprostatic 3 $\beta$ Adiol levels. In paper III we demonstrated increased CYP7B1 protein expression in malignant areas in the prostate and that the CYP7B1/ER $\beta$  mRNA ratio was increased in prostate cancer compared to benign tissue. The transcriptional activity of the CYP7B1 gene is regulated by methylation.

Many studies have investigated the association between circulating sex hormones and prostate cancer risk, but the conclusion from these studies may be wrong since the correlation between intraprostatic androgen metabolism and systemic androgens is poor. In paper IV we measured peripheral and local peri-prostatic androgen and gonadotropin levels in men undergoing radical prostatectomy (RP) for localized prostate cancer. There was an almost two-fold higher DHT concentration and a fifteen percent lower luteinizing hormone (LH) concentration in local prostatic serum compared to peripheral serum levels. A significant positive correlation between prostate weight and local DHT levels was observed. The correlation studies also indicate that 28 % of the systemic DHT variance can be explained by prostatic DHT production.

In paper V we investigated the serum and urine hormonal changes after RP. We measured serum levels of testosterone, DHT, sex hormone binding globulin (SHBG), luteinizing hormone LH, follicle stimulating hormone (FSH) and inhibin B preoperatively and 90 days postoperatively. Steroid urine profile was also determined pre- and postoperatively in 18 patients. There were significant increases in serum LH and FSH and a 13 % decrease in DHT levels. Urinary levels of DHT glucuronides (DHT-G) decreased by 67% while Androsterone-G and 3 $\alpha$ Adiol-G increased. Inhibin B levels correlated inversely with both FSH and LH. Thus RP leads to significant DHT decrease in both serum and urine and we conclude that the observed increase in gonadotropin levels is a consequence of the DHT changes.

# LIST OF PUBLICATIONS

This thesis is based on the following papers, referred to in the text by their Roman numerals:

- I. Karypidis AH, **Olsson M**, Andersson SO, Rane A, Ekström L. Deletion polymorphism of the UGT2B17 gene is associated with increased risk for prostate cancer and correlated to gene expression in the prostate. *Pharmacogenomics J.* 2008; 8:147-51
- II. **Olsson M**, Lindström S, Häggkvist B, Adami HO, Bälter K, Stattin P, Ask B, Rane A, Ekström L, Grönberg H. The UGT2B17 gene deletion is not associated with prostate cancer risk. *The Prostate* 2008; 68:571-5
- III. **Olsson M**, Gustafsson O, Skogastierna C, Tolf A, Du Rietz B, Morfin R, Rane A, Ekström L. Regulation and expression of human CYP7B1 in prostate. Overexpression of CYP7B1 during progression of prostatic adenocarcinoma. *The Prostate* 2007; 67:1439-46
- IV. **Olsson M**, Ekström L, Belanger A, Rane A, Gustafsson O. Correlation between circulatory, peri-prostatic and intra-prostatic androgen levels. Manuscript 2009.
- V. **Olsson M**, Ekström L, Schulze J, Kjellman A, Akre O, Rane A, Gustafsson O. Radical prostatectomy – influence on serum and urinary androgen levels. Manuscript 2009.

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## LIST OF ABBREVIATIONS

ADT	Androsterone
AR	Androgen receptor
CAPS	Cancer of the Prostate in Sweden
CYP	Cytochrome P450
DEL	Deletion
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
ED	Erectile dysfunction
ER	Estrogen receptor
FSH	Follicle-stimulating hormone
G	Glucuronide
GnRH	Gonadotropin-releasing hormone
HGPIN	High-grade prostatic intraepithelial neoplasia
HSD	Hydroxysteroid dehydrogenase
INS	Insertion
LH	Luteinizing hormone
mRNA	messenger ribonucleic acid
PCPT	Prostate Cancer Prevention Trial
PCR	Polymerase chain reaction
PSA	Prostate specific antigen
RP	Radical prostatectomy
SHBG	Sex hormone binding globulin
UGT	UDP-glucuronosyl transferase
3 $\alpha$ Adiol	5 $\alpha$ -Androstane-3 $\alpha$ ,17 $\beta$ -diol
3 $\beta$ Adiol	5 $\alpha$ -Androstane-3 $\beta$ ,17 $\beta$ -diol



# 1 INTRODUCTION

## HISTORY OF ANDROGENS

The first androgen, isolated from human urine in 1929, was androsterone. However, it soon became apparent that this was not the testicular hormone and in 1935 David and Laqueur proudly reported the isolation of testosterone from several tons [sic] of bull testes. In 1939 the Nobel Prize in chemistry was, somewhat unjustly one might reflect, awarded to Butenandt and Ruzicka for the characterization and synthesis of testosterone. From 1935-68, testosterone secreted by the testes and the major androgen in the circulation of men, was considered to be the only male hormone. By injecting radioactive testosterone into rats and removing their prostates, Bruchovsky and Wilson were able to demonstrate that the predominant steroid recovered from nuclei was not testosterone, but its  $5\alpha$ -reduced derivative, dihydrotestosterone (DHT).

## PROSTATE CANCER

### Incidence

Prostate cancer is the most common malignancy among Swedish men with 8870 new cases diagnosed during 2007, accounting for 34.2 % of all male cancers. Between 1990 and 2004 there was a dramatic increase in the incidence of prostate cancer from 4788 to 9962 new cases per year, but since then the number of new cases has been relatively constant (Cancer Incidence in Sweden 2007, National Board of Health and Welfare, ([www.sos.se](http://www.sos.se))). In contrast to the increase in incidence, the age-adjusted mortality of the disease has remained relatively constant, ranging between 60-75 deaths/100 000 men and year during 1987-2006. In the year 2006, 2473 Swedish men died from prostate cancer and 1421 of them were 80 years or older at the time of death (Cause of death 2006, National Board of Health and Welfare). With the wide-spread use of prostate specific antigen (PSA)-testing younger patients are being diagnosed at an earlier stage which results in a stage migration towards smaller, organ confined tumours [1, 2].

### Staging and Grading

The clinical stage of the tumour extension in prostate cancer patients is assessed using the tumour-node-metastases (TNM)-classification (AJCC/UICC 1997). In short, primary tumour category (T) is estimated with digital rectal examination and classified into either of 4 stages: T1; no palpable tumour, diagnosis based upon specimen after transurethral resection (T1a-b) or biopsy after elevated PSA (T1c), T2; palpable tumour confined to the prostate, T3; palpable tumour extending outside the prostate capsule or seminal vesicles and T4; tumour extending on other organs such as the rectum or the urinary bladder. The N category is defined as either N0; no pelvic lymph-node involvement, N1; pelvic lymph-node involvement or NX; not examined. The M category is defined as either M0; no metastasis, M1; evidence of metastasis (usually bone involvement) or MX; not examined.

In Sweden, fine needle aspiration cytology was the traditional method for establishing prostate cancer diagnosis. The material obtained can be classified according to cytological criteria into well-, moderately-, or poorly differentiated (high-grade) adenocarcinoma (WHO-grading). However, with the introduction of core biopsies conventional histological techniques for diagnosis can be applied. Gleason's grading system is by far the most wide-spread classification. It defines five patterns of glandular

morphology and its relation to the surrounding stroma. Each cancer is awarded a primary grade for the predominant cancer grade in the specimen and a secondary grade, both ranging from 1 to 5. The 2 grades are added to provide the final Gleason score ranging from 2 to 10 [3]. In the Swedish national prostate cancer register less than 1 % had a missing Gleason score in patients with localized tumour in the year 2005. During the period 1996-2005 the category with Gleason score 2-4 has decreased dramatically, probably due to a change in the perception among pathologists. This change in grading diminishes the utility of the Gleason score as a prognostic marker and makes comparative studies over time more difficult [2].

## **Prostate specific antigen**

The glycoprotein PSA has been used as a serum marker in the management of prostate cancer since the 1980's. Since other prostatic diseases such as inflammation and benign prostatic hyperplasia can increase PSA and prostate cancers with poor differentiation can present without PSA elevation the diagnostic and prognostic reliability is limited. PSA-kinetics, i.e. PSA changes with time, has been proposed to overcome these limitations. PSA-velocity is based on the assumption of a linear PSA increase, PSA-doubling time assumes an exponential increase [4]. Combinations of clinical stage, Gleason score and PSA are commonly used to stratify patients into risk groups and to predict outcome after treatment [5].

## **Risk factors**

Established risk factors for prostate cancer are age, ethnicity and family history. The incidence of prostate cancer varies widely between ethnic populations and rates of this disease differ by as much as 60-fold between populations. The lowest rates are usually in Asia (China 2.3 per 100 000 per year) and the highest rates are in North America and Scandinavia, especially in African-American people in the USA (137 per 100 000 per year) [6]. These differences has been attributed to a combination of underlying differences such as genetic susceptibility, exposure to unknown external risk factors, or reasons such as cancer registration [7]. First-degree relatives of patients with prostate cancer have a 2-3-fold increased risk for developing this disease. The risk of developing prostate cancer in relatives increases with an increase in the number of affected individuals in the family and with a decrease in the age at diagnosis of the index prostate cancer case [7].

## **SEX HORMONES**

### **Androgens**

The male hormone testosterone and its  $5\alpha$ -reduced derivative  $5\alpha$ -dihydrotestosterone (DHT) exert different actions during embryogenesis and puberty. Analysis of the rare autosomal recessive disorder that impairs the  $5\alpha$ -reductase type 2 enzyme was essential for elucidating the physiological roles of the two androgens [8]. Testosterone is responsible for i) regulation of luteinizing hormone (LH) secretion, ii) regulation of spermatogenesis and iii) the differentiation of the embryological Wolffian duct system to the epididymis, vas deferens and seminal vesicles. DHT stimulates the differentiation of the urogenital sinus and urogenital tubercle to male external genitalia and prostate. It is also necessary for most of the virilizing events at male puberty [9, 10]. Testosterone is synthesized primarily in the testes and to some extent via testosterone precursors originating from the adrenals, including androstenedione and dihydroepiandrosterone (DHEA). In the circulation the majority of the total testosterone is bound to carrier proteins: 45 % is bound to sex hormone binding globulin (SHBG), 50 % to albumin

and < 4 % is unbound (free testosterone) [11]. Testosterone is one of the major circulating androgens with a serum T/DHT-ratio of approximately 10:1 [12]. Unlike testosterone, DHT cannot be aromatised into estrogen. The irreversible conversion of testosterone to DHT is catalyzed by the enzyme 5 $\alpha$ -reductase (fig 1), which is present in at least 2 isoforms designated types 1 and 2, encoded by 2 different genes SRD5A1 and SRD5A2 [9]. The 5 $\alpha$ -reductase type 2 is the predominant enzyme in male urogenital tract, whereas type 1 is the major enzyme in liver and non-genital skin [13]. Recently a novel 5 $\alpha$ -reductase enzyme, designated type 3, was expressed in hormone refractory prostate cancer [14].

Testosterone and DHT act via the same intracellular receptor, the androgen receptor (AR) that is a ligand dependent transcription factor. The AR-ligand complex binds to androgen-response elements in the DNA and regulates transcription of androgen-regulated genes [15]. DHT has a greater affinity and a decreased rate of dissociation to the AR compared to the testosterone-AR complex [9]. The DHT-AR complex also appears to be more transcriptionally active [10]. It is not clear how the two androgens, via the same receptor, can mediate different physiological actions, but different mechanisms have been discussed. The two hormones may have different allosteric effects on the AR resulting in transcription of different genes or interaction with different coactivators and corepressors. Alternatively, DHT formation could serve to enhance a weak hormone signal in tissues with low androgen content [10, 16]. Non-genomic androgen pathways, i. e. steroid actions not mediated through the classical AR, offer a third alternative for differentiated androgen effects. Criteria indicating non-genomic effects include a rapid onset of action, effects elicited by androgen analogues that do not enter the cell and actions that are not blocked by AR-inhibitors. Non-genomic androgen effects have previously been demonstrated in the smooth musculature of coronary arteries [17, 18] and recently also in human cavernous tissue [19].

#### *Androgen regulation of the prostate*

The most striking evidence of the role of androgens in prostate pathology is the beneficial clinical effect of androgen deprivation in patients with advanced prostate cancer that was first described by Huggins and Hodges in the 1940's [20] and castration remains to be the first line treatment of metastatic prostate cancer. In animal models testosterone induces prostate tumors supporting the role of androgens in prostate cancer growth, proliferation and progression [21, 22]. Subsequently, the androgen hypothesis has been the most widely tested for prostate cancer in various epidemiological studies. However, in a recent collaborative analysis, data on serum concentration of sex hormones from 18 prospective studies, including 3886 cases of prostate cancer and 6438 control subjects, no association with the risk of prostate cancer was found. The analysis included serum concentration of androgens (testosterone, free testosterone, DHT), androgen precursors (dihydroepiandrosterone, androstenedione), androgen metabolites (androstenediol glucuronide) and estrogens [23]. The pooled analysis reported a modest inverse association between SHBG-levels and prostate cancer risk. Moreover, in a study from our group, patients with serum DHT above the median had a significant better prostate-cancer-specific survival than those with DHT below the median [24]. These contradictory associations between circulating sex hormones and prostate cancer risk have prompted the need for measuring intraprostatic tissue hormones and determine the relationship to their circulating correlates [16, 23].

### *Phase 1 intraprostatic androgen metabolism*

The testosterone in the prostate is rapidly converted to the more potent androgen DHT by the 5 $\alpha$ -reductase enzyme [25] thus amplifying the androgen signal. DHT is further metabolised to 5 $\alpha$ -androstane-3 $\alpha$ ,17 $\beta$ -diol (3 $\alpha$ Adiol), 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol (3 $\beta$ Adiol) and androsterone (ADT) (figure 1). All of these reactions are considered reversible [26]. ADT and 3 $\alpha$ Adiol are weak androgens [27], whereas 3 $\beta$ Adiol exert anti-androgenic activity via the estrogen receptor  $\beta$  [28].

In 1968 Bruchovsky and Wilson demonstrated that DHT is the major active androgen within the prostate [29]. Current methods of quantification of androgens in prostate tissue are based on radioimmunoassay or mass spectrometry methods. In prostate tissue the typical DHT/T ratio is 6:1 based on 14 studies reviewed by Marks et al. [30]. The relationship between circulating androgen levels and intra-prostatic androgen levels has not been extensively studied. There was no correlation between serum androgens and intra-prostatic androgens in studies on men with benign prostatic hyperplasia and prostate cancer [31], men with androgen deprivation treatment [32], men with testosterone replacement therapy [33] or men undergoing radical prostatectomy (RP) (Paper IV). Mohler et al studied 22 patients with prostate cancer undergoing androgen deprivation treatment and despite castrate levels of testosterone in serum residual androgens in prostate tissue were found at levels sufficient to activate the androgen receptor [32].

The adrenal biosynthetic androgen pathway has been implicated to play a role in castrated prostate cancer patients [34, 35] and led to the concept of total androgen blockade suggesting an efficacy of anti-androgen receptor blocker in combination with androgen ablation. Meta-analyses of 27 prospective randomized trials comparing total androgen blockade versus androgen ablation alone have however only showed a modest absolute 5-year survival benefit of 2-5 % [36-38].

### **Estrogen regulation of the prostate**

Estrogens were proven effective in the hormonal treatment of advanced prostate cancer at the same time as the benefits of castration were described [20]. Estrogen action may be viewed in two different ways, the systemic endocrine effects via the pituitary that indirectly lower androgen levels and the direct local effects exerted via intra-prostatic estrogen receptors. The human prostate expresses two different estrogen receptors, most commonly designated alfa (ER $\alpha$ ) and beta (ER $\beta$ ). In the normal prostate the ER $\alpha$  is restricted to stromal cells while the ER $\beta$  is predominantly expressed in luminal cells [39]. The ER $\alpha$  was expressed in 10-30% of the patients with high-grade prostatic intraepithelial neoplasia (HGPIN), a putative precursor of prostate cancer, and the most significant expression was found in metastatic lesions [40]. In a randomized phase IIb study on men with HGPIN, toremifene, an ER $\alpha$ -antagonist, reduced the prostate cancer incidence at one year compared to placebo [41].

The ER $\beta$  was cloned in 1995 by Gustafsson and co-workers [42]. Studies on ER $\beta$ -knockout mice indicate that the ER $\beta$  exhibits pro-differentiative and anti-proliferate actions in the prostate, counteracting the stimulatory effect of the AR [43]. Weihua et al [28] demonstrated that 3 $\beta$ Adiol is an endogenous ER $\beta$  ligand (fig 1). 3 $\alpha$ -Hydroxysteroid dehydrogenase (3 $\alpha$ -HSD), 3 $\beta$ -Hydroxysteroid dehydrogenase (3 $\beta$ -HSD) and Cytochrome P450 7B1 (CYP7B1) are three enzymes that are putative

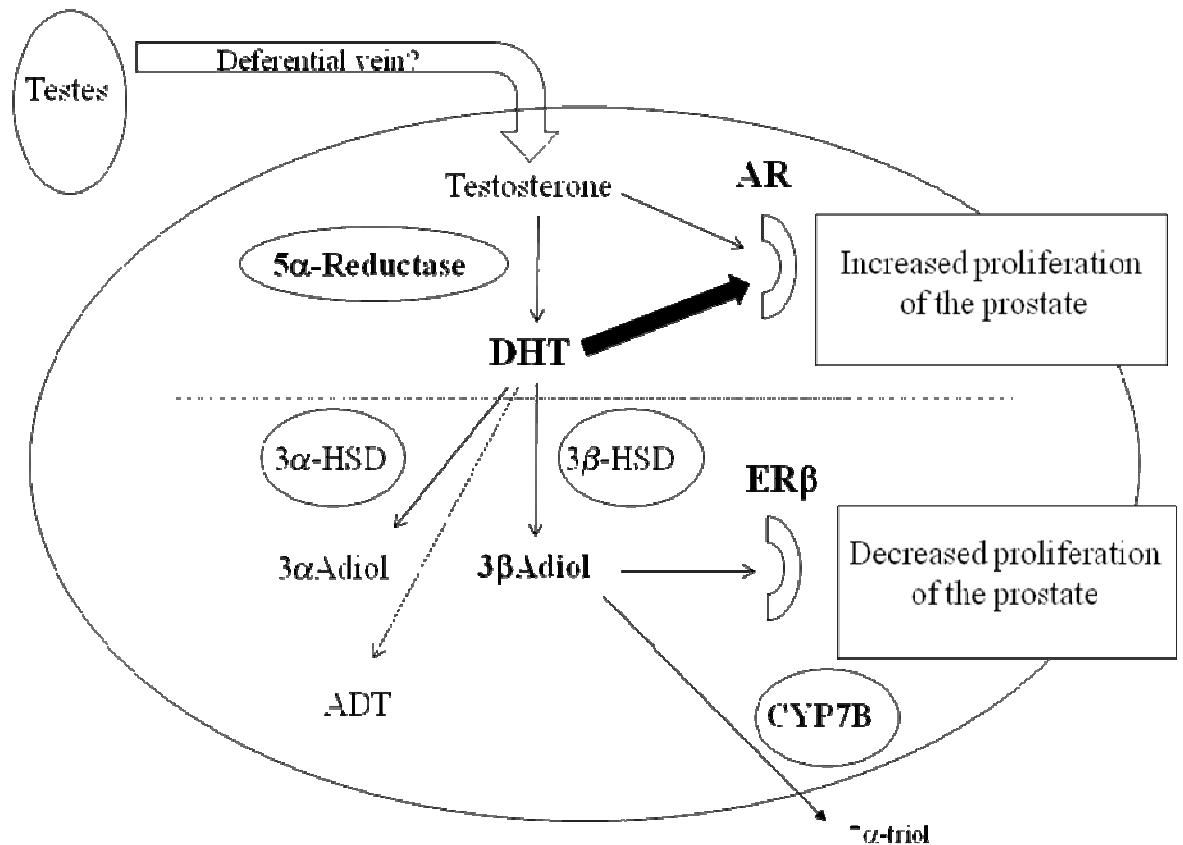


Figure 1: Phase 1 androgen metabolism within the prostate gland. Testosterone enters the prostate and is rapidly converted to DHT by 5 $\alpha$ -reductase. Testosterone and DHT exert their androgenic effects via the androgen receptor (AR) whereas the DHT metabolite 3 $\beta$ -Adiol functions as an estrogen agonist via the estrogen receptor  $\beta$  (ER $\beta$ ).

determinants of the amount of 3 $\beta$ Adiol available for ER $\beta$  and hence affect the regulation of prostate proliferation (figure 1). During recent years an increasing number of studies on ER $\beta$ -expression in human prostatic tissue have been published. In the initial studies the overall impression was that ER $\beta$  mRNA and protein expression decreases in cancer tissue compared to normal tissue [44-46]. Leav et al demonstrated loss of ER $\beta$ -expression in HGPIN, expression in the majority of Gleason 3 cancers whereas the expression was lost with increasing Gleason score. Moreover, in both lymph node and bone metastasis the ER $\beta$  was once again expressed [47]. In contrast, Fixemer et al reported strong ER $\beta$ -expression in 23% of their HGPIN-cases and a strong expression in the majority of the primary cancers and metastases. However, a markedly decrease in protein expression in androgen-insensitive cancer was noted in their study [48]. The ERs can form homo- or heterodimeric signalling complexes and may interact with the AR and further studies are needed to clarify their biological functions and their role in prostate cancer development and progression.

## GENETICS

### Genetic variation

The human genome contains approximately 2.85 billion base pairs encoding roughly 25,000 genes but only 0.1% differs between individuals. The most frequent genetic alterations are single nucleotide polymorphisms (SNPs) but deletion and insertions of various lengths are also common. It has been proposed that the etiology to common

diseases with a heritable component (e.g. prostate cancer) is likely to involve multiple genetic variants with low to moderate risk combined with environmental factors [49]. Interestingly several independent studies on different populations have shown that SNPs situated at chromosome region 8q24 are associated with prostate cancer (as well as other cancers) risk [50-54]. The phenotypic consequences of these genotypes have yet to be explored.

Several genetic association studies on prostate cancer and genes involved in the metabolism of sex-hormones have been performed during the last decade. The androgen receptor (AR) contains two polymorphic tri-nucleotide repeats located in the transactivation domain, one poly-glutamine (CAG) repeat where fewer repeats results in higher transcriptional activity [55] and one polyglycin (CGN) repeat. The CAG-repeat polymorphism has been associated with prostate cancer risk in several studies but in a recent meta-analysis of 19 case-control studies only a modest association (OR=1.19; 95%CI 1.07-1.31) was found and the biological impact was questioned [56]. Similar results were found for the CGN-repeat polymorphism. In a case-control study within the Cancer Prostate in Sweden (CAPS) study 46 selected polymorphisms previously reported to alter prostate cancer risk were assessed. Significant associations were found in 6 polymorphisms in 5 different genes of which 3 were key genes in androgen metabolism; the AR-CAG repeat, the CYP17 gene encoding cytochrome P450 17 $\alpha$ -hydroxylase and the SRD5A2 gene [57]. These associations were not consistent in different populations according to a recent review [58].

We investigated the association between the UGT2B17 deletion polymorphism (an important androgen metabolizing enzyme, see below) and prostate cancer risk in paper I and II.

## **Epigenetics**

The definition of epigenetic changes is heritable changes in gene expression that occur without changes in the DNA sequence. One type of epigenetic aberration is DNA methylation – the addition of a methyl group to the 5'-carbon of cytosine in CpG sequences – catalyzed by DNA methyltransferases [59]. DNA-regions including several CpG-sites are known as CpG-islands. Hypermethylation of CpG islands is a common feature of human cancer and is often associated with a decrease in transcription activity. Altered methylation frequencies of several gene promoters, e.g. the glutathione transferase P (GSTP1) [60], CYP1B1 [61], multidrug resistance 1 (MDR1) [62] and androgen receptor genes [63] have been associated with pathogenesis and progression of prostate cancer. GSTP1 is the most frequently methylated gene in prostate cancer. Hypermethylation of ER $\beta$  and CYP1B1 are main mechanisms for inactivation of ER $\beta$  and CYP1B1 expression in prostate cancer. DNA hypermethylation of a few genes have given promising results as potential tumour biomarkers for detection and risk assessment of prostate cancer [59].

## **ANDROGEN METABOLIZING ENZYMES**

### **CYP7B1**

The enzyme Cytochrome P450 7B1 (CYP7B1) is mainly expressed in the liver, but high levels are also found in the prostate, kidney and brain [64]. CYP7B1 is co-expressed with ER $\beta$  in the prostate [65] and may determine the 3 $\beta$ Adiol levels available at the ER $\beta$  and hence affect the regulation of prostate proliferation. A lower proliferation rate of the prostate has been found in CYP7B1-knockout mice compared



to the wild-type mice [66]. CYP7B1 also displays high catalytic activity towards the androgen precursor DHEA and via this pathway may be important for controlling intraprostatic androgen levels [65]. The CYP7B1 promoter includes a CpG island and it is possible that methylation-mediated regulation is involved in gene silencing [67]. In paper III we investigate the expression of ER $\beta$  and CYP7B1 in human prostatic tissue and show that CYP7B1 promoter methylation is a regulatory mechanism in the prostate.

### Androgen Phase-2 enzymes

Phase II catabolism, through sulfonation and glucuronidation by the liver or the kidneys, is the well accepted pathway for the inactivation and elimination of several endogenous and exogenous compounds (figure 2). The metabolic inactivation of steroids by sulfoconjugation is important in the regulation of intracellular steroid activity and the conversion of steroidal precursors to more potent steroids. The resulting conjugated products are more polar, generally less toxic and more easily excreted from the body than the parent compound [26]. Human cytosolic sulfotransferases (SULTs) are the major superfamily responsible for the sulfonation of numerous compounds [68]. The isoform SULT2B1b sulfoconjugates 3 $\beta$ -hydroxy steroids such as DHEA and 3 $\beta$ -Adiol [68] and is expressed in the epithelial cells of normal prostate, BPH and prostate cancer [69].

Conjugation by glucuronidation corresponds to the transfer of the glucuronosyl group from uridine diphospho (UDP)-glucuronic acid to small hydrophobic molecules. To date 18 human UDP-glucuronosyl transferases (UGTs) have been described. The UGT proteins have been categorized into two families UGT1 and UGT2, based on homology of primary structure, with UGT2 being subdivided into two subfamilies, UGT2A and UGT2B [26]. The human UGT2B enzymes, in particular UGT2B7, 2B15, 2B17 and 2B28 are currently thought to be the major enzymes for androgen conjugation [26]. Of these UGT2B15 and 2B17 have been identified in the prostate [70]. The intraprostatic glucuronidation of androgens leads to inactive metabolites and probably plays an important role in regulating intracellular androgen concentrations [70]. In paper V we investigate the role of the prostate in androgen glucuronidation.

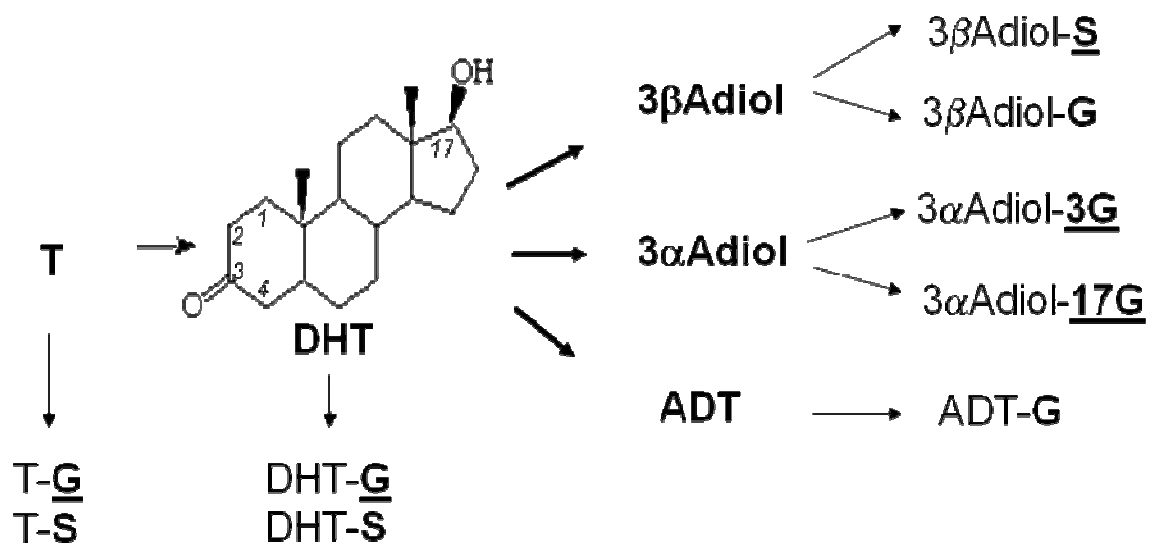


Figure 2. Phase II metabolism of androgen metabolites. Sulfonation and UGT conjugation occurs at the free hydroxy (-OH) group at position 3 and/or position 17.

### *UGT2B15*

UGT2B15 specifically conjugates the 17 $\beta$ -hydroxy position of 3 $\alpha$ -Adiol (high capacity) and DHT (moderate capacity). It is also capable of conjugating testosterone, but at a low capacity [71]. A polymorphism in the UGT2B15 gene, resulting in an aspartate (D) to tyrosine (Y) amino acid change at position 85, has been described [72]. Previous in vitro studies showed increased glucuronidation of 3 $\alpha$ -Adiol for the D85 variant [73] whereas Levesque et al demonstrated increased DHT conjugation of the Y85 variant [72]. Subsequently several case-control studies have investigated the association between the D85Y polymorphism of the UGT2B15 gene and the risk for prostate cancer. Three studies with different populations have demonstrated an increased risk for prostate cancer for individuals homozygous for the D85 allele compared to carriers of the Y85 allele [74-76]. Hajdinjak et al found no significant difference between the D and Y85 allele frequency in prostate cancer cases and controls, but the frequency of DD homozygotes increased with increasing Gleason score [77]. Gsur et al however demonstrated no association between the D85Y polymorphism and prostate cancer risk [78]. In paper IV the association between the UGT2B15 polymorphism and intraprostatic levels of glucuronide metabolites was investigated.

### *UGT2B17*

UGT2B17 shares 96% homology with UGT2B15 [71], but 2B17 has the capacity to glucuronidate both at the 17 $\beta$ -hydroxy and the 3 $\alpha$ -hydroxy position [70]. UGT2B17 has a higher capacity to conjugate testosterone and DHT than UGT2B15. UGT2B17 is highly expressed in the basal cells of the prostate [79]. A deletion polymorphism spanning the whole UGT2B17 gene has been identified [80]. In studies from our group the deletion genotype was seven times more common in a Korean population (66.7%) compared to a Swedish population (9.3%) and was associated with markedly decreased amounts of testosterone and DHT in urine [81]. In paper I and II the association between the UGT2B17 deletion polymorphism and prostate cancer was investigated and in paper IV the association between the deletion polymorphism and intraprostatic levels of glucuronide metabolites was determined.

## **5 $\alpha$ -reductase inhibitors**

In the 1970's the 5 $\alpha$ -reductase deficiency syndrome was described and out of this knowledge a selective 5 $\alpha$ -reductase type 2 inhibitor, N-(2-methyl-2-propyl)3-oxo-4-aza-5 $\alpha$ -androst-1-ene-17 $\beta$ -carboxamide (thankfully commonly known as finasteride), was developed in the late 1980's. Finasteride treatment decreases plasma DHT levels by 65 % without a significant effect on plasma testosterone levels. Concurrently the intra-prostatic DHT levels decreases by 85 % while the testosterone levels increases 6-10 fold [82]. The therapeutic effect relies on a reduction of prostate volume. In Sweden, finasteride is currently approved for treatment of benign prostatic hyperplasia and androgenetic alopecia. Recently a dual 5 $\alpha$ -reductase type 1 and 2 inhibitor, dutasteride, was developed. Dutasteride suppresses serum DHT levels by approximately 90 % and intra-prostatic DHT levels by the same degree. The serum testosterone levels increases by 16 % and after 4 months treatment the intra-prostatic testosterone levels equals the DHT levels prior to treatment [83]. The most frequently reported adverse effect for 5 $\alpha$ -reductase inhibitors is sexual dysfunction [84]. The reported incidence of impotence was 8 %, decreased libido 6 % and ejaculation disorders 1 %.

### *The Prostate Cancer Prevention Trial*

The Prostate Cancer Prevention Trial (PCPT), involving nearly 19,000 men, randomized two groups to either finasteride or placebo [85]. The published results demonstrated a nearly 25 % reduction of prostate cancer prevalence in the finasteride group compared to men treated with placebo after 7 years. However a 25% increase in the more aggressive high-grade cancers (Gleason 8-10) was observed in the finasteride group. The question whether finasteride induces high grade cancer or if the results were due to bias has been much debated. Detection bias due to effects of finasteride on prostate volume has been proposed. Among patients who had prostatectomy in the PCPT trial (n=222, finasteride; n=306, placebo) the finasteride-associated increase in high-grade disease (Gleason score  $\geq 7$ ) remained (46.4% finasteride vs 38.6% placebo, p=0.10) [86]. Changes in AR and ER $\beta$  signalling have been presented as an alternative explanation. Finasteride treatment increases the intra-prostatic testosterone levels. Moreover, by reduction of intra-prostatic DHT levels, a reduction of the anti-proliferative ER $\beta$ -ligand 3 $\beta$ -Adiol has been predicted [66, 87]. The debate will surely continue but to date no evidence that finasteride prevents from high-grade prostate cancer has been produced. Currently a study investigating the prostate cancer preventive effect of dutasteride in a high risk population is ongoing but the final results have not yet been published [88].

### **HYPOTHALAMIC-PITUITARY AXIS**

The reproductive axis is generally organized into three levels: the hypothalamus of the brain, the pituitary gland, and the testis (figure 3). Hypothalamic neurons secrete gonadotropin-releasing hormone (GnRH) in a pulsatile fashion into a portal system leading to the anterior pituitary, stimulating the release of the 2 gonadotropins: luteinizing hormone (LH) and follicle-stimulating hormone (FSH). The gonadotropins act on the testis, where LH stimulates testosterone production by Leydig cells while FSH, through stimulation of Sertoli cells, supports spermatogenesis [89]. The knowledge of the androgen transport from the testes to the prostate in humans is limited and is discussed in paper V. The secretion of gonadotropins is controlled by the negative feedback action of gonadal steroids on the hypothalamus and the pituitary. Testosterone or its metabolites (e.g. estradiol) exert their action by slowing the hypothalamic pulse generator and consequently decrease the frequency of LH pulsatile release [90].

Apart from the gonadal steroids other factors influencing the hypothalamic-pituitary axis have been discussed. Inhibin is a disulphide-linked heterodimeric glycoprotein hormone consisting of  $\alpha$ - and  $\beta$ -subunits and is closely related to activin proteins. Inhibin A is undetectable in men with contemporary methods [91]. Inhibin B is mainly produced from testicular Sertoli cells in men and has been implicated as a marker for spermatogenesis and inhibits the production of gonadotropins, preferentially FSH [92, 93]. Activin stimulates gonadotropin secretion. The prostate has been suggested as a rich extragonadal source of inhibin B since the levels were increased in patients with benign prostatic hyperplasia and decreased after surgery [94]. Further, the inhibin  $\alpha$ -subunit has been suggested to be a tumour suppressor in the prostate [95]. Inhibin B levels in relation to RP and the potential influence of DHT on the HP-axis are discussed in paper V.

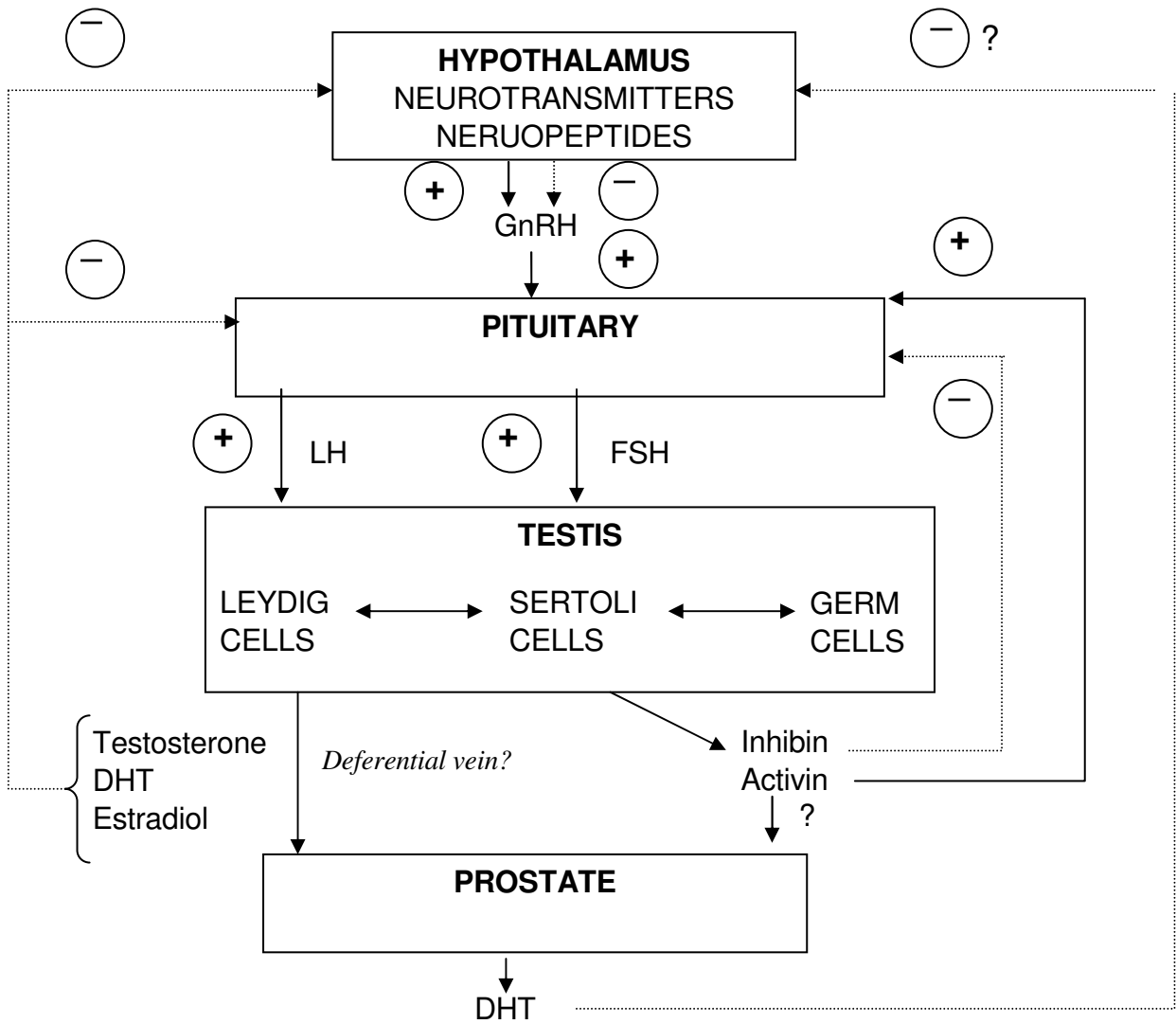


Figure 3: Diagram of the hypothalamic pituitary testis axis. (Modified from Schlegel PN, Hardy MP, Goldstein M: Male reproductive physiology, in Wein AJ, Kavoussi L, Novick AC, Partin AW, Peters CA: Campbell’s Urology, 9<sup>th</sup> Edition, WB Saunders, 2007)

## **MANAGEMENT OF LOCALIZED PROSTATE CANCER**

The first perineal prostatectomy was performed by Billroth in the late 19<sup>th</sup> century and was developed in early 20<sup>th</sup> century by Hugh Hampton Young. In 1947 the retropubic approach was introduced by Millin [96]. Although the operation offered excellent cancer control it did not gain popularity due to excessive blood loss and significant side effects such as urinary incontinence and erectile dysfunction (ED). In the 1980's Walsh described the anatomical approach to radical prostatectomy (RP), emphasizing 3 key points: i) control of the dorsal venous complex, ii) preservation of urethral sphincter complex and iii) preservation of the erectile nerves situated between the levator and prostatic fascia at the lateral border of the prostate [97]. Further developments including loupe-magnification, laparoscopic and robotic-assisted approaches have strived for minimizing side effects without losing oncological control.

Parallel with the improvement in surgery, radiation therapy became available as an alternative curative treatment. It was first given in 1909 using intraurethral radium capsules and a few years later perineal radium needles were introduced. Both of these methods used the concept of placing the radioactive source close to the target, commonly known as brachy therapy. In the 1950's external beam radiation therapy (EBRT) was introduced. Currently in Sweden the most common form of radiation therapy is either EBRT in combination with high dose-rate brachy therapy using temporary iridium isotope for larger or poorly differentiated tumours or low dose-rate brachy therapy using permanent iodine or palladium isotope for low risk tumours [98].

Since low grade, low volume prostate cancer often has a slow progression rate the option of watchful waiting has long been advocated in these patients. In recent years this has been replaced by the concept of active surveillance where patients are monitored with PSA, PSA-kinetics and repeated prostate biopsies. Delayed definitive therapy is offered if signs of progression occur or on patients request [99, 100]. However in large prospective trials a significant proportion of patients (21 to 39%) required further interventions and no consensus on the optimal active surveillance protocol is currently present [101].

The randomized Scandinavian prostate cancer group-4 trial showed a 5.4% prostate cancer specific survival benefit for surgery compared to watchful waiting at a median follow-up of 12 years [102]. No other randomized trial comparing the different modalities is currently at hand making the management of this patient group a clinical challenge. In Sweden, during the period 1996-2005 the absolute number of RP increased from 215 to 2363 and the age-standardized incidence rate for the procedure showed a fivefold increase [2].

### **Androgens and sexual function**

Despite advances in surgical technique erectile dysfunction (ED) is still a common side effect after RP. In a recent review from the American Urological Association Prostate Cancer Guideline Update Panel the conclusion was that clinical studies reporting erectile function outcomes after localized prostate cancer treatment often demonstrate poorly interpretable and inconsistent manners of assessment as well as widely disparate rates of ED [103]. Complete or partial ED following RP was reported in a wide range between 16-100 %. Our clinical observation is that apart from the pure ED many patients also suffer from a reduced libido. It is generally believed that the loss of sexual function after RP is mainly due to surgical damage to the neuro-vascular bundles, leading to hypoxia-induced fibrosis of the corpora cavernosa and that androgen

deficiency plays a minor role [104, 105]. In paper IV and V we investigate the circulatory and local peri-prostatic hormonal changes in relation to RP.

## 2 THE PRESENT STUDY

### AIMS

With this background the aims of this PhD work were as follows:

- To characterize the functional consequences of the UGT2B15 Y85D and the UGT2B17-deletion polymorphism in human prostatic tissue (papers II and IV)
- To test the UGT2B17-deletion polymorphism for its association to prostate cancer risk in two case-control studies (papers I and II)
- To evaluate the role of the ER $\beta$ -receptor and its internal ligand 3 $\beta$ -Adiol in human prostatic tissue (papers III and IV)
- To identify potential biomarkers for intra-prostatic androgenicity (papers III and IV)
- To evaluate the role of the prostate gland in:
  - DHT formation and excretion (papers IV and V)
  - its influence on systemic endocrine effects (papers IV and V)

### 3 MATERIAL AND METHODS

#### Study population:

##### *Case-control study 1 (Paper I):*

This study was a population-based case–control study described in detail elsewhere [106]. Incident prostate cancer cases were recruited consecutively between May 1994 and February 1996 from either of three hospitals in the county of Örebro in Sweden. In total 176 cases (86%) agreed to participate. Controls were randomly selected every 3 months from the County population register and frequency matched for age (50–59, 60–69 and 70–79 years). They were asked to participate by mail and 161 (79%) individuals agreed to participate. Controls were not screened for prostate cancer. Characteristics of the study population are summarized in table 1.

Table 1: Characteristics for prostate cancer cases and controls in paper I.

	Cases (n=176 (%))	Controls (n=161 (%))
Mean age (range)	71 (51-79)	71 (51-79)
Moderately + well differentiated	154 (87.5)	
Poorly differentiated	22 (12.5)	
M0	132 (75)	
M1	36 (20.5)	
T1+T2	83 (47.2)	
T3+T4	90 (51.1)	
PSA≤19 (ng/ml)	80 (45.5)	
PSA≥20 (ng/ml)	95 (52.3)	

##### *Case-control study 2 (Paper II)*

Cancer of the Prostate Sweden (CAPS) is a population based case–control study that has been described in detail elsewhere [107]. The study base for CAPS included all men under the year of 80 living in central and northern part of Sweden and all men under the year of 65 living in south-eastern part of Sweden and Stockholm. All incident prostate cancer cases between March 2001 to October 2003 were identified. In total, 3,648 prostate cancer patients were identified and invited to the study and 3,161 (87%) agreed to participate. Control subjects were recruited concurrently with the cases and frequency matched according to the expected age distribution of cases (group of 5-year interval) and geographical region. A total of 3,153 controls were invited to the study and 2,149 (68%) agreed to participate. A blood sample and a questionnaire concerning risk factors for prostate cancer were collected for each participant. At the time of this study, DNA was available for 2,779 cases and 1,722 controls. We collected information about prostate cancer specific mortality for each case subject in CAPS through record linkage to the Swedish Cause of Death Registry. Subjects were followed until March 1st, 2007. The average follow-up time was 4.4 years (range 0.3 to 6.5 years). Characteristics of the study population are summarized in Table 2.



Table 2: Characteristics for prostate cancer cases and controls enrolled in paper II (CAPS).

CHARACTERISTICS*	CASES		CONTROLS	
	N= 2,779	%	N= 1,722	%
Age (years)				
≤ 59	555	20.0	282	16.4
60-69	1337	48.1	733	42.6
≥ 70	887	31.9	707	41.1
PSA levels, ng/ml				
< 4	141	5.2	1,418	82.4
4-9.99	950	35.4	238	13.8
10-19.99	635	23.6	40	2.3
20-49.99	439	16.3	20	1.2
50-99.99	217	8.1	3	0.2
≥ 100	304	11.3	2	0.1
T stage				
T0/TX	77	2.7		
T1	1,042	37.5		
T2	867	31.2		
T3	690	24.8		
T4	103	3.7		
N stage				
N0/NX	2,686	96.7		
N1-N3	93	3.3		
M stage				
M0/MX	2,513	90.4		
M1	266	9.6		
Gleason Score				
≤ 4	98	3.9		
5	280	11.0		
6	953	37.5		
7	766	30.1		
8	245	9.6		
9	177	7.0		
10	25	1.0		
Differential Grade				
GI/GX	1,898	68.3		
GII	571	20.5		
GIII	310	11.2		
Prostate Cancer Stage**				
Localized	1,593	57.3		
Advanced	1,186	42.7		

\* Characteristics were not available for all study participants.

\*\* Case subjects were classified as advanced cases if they met at least one of the following criteria: T3/T4, N+, M+, Gleason score of 8-10 or PSA level ≥ 50 ng/ml.

Both study population I and II are mainly of Caucasian ethnicity and the age distribution appears to be similar. However, there is a greater proportion of advanced prostate cancer cases in study population I compared to II with regard to primary tumour stage (T3+T4; 51.1 vs 28.5%), PSA above 20 ng/ml (52.3 vs 35.7%) and the presence of bone metastases (M1; 20.5 vs 9.6%). The proportion of poorly differentiated cancers is essentially the same. The most likely explanation to this is the time period for recruiting cases. The patients in study population I was recruited before the wide spread use of PSA-testing in Sweden (1994-96 vs 2001-2003) that resulted in a stage migration [2].

*Patients undergoing radical prostatectomy (Paper III, IV and V)*

Fifty-five healthy men, 54 to 73 years old, undergoing open radical prostatectomy for localized prostate cancer from March 2004 to June 2008 were enrolled in this prospective study. None of the patients had received any radiation or hormonal therapy before surgery. Patients with prescribed medication that might interfere with hormone levels were excluded (e.g. prednisone and 5- $\alpha$ -reductase inhibitors). Surgery was performed by either Ove Gustafsson or Mats Olsson. Descriptive clinical data for the full study group is presented in table 3.

Table 3. Clinical and pathological data for 55 patients undergoing radical prostatectomy

<b>Patient characteristics (n=55)</b>	
<b>Age</b>	
Mean (range)	63 (54-73)
<b>Preop. PSA concentration (ng/ml)</b>	
Mean (range)	10.04 (0.9-31)
<b>Postop. Gleason score</b>	
≤6	22
7	30
8-10	3
<b>Pathological stage</b>	
T2	32
T3	23

## **Genotyping and gene expression (I, II, III and IV)**

Genotyping of the study participants was performed by extracting genomic DNA from leukocytes. Total RNA was extracted from the prostate and converted to complementary DNA. The genomic DNA and complementary DNA were used as templates in real-time PCR analysis, using gene specific primers (UGT2B17 in paper I, IV and V; CYP7B1 and ER $\beta$  in paper III) and probes (for UGT2B17), and an endogenous control gene ( $\beta$ -actin in paper II, 18s in paper III and albumin in paper IV). The relative mRNA expression and the UGT2B17 copy number were calculated employing the  $\Delta\Delta$ CT formula [108]. Detailed information is provided in respective paper.

## **Methylation PCR**

In order to quantify the grade of methylation on CpG sites, methylation and unmethylation specific PCR (MSP and USP respectively) were employed. First prostatic genomic DNA was extracted and subjected to sodium bisulphite treatment, i.e. all C are converted to T unless methylated, and used as a template in PCR reactions including primers that distinguish between methylated (C) and unmethylated (T) cytosines. The PCR products were run on an agarose gel and quantified on a gel scanner. Using this approach only the methylation grade of 2 CpG sites (those corresponding to the 3'-end of each PCR primer) are determined. There are other methods (i.e. pyrosequencing) that can determine the methylation frequency in all the CpG sites included in the amplified product.

## **Immunohistochemistry**

In order to quantify and identify the location of the CYP7B1 protein in the prostate immunohistochemistry analysis was performed. We used a CYP7B1 specific antibody produced in mice with no cross-reaction with other human P450s [109]. Serial 2.5-mm thick sections of radical prostatectomy specimens from the patients described above were used. The immunohistochemical detection was performed at the Division of Pathology using a standard protocol as described in detail in paper III. Negative controls were performed using the same procedure but without adding the CYP7B1 antibody.

## **Serum sampling and analyses (IV and V)**

Serum samples were collected the day before and 90 days postoperatively in all men undergoing RP, except for inhibin B that was only analyzed in 44 men due to lack of serum. All the samples were collected between 8 and 12 am and stored immediately at -20°C. Additionally, we collected local prostatic serum samples during surgery from 25 men. We sampled the veins on the lateral border of the prostate, and not the dorsal venous complex, aiming to obtain serum representing intra-prostatic metabolism. These samples were also collected before any manipulation of the prostate had occurred in order to obtain basal androgen levels. Postoperatively the samples were stored at -20°C.

Testosterone and DHT was determined by radioimmunoassay (RIA) using commercial kits. SHBG, LH and FSH were determined by chemoluminescence enzyme immunoassay using commercial Immulite® kits. Concentrations of free testosterone were calculated from values for total testosterone, SHBG and a fixed albumin concentration of 40 g/l. Detailed information is provided in paper IV and V.

## **Urine analyses (V)**

Urine samples were collected the day before and 90 days postoperatively in 18 men undergoing RP. All the samples were collected between 8 and 12 am and stored immediately at - 20°C. Urinary unconjugated steroids (typically < 1% of glucuronide fraction) + steroid glucuronides were determined by gas chromatography-mass spectrometry after hydrolysis with  $\beta$ -glucuronidase as described [110] with minor modifications.[111] Within- and between-assay coefficients of variation were less than 7% and 10% respectively for all steroids analyzed.

## **Statistical methods**

The genotype associations were calculated with statistics based on the logistic regression analysis. A likelihood ratio test of a covariate based on the Cox proportional hazard model was utilized to test for prostate cancer specific death. The paired Student's t-test was used when comparing two related groups and the Mann-Whitney U test for unrelated groups. Correlation coefficients were calculated using the Spearman's rank-correlation or the Pearson correlation coefficient test depending on whether the variables assumed normal distribution or not.

## 4 RESULTS AND DISCUSSION

**Paper I. Deletion polymorphism of the UGT2B17 gene is associated with increased risk for prostate cancer and correlated to gene expression in the prostate.**

**Paper II. The UGT2B17 gene deletion is not associated with prostate cancer risk.**

In paper I and II the principal aim was the same: to test the UGT2B17 deletion polymorphism for its association to prostate cancer. Prostate cancer is generally considered a complex disease with a strong hereditary component and the hypothesis is that multiple genetic variants contribute with low to moderate risks. Subsequently the ideal design of a genetic association study would constitute of testing for multiple genetic variants in well powered, large scale studies. Another general problem with genetic association studies is that far from all polymorphic variants have well characterized phenotypic consequences. There were several reasons for regarding the UGT2B17 deletion polymorphism a good candidate gene for prostate cancer association:

- The UGT2B17 is a main enzyme for glucuronidation of androgens and their metabolites
- It is highly expressed in the prostate and may therefore be involved in regulating the intra-prostatic androgen load
- Recently our group showed that the deletion variant is associated with markedly decreased levels of urinary testosterone and DHT glucuronides [81]

In paper I we performed genotyping for the UGT2B17 deletion polymorphism in 176 cases and 161 controls. We used binary logistic regression for genotype association and found a significantly increased risk of prostate cancer for individuals either lacking one (OR 2.23, 95% CI 1.33-3.74) or two alleles (OR 2.12, 95% CI 1.34-3.34) compared to the reference group (homozygous for the insertion allele). Additionally, in order to determine the effect of the UGT2B17 deletion polymorphism in prostatic tissue specimen from 15 patients undergoing radical prostatectomy was collected and UGT2B17 mRNA expression was determined by real-time PCR. As expected, heterozygous individuals (ins/del) expressed significantly lower UGT2B17 mRNA in prostate tissue compared to homozygotes (ins/ins).

With the aim to confirm the findings in paper II, we genotyped the UGT2B17 deletion polymorphism in a population-based case-control study (CAPS) comprising 2,682 cases and 1,672 controls. We used a likelihood ratio test of a covariate describing genotype status (0 if at least one copy of the gene was present, 1 otherwise) based on an unconditional logistic regression to test for association between prostate cancer risk and UGT2B17 deletion.

In contrast to paper I there was no evidence of association between prostate cancer risk and deletion of the UGT2B17 gene (OR=1.01, 95% CI 0.83-1.23, P=0.91). Stratifying for disease severity or age of onset did not alter the results. Furthermore, the UGT2B17

gene deletion did not affect the risk for prostate cancer specific death (HR 1.25, 95% CI 0.91-1.73, P=0.18).

To date at least 4 studies have been published investigating the association between the UGT2B17 deletion polymorphism and prostate cancer risk, including paper I and II in this thesis [112, 113]. The results are summarized in table 4. Although the first studies demonstrated an increased risk for prostate cancer, with an OR of 1.7-2.2, the more recent studies with considerable larger study populations were unable to confirm this association. The most likely explanation for the contradictory results between study I and II is the large number of participants in the CAPS-study. CAPS has a 95 % power ( $\alpha=0.05$ , two-sided test) to detect a minimum OR of 1.4 for the UGT2B17 deletion polymorphism. In summary the UGT2B17 deletion polymorphism does not play a major role in prostate cancer susceptibility as previously indicated. This illustrates the importance of testing indicative results in large scale studies.

Table 4: Comparison between four different studies of the association between deletion of the UGT2B17 gene and prostate cancer risk.

Author	Genotype	Cases	Controls	OR (95%CI)	P
		Frequency (%)	Frequency (%)		
Paper I	Ins/Ins	82 (47)	104 (64)	1.00 (Reference)	
	Del/Ins	85 (48)	52 (32)	2.23 (1.33-3.74)	n. a.
	Del/Del	7 (4.0)	5 (3.1)	1.77 (0.40-7.82)	n. a.
	Del/Ins + Del/Del	92 (53)	57 (35)	<b>2.12</b> (1.34-3.34)	n. a.
Park et al. [112]	Ins/Ins + Ins/Del	331 (82)	426 (88)	1.0 (Reference)	
	Del/Del	75 (18)	56 (12)	<b>1.7</b> (1.2-2.26)	0.004
Gallager et al [113]	Ins/Ins	207 (49)	190 (48)	1.00 (Reference)	
	Ins/Del	168 (41)	159 (40)	0.99 (0.73-1.35)	n.s.
	Del/Del	42 (10)	48 (12)	0.89 (0.55-1.45)	n.s.
Paper II	Ins/Ins + Ins/Del	2,391 (89)	1,491 (89)	1.00 (Reference)	
	Del/Del	291 (11)	181 (11)	<b>1.01</b> (0.83-1.23)	0.91

n.a. not available, n.s. not significant

Another study from our group indicates that UGT2B17 del/del subjects express more hepatic UGT2B15 mRNA [114]. It is possible that a lack of UGT2B17 enzyme is compensated for by an increase in UGT2B15 transcription. The UGT2B15 D85 allele is less protective for intra-prostatic DHT levels due to decreased DHT conjugation in vitro [72] and since carriers of the D85 allele have increased risk for prostate cancer in some studies [74-76] it is possible that individuals with disadvantage genotypes in both the UGT genes may exhibit an increased prostate cancer risk. To date there is no case-control study that has investigated both the 2B15 and 2B17 polymorphisms. So it is possible that the UGT2B17 deletion polymorphism, in combination with other genetic variations, may play a role in prostate cancer susceptibility.

### **Paper III. Regulation and expression of human CYP7B1 in prostate. Overexpression of CYP7B1 during progression of prostatic adenocarcinoma.**

Cytochrome P450 (CYP) 7B1 is involved in many metabolic processes including androgen metabolism. CYP7B1 is expressed within the prostate and may determine the levels of the natural estrogen receptor  $\beta$  (ER $\beta$ ) ligand 5 $\alpha$ -androstane-3 $\beta$ ,17 $\beta$ -diol (3 $\beta$ Adiol) available and hence affect the regulation of prostate proliferation. We hypothesized that CYP7B1 expression is increased in prostate tumours and that promoter methylation contributes to the regulation of CYP7B1 expression in human prostate tissue. We investigated expression of the CYP7B1 gene and protein in clinical prostate tissue and prostate cancer cell lines using real-time PCR and immunohistochemistry. The methylation status of the CYP7B1 gene was analyzed using methylation-specific PCR (MSP). The difference in relative mRNA expression of CYP7B1 and ER $\beta$  was performed with a paired Student t-test. Spearman's rank-correlation coefficient was used to assess the association between the methylation frequency and CYP7B1 mRNA expression. The Mann-Whitney test was used for comparison between 5-aza and non-treated cells.

The immunohistochemical results demonstrate that high expression of CYP7B1 protein occurs in high-grade prostatic intraepithelial neoplasia (PIN) and adenocarcinomas. The ER $\beta$ /CYP7B1 mRNA ratio was significantly lower in tumour compared to the non-tumour area. The MSP analysis indicate that local methylation of CYP7B1 promoter region is an important mechanism involved in down-regulation of CYP7B1 in human prostate tissue. This is the first report showing that CYP7B1 is overexpressed in high-grade PIN and in prostate cancer and that local methylation of CYP7B1 promoter region may have significant effect on gene transcription. Methylation status of key regulatory genes has been proposed as prognostic markers for prostate cancer progression. Further investigations are needed to determine whether methylation status of the CYP7B1 gene may be useful in this aspect.

Preliminary analysis of our data indicates that CYP7B1 is a determinant of 3 $\beta$ Adiol levels. Correlation studies revealed that the CYP7B1 mRNA levels are significantly inversely associated with 3 $\beta$ Adiol concentration in the urine (figure 4) and in the prostate (figure 5).

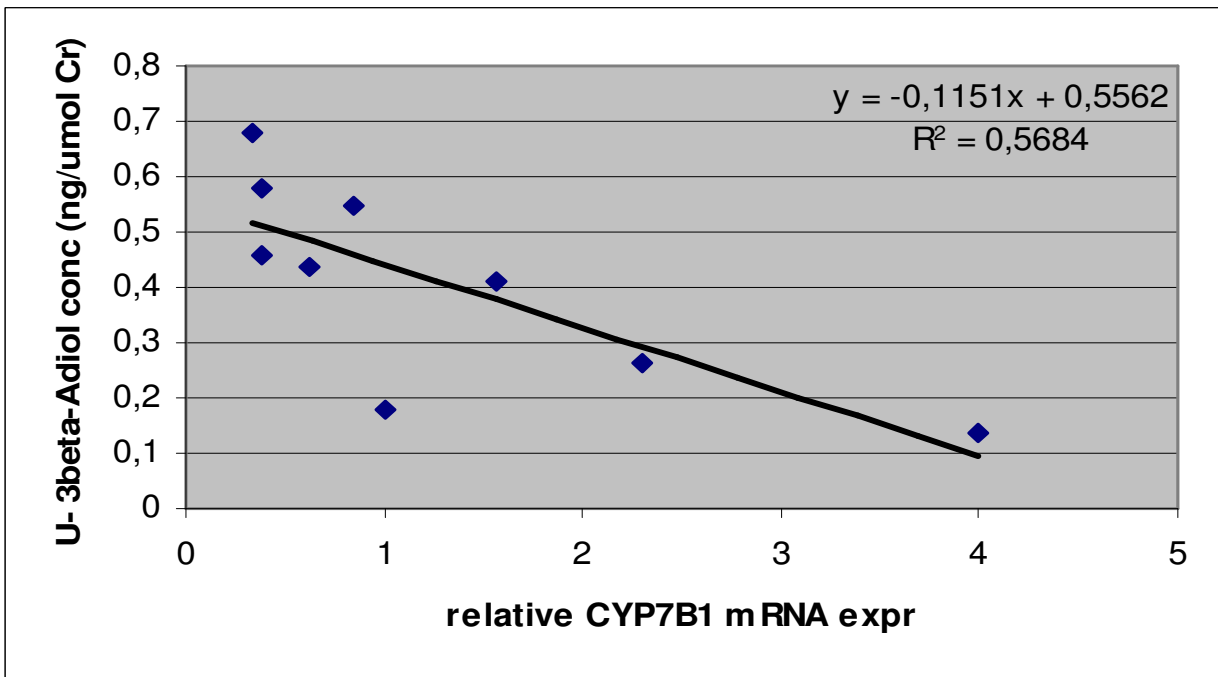


Figure 4: Correlation analysis of intra-prostatic CYP7B1 mRNA levels and 3βAdiol concentration in the urine (p=0.003). The 3βAdiol data are from paper V.

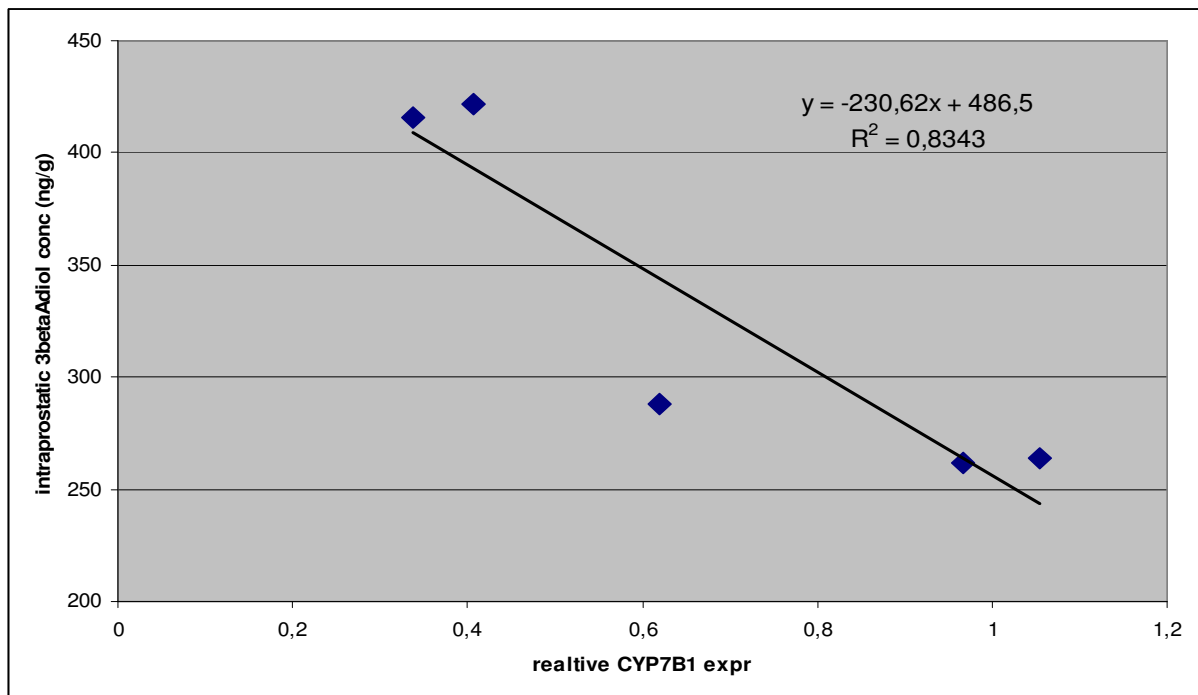


Figure 5: Correlation analysis of intra-prostatic CYP7B1 mRNA levels and 3βAdiol levels (p=0.0002). The 3βAdiol data is from paper IV.



## **Paper IV. Correlation between circulatory, peri-prostatic and intra-prostatic androgen levels**

In paper IV and V the overall aim was to investigate the hormonal changes during and after RP. The idea originated from an interest in prostatic diseases and a couple of clinical observations of common patients in the urology department:

- The prostate is generally considered a major site for DHT formation, the most potent androgen in the human body.
- Patients with enlarged prostates and obstructive voiding symptoms can be effectively treated with 5 $\alpha$ -reductase inhibitors, reducing the prostate volume and hence the intra-prostatic DHT concentration. In these patients decreased sexual function is the most commonly reported adverse event.
- In patients undergoing RP for prostatic cancer, erectile dysfunction is a common side effect. Our clinical observation, not systematically confirmed, is that additional to this many of the patients also have a reduced libido postoperatively.

During literature search it was evident that the hormonal changes after RP were not extensively studied. Historically, the prostate gland has been considered the target organ for testicular and non-testicular androgens and its systemic effect of endocrine activity has up to now been considered of minor importance. Furthermore the correlation between intra-prostatic and circulating androgen levels was generally considered weak.

The specific aim in paper IV was to test the role of the prostate gland for its influence on the systemic levels of DHT. We collected peripheral serum, serum from the local prostatic veins and prostatic tissue from 25 patients undergoing RP for localized prostate cancer. Our study design also permitted us to determine the association between UGT2B15 and 2B17 polymorphisms and the intra-prostatic levels of glucuronidated androgen metabolites.

There was an almost two-fold higher DHT concentration and a fifteen percent lower LH concentration in local prostatic serum compared to peripheral serum levels. There was no difference between local prostatic and peripheral serum for any other hormone measured, including testosterone. We found a positive correlation between local prostatic and peripheral serum DHT levels ( $r=0.53$ ,  $r^2=0.28$ ,  $p<0.006$ ). A significant positive correlation between prostate weight and local DHT levels was also observed ( $r=0.47$ ,  $r^2=0.221$ ,  $p=0.02$ ). No correlation was found between prostate weight and local free testosterone levels. There was no correlation between prostate weight and peripheral serum DHT.

This is the first study showing an almost two-fold higher DHT concentration in local prostatic serum compared to peripheral serum. The significant positive correlation between prostate weight and local DHT levels but not to local testosterone levels supports the evidence that the prostate gland is a main testosterone to DHT converter. The most significant finding in this study is the correlation that we found between local and peripheral DHT levels. We estimated that 28 % of the variance in peripheral DHT serum levels depends on prostatic DHT production. Thus, in contrast to previous conceptions [31, 33, 115], our data clearly indicates that local prostatic DHT production has an influence on systemic serum DHT levels.

The mean tissue androgen concentration are presented in table 3, paper IV. An interesting observation is that  $3\beta$ Adiol is expressed at 50% levels of ADT in the prostate, i. e. approximately in the same relation as observed in serum[116]. Our findings of high intra-prostatic concentration further support the theory that DHT metabolism generates estrogen-like activity and hence the inhibition of  $5\alpha$ -reductases not only protects from androgenic load but also inhibit the protective anti-androgen properties.

We found an association between the UGT2B17 deletion polymorphism and  $3\alpha$ Adiol-17G levels and between the UGT2B15 Y85D polymorphism and  $3\alpha$ Adiol-3G levels. Carriers of the Y85 allele had significantly higher intra-prostatic levels of  $3\alpha$ Adiol-3G. This result is not in agreement with the in-vitro findings that UGT2B15 displays preferable activity against 17-hydroxy androgens [71].

On the basis of the results in paper IV we conclude that the adult prostatic gland maintains an importance in DHT production and postulate that androgen metabolism in the prostate not only has an intracrine role but, more importantly, serves as a systemic and loco-regional androgen regulator.

## Paper V. Radical prostatectomy - influence on serum and urinary androgen levels

In paper V we investigated the influence of RP on serum and urinary androgen levels. The changes in hormonal serum levels before and one year after RP have previously been studied demonstrating a loss of feed-back regulation of the hypothalamic-pituitary axis (e.g. increased LH and FSH levels) [104, 117, 118]. The reason for the change in the hormone profile after RP is not known but Miller et al concluded that it was unlikely that DHT had an important role in the endocrine regulation of LH. Since the prostate has been suggested as a rich extragonadal source of inhibin B [94], this glycoprotein was suggested to cause the increase in gonadotropins levels observed after RP [104]. We determined serum hormone levels, including inhibin B prior to and after RP in 55 men undergoing RP for localized prostate cancer. In addition to DHT formation, the prostate gland is also important in androgen metabolism and inactivation by glucuronidation according to the intracrinology concept. Therefore we measured glucuronidated metabolites of testosterone and DHT in urine before and after RP.

Average change in hormone levels is shown in table 5. There were statistically significant increases in serum LH and FSH and 13 % decrease in serum DHT levels. There were no significant or important changes in total, free testosterone, inhibin B or SHBG levels.

Hormone (Mean ± SE)	Preop(N=55)	Postop(N=53)	Change (%)	p value
Testosterone (nmol/L)	13.7 ± 0.62	12.7 ± 0.61	0.89 ± 0.47 (-6.8)	n.s.
Free testosterone(nmol/L)	0.28 ± 0.012	0.26 ± 0.011	-0.019 ± 0.010 (-7.2)	n.s.
DHT (pg/ml)	482 ± 39.7	419 ± 35.7	-59.5 ± 26.3 (-13.0)	p=0.028
SHBG (nmol/L)	37.9 ± 1.9	37.3 ± 1.9	0.62 ± 0.85 (-1.6)	n. s.
LH (mIU/ml)	3.24 ± 0.32	4.97 ± 0.48	1.74 ± 0.29 (53.4)	p<0.0001
FSH (mIU/ml)	6.62 ± 0.88	8.04 ± 1.1	1.48 ± 0.25 (21.5)	p<0.0001
Inhibin B (ng/L) (N=44)	166.3±13.5	167.7 ±12.1	2.7±5.5 (1.6)	n.s.

**Table 5.** Preoperative, 90-days postoperative and overall change in hormone levels.

Four independent studies, including this study, have now demonstrated that RP will induce increased gonadotropin levels, indicating loss of feed-back regulation on the hypothalamic-pituitary axis [104, 117, 118]. The results from these studies are summarized in table 6.

Ref.	n	Months after RP	Hormone						
			LH	FSH	DHT	Testo	Estradiol	SHBG	Inhibin B
[104]	63	12	↑	↑	↓	↑	↑	UC	n.d.
[117]*	70	12	↑	↑	↓	↑	n.d.	n.d.	n.d.
[118]	49	12	↑	↑	n. d.	UC	n.d.	n.r.	n.d.
Paper V	55	3	↑	↑	↓	UC	n.d.	UC	UC

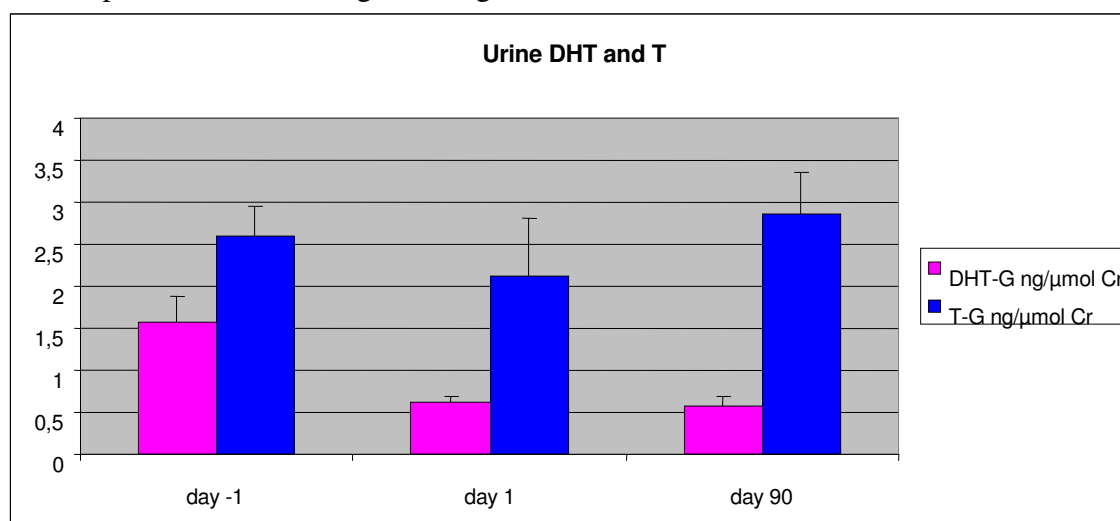
Table 6. Serum hormonal changes 3 and 12 months after radical prostatectomy in 4 independent studies: ↑ = significant increase, ↓ = significant decrease, UC = no significant change, n.d. = not determined, n.r. = not reported. \* Only abstract available in English language.

In contrast to 2 previous studies, demonstrating an increase in testosterone after RP [104, 117], we and Madersbacher et al [118] found no changes in testosterone levels after prostatectomy. Postoperative levels were measured one year after RP in the 3 other studies, whereas we collected our samples after 90 days (table 6). This difference

could explain the contradictive results, although the LH regulation of testosterone production is generally considered an early onset feed-back function. The reason for the possible long lag-time (months) before the testosterone level increase is not known. As a limitation in our study no serum samples were collected at 1 year after RP.

This is the first study to measure serum levels of inhibin B before and after RP, and we found that a prostatectomy appears to leave the inhibin B level unchanged. Furthermore, our and previous studies [119-122] support the fact that DHT affects the hypothalamic pituitary axis (as discussed in paper V). Therefore, we hypothesize that the minor reduction (15 %) in circulatory DHT after RP may lead to a decrease of negative feed-back on the hypothalamic and/or pituitary level. This will lead to a compensatory increase in the pulsatile release of gonadotropin releasing hormone (GnRH) and explains the subsequent increase in both the LH and FSH levels observed (figure 3). Thus, our data are in agreement with the results of Miller et al, but in conflict with their interpretation.

Urinary levels of DHT glucuronides (DHT-G) decreased by 66 % after prostatectomy (figure 6) whereas the DHT metabolites ADT-G and 3 $\alpha$ -Adiol-G both increased significantly by 37% and 22%, respectively. There were no significant alterations by RP in the urinary levels of the other steroids investigated, including testosterone (figure 6). The urinary and serum data together indicate that glucuronidation of DHT (into DHT-G) occurs mainly in the prostate. The large decrease of urinary DHT-G cannot solely be explained by the decrease in serum DHT levels. This is the first report of an extra-hepatic tissue with a significant glucuronidation in vivo.



**Figure 6.** Preoperative and 1 and 90-days postoperative urinary levels of DHT and testosterone glucuronides.

It is generally believed that androgen deficiency plays a minor role in the loss of sexual function after RP since the testosterone levels are normal or increased [104, 105]. RP will however induce a circulatory androgen pattern resembling men treated with 5 $\alpha$ -reductase inhibitors where the DHT levels are decreased but the testosterone levels are normal or increased. In these men sexual dysfunction is the most frequently reported adverse effect [82, 84]. This iatrogenic DHT deficiency may have a negative effect on local androgen dependent tissues such as erectile nerves and corpora cavernosa or libido centra. Subsequently, in contrast to previous authors [104], we hypothesize that the DHT deficiency observed after RP may partly explain the loss of sexual function.

We found that the prostate is important in DHT formation and excretion and that RP leads to a significant decrease of DHT concentrations in serum as well as in urine. We also conclude that the observed increase in gonadotropin levels may be a consequence of the DHT changes.

## 5 CONCLUSIONS

- The UGT2B17 deletion polymorphism is associated with decreased UGT2B17 mRNA expression in prostatic tissue
- The UGT2B15 D85Y and the UGT2B17 deletion polymorphisms are associated with androgen glucuronide levels in prostatic tissue
- The UGT2B17 deletion polymorphism does not have a major impact on prostate cancer susceptibility as previously indicated
- The CYP7B1 mRNA and protein are over-expressed in premalignant and malignant prostatic tissue and its transcriptional expression is regulated by DNA-methylation
- 3 $\beta$ Adiol, an anti-proliferative ER $\beta$ -ligand, is present at 50 % levels of ADT in human prostatic tissue and the enzyme CYP7B1 appears to be a determinant of 3 $\beta$ Adiol levels
- The prostate is a major site for DHT formation and maintains an important loco-regional and systemic function for regulation of androgens in adult life
- The peri-prostatic DHT levels are correlated to prostate weight as well as systemic DHT levels
- Complete removal of the prostate gland results in loss of negative feed-back on the hypothalamic-pituitary axis presumably due to decreased circulatory DHT levels
- The prostate is the major DHT glucuronidation “factory” in humans

## **6 GENERAL DISCUSSION AND PROPOSED FUTURE DIRECTIONS**

### **EPIGENETIC STUDIES**

To further evaluate the role of CYP7B1 methylation in prostate carcinogenesis, intra-prostatic and inter-individual comparison of the methylation degree will be studied in normal and cancer tissues obtained from whole mount section paraffin embedded tissues. Prostatic genomic DNA will be extracted followed by quantitative methylation PCR/sequences analysis. We will correlate differences in methylation degree of the CYP7B1 promoter to Gleason score and survival to evaluate if the methylation degree may have prognostic value.

Prostatic DNA can also be prepared from urine after digital rectal palpation. The methylation grade of several genes from urinary DNA has been shown to function as promising prognostic biomarkers [59]. Subsequently we will determine the CYP7B1 (as well as other androgen metabolising enzyme genes) methylation status in urine from prostate cancer patients that are eligible for RP.

### **THE PROSTATE AS AN ANDROGEN PROCESSOR**

The concept of the prostate gland as a processor of androgens needs further investigations. Surprisingly, we found a 15 percent lower mean level of LH in local prostatic serum compared to peripheral serum indicating that LH may act as a ligand inside the prostate or in periprostatic tissue (paper IV). Expression of LH receptors within the prostate has been described, although the exact biological role is still unknown [123]. Our findings indicate that intra-prostatic androgen metabolism may be regulated from the pituitary.

Knowledge of the androgen transport from the testes to the prostate in humans is limited. Previous studies in dogs have indicated that the concentration of androgens in the deferential vein is 15 times higher compared to peripheral plasma concentration and that this may serve as a direct transporting system of androgens from the epididymis to the prostatic complex [124, 125]. The androgen concentration in the human deferential vein has never been measured. The decreased levels of DHT-G in urine (paper V) together with the absence of a testosterone gradient between local prostatic and peripheral serum (paper IV) indicate that testicular androgens may be processed in the prostate similar to a first-pass metabolism. If humans exhibit the same high concentration androgen transport via the deferential vein, our findings indicate that the prostate serves as a determinant for systemic testosterone levels.

### **INTRAPROSTATIC ANDROGENICITY**

Given the complexity of the intra-prostatic androgen metabolism it is evident that androgenic action within the prostate is determined not only by the concentration of androgens. The intracrinology concept suggests that locally produced androgens might exert their action in the same organ in which their synthesis takes place without significant diffusion into the circulation. Factors such as 5 $\alpha$ -reductase activity, androgen receptor modulating proteins, the estrogenic activity of DHT metabolites and the activity of androgen inactivation enzymes must be taken in account. Therefore,

serum concentration of the glucuronidated androgen metabolite 3 $\alpha$ Adiol-G is commonly used as an indirect measure of intra-prostatic androgenicity. The concentration of 3 $\alpha$ Adiol-G in serum correlates with 5 $\alpha$ -reductase activity in genital skin. In men treated with 5 $\alpha$ -reductase type 2 inhibitor the serum levels of DHT and 3 $\alpha$ Adiol-G decreased concomitantly, indicating that serum levels of 3 $\alpha$ Adiol-G may predominantly reflect 5 $\alpha$ -reductase type 2 activity [58]. In a recent study serum levels of 3 $\alpha$ Adiol-17G were correlated to prostate volume, whereas neither serum testosterone nor DHT reflected the prostate volume [126]. Androgen analysis in prostate tissue samples may not reflect the total prostatic androgenicity. Such analyses may be associated with analytical problems, i.e. the texture, the amount of fibromuscular component, the proportion of epithelial cells and sample processing (as discussed by Hsing 2001 [127]). This could explain the lack of correlation between intra-prostatic and circulatory androgens (see above and paper IV).

In order to evaluate periprostatic androgens and androgen metabolites as biomarkers for intra-prostatic androgenicity we will collect periprostatic serum preoperatively by transrectal ultrasound and peroperatively in patients eligible for RP. We will analyze testosterone, DHT and relevant glucuronidated metabolites. Correlation between pre- and peroperative samples and between peri-prostatic androgenicity and severity of prostate cancer will be assessed.

## **DHT SUPPLEMENTATION**

DHT deficiency as a cause for sexual dysfunction after RP needs to be further investigated. Since the testosterone levels are unchanged or increased after RP, androgen deficiency has previously not been implicated in the pathophysiology of ED after RP [104, 105]. The DHT deficiency may be viewed in at least two different levels, the local depletion and the systemic endocrine effects. The prostate gland is generally considered a major site for DHT formation [13] and in a paper IV we demonstrated an almost 2-fold higher DHT level in local periprostatic serum compared to peripheral serum. A RP would thus leave androgen dependent tissues such as erectile nerves and cavernous tissue in a DHT depleted milieu. Accumulating experimental data provides evidence of androgen dependence for peri-prostatic tissues such as erectile nerves and cavernous tissue. Androgens are considered to control the expression and activity of nitric oxide synthase (NOS) isoforms and phosphodiesterase (PDE)-5 in penile erectile tissue [128, 129]. Both testosterone and DHT have been shown to induce non-genomic relaxation of human cavernosal arteries and corpus cavernosum [19]. In rats, DHT was responsible for maintenance of nitric oxide-mediated penile erections [130] and when castrated, the number of NOS-containing nerve fibers in corpora cavernosal and penile dorsal nerve was decreased [131].

A clinical observation is that, apart from pure erectile dysfunction, many of the patients have a reduced libido postoperatively and we postulate that the perturbed hormonal profile (paper IV and V) may play a significant role in this regard. In men with late-onset hypogonadism (LOH) reduced libido and ED are common features [132]. It was recently demonstrated that in these patients loss of libido occurs when testosterone levels fall below 15 nmol/L, while ED becomes manifest only when testosterone levels fall below 8 nmol/L [133]. It is well accepted that this loss of sexual function can be improved by testosterone replacement therapy [134]. In figure 7 we propose a new pathophysiological model for the sexual dysfunction after RP.



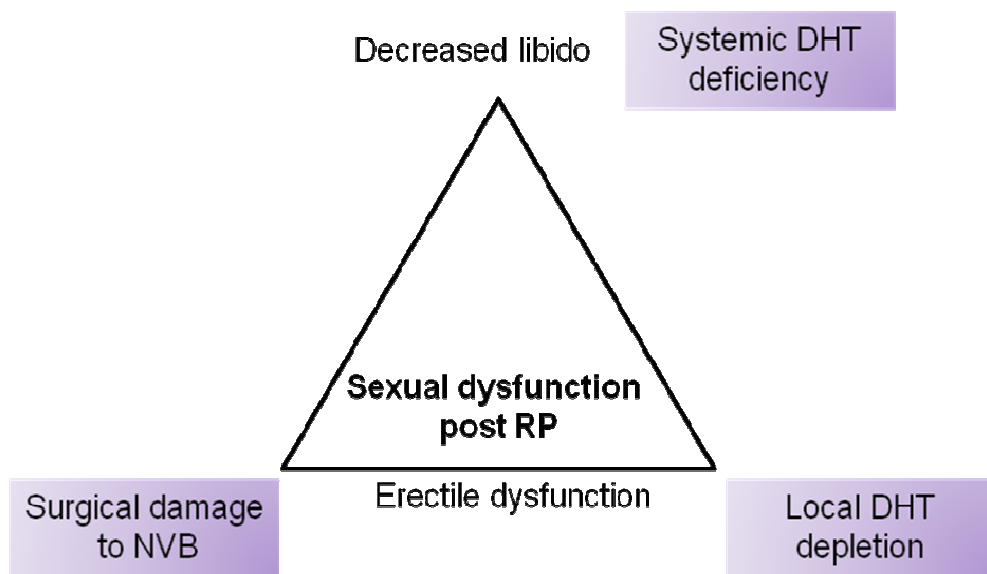


Figure 7: Proposed model for sexual dysfunction after RP. NVB= neuro vascular bundles.

To evaluate if RP-patients may benefit from DHT supplementation for restoring sexual function a clinical trial is planned.

## THE FINASTERIDE STUDY

The results from the Prostate Cancer Prevention Trial still remain puzzling, i.e. a 25 % reduction of low-grade prostate cancer but an increase in high-grade cancers. The proposed mechanism for changes in intra-prostatic AR and ER $\beta$  signalling are demonstrated in figure 8a and b.

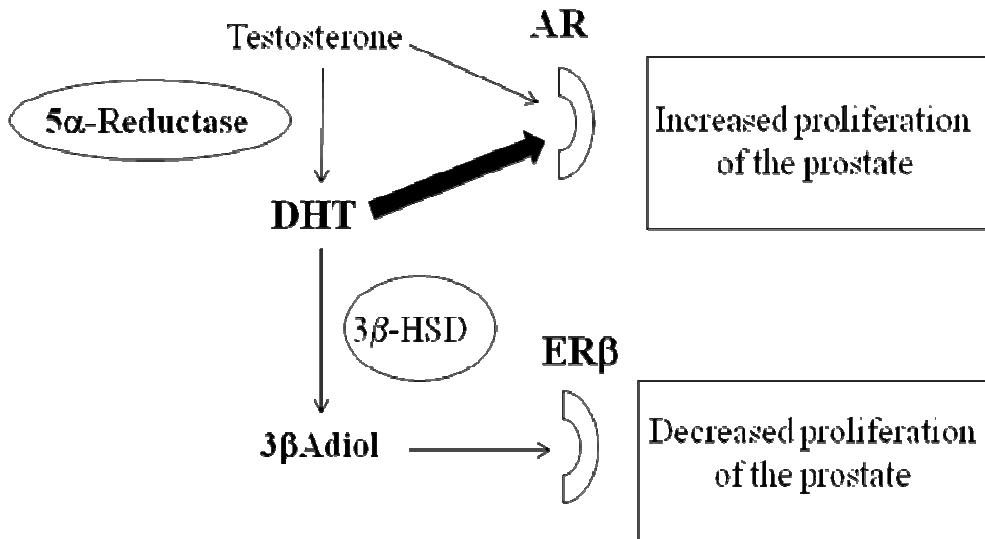


Figure 8a: Placebo treatment.

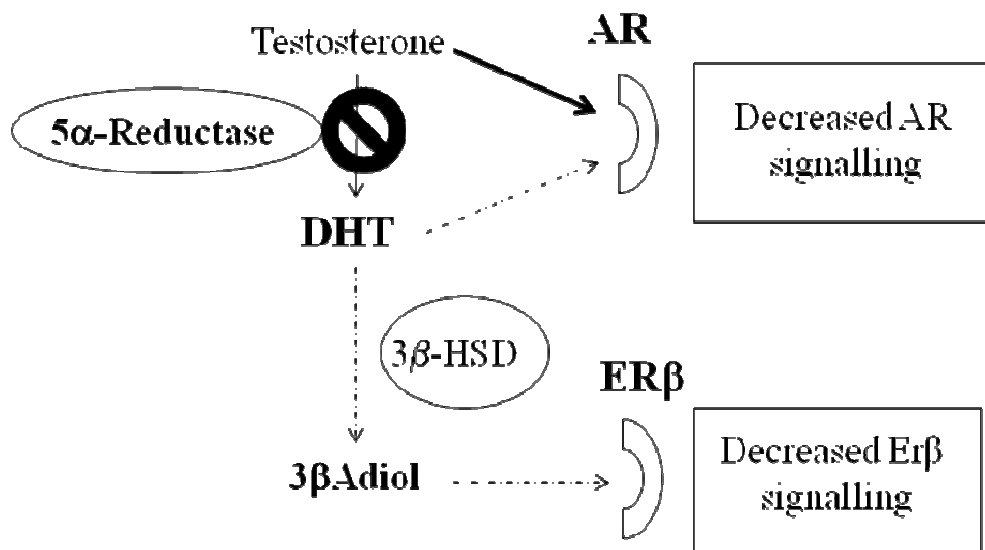


Figure 8b: Finasteride treatment inhibits 5 $\alpha$ -reductase and results in markedly decreased DHT levels but will also lead to increased testosterone levels and presumably decreased 3 $\beta$ Adiol levels. The net effect may explain the contradictory results in the PCPT-trial.

To further investigate and establish the role of the 3 $\beta$ Adiol we will conduct a clinical study in order to assess how finasteride treatment affects the 3 $\beta$ Adiol as well as other metabolites levels in the prostate in patients undergoing RP.

## 7 SAMMANFATTNING (SUMMARY IN SWEDISH)

Prostatakörteln är en accessorisk könskörtel belägen under urinblåsan, runtomkring den första delen av urinröret. De nerver som styr erektionsförmågan löper lateralt om prostatan ut till penis och kan skadas vid en operation av prostatan. Prostata körteln är beroende av de manliga könshormonerna (androgenerna) för sin normala utveckling men de har även stor betydelse vid utveckling av sjukdomar i prostatan, tex prostatacancer. Androgenerna utövar sin effekt via bindning till särskilda målmolekyler, så kallade androgenreceptorer. Prostatacancer är den vanligaste cancerformen hos svenska män. Denna avhandling handlar om hur prostatakörteln tar hand om och omvandlar androgenerna och hur detta kan påverka risken att utveckla prostatacancer. I prostata omvandlas den vanligaste androgenen testosteron till en betydligt starkare androgen som kallas dihydrotestosteron (DHT), vilket har en avgörande betydelse för normal manlig fosterutveckling. Hos den vuxna mannen har prostatans hormonella påverkan på övriga kroppen fram tills nu ansetts ha mindre betydelse.

I delarbete I och II undersöker vi ett enzym i prostatan som genom så kallad glukuronidering ser till att androgenerna transporteras bort från prostatan och slutligen utsöndras i urinen. Detta enzym betecknas UGT2B17. Som individ föds man med antingen 1, 2 eller helt utan arvsanlaget (genen) för detta enzym. Dessa så kallade genetiska variationer anses ha stor betydelse för risken att drabbas av prostatacancer. I delarbete I fann vi att risken att drabbas av prostatacancer var nästan 2-faldigt ökad hos individer som saknade genen. I delarbete II upprepade vi samma undersökning i en studie med betydligt fler deltagare och då fann man att risken inte längre kvarstod. I detta fall förlitar vi oss mer på resultaten från delarbete II.

Det finns även målmolekyler (receptorer) för kvinnliga könshormoner (östrogener) i prostatakörteln. Nedbrytningsprodukter av androgener har visats verka på dessa östrogenreceptorer och ha en bromsande, balanserande effekt på tillväxten av prostatacancer. I delarbete III undersökte vi ett enzym som är involverat i denna process, kallat CYP7B1. Vi fann att det fanns en ökad mängd av detta enzym i område med prostatacancer jämfört med den friska vävnaden. I framtiden vill vi undersöka om detta kan användas som en markör för att skilja aggressiva tumörer från mer långsamt växande tumörer. Det finns ett stort behov av förbättrade prognostiska markörer för prostatacancer.

I de avslutande delarbeten IV och V undersökte vi den hormonella omställningen lokalt och generellt i kroppen efter kirurgiskt avlägsnande av hela prostatakörteln. Detta visade sig ha större betydelse än vad vi tidigare trott. Vi fann att de lokala koncentrationerna av den starka androgenen DHT var nästan dubbelt så höga bredvid prostatan jämfört med i den allmänna cirkulationen. Vi fann att prostatans DHT produktion står för nästan en tredjedel av den cirkulerande DHT nivån. Utöver detta fann vi att den centrala styrningen av könshormoner påverkades vid borttagande av prostatakörteln. Vi sätter detta i samband med att DHT nivåerna i cirkulationen sjunker då övriga undersökta hormoner, inklusive testosteron, var opåverkade. Vi tror att dessa hormonella omställningar, utöver kirurgisk skada på erektionsnerverna, kan ha betydelse för den sexuella dysfunktion som drabbar många män i samband med prostata cancer kirurgi. Vi planerar att studera om substitution med DHT medicin i gel form kan förbättra återhämtningen av sexuellförmågan hos patienter efter operation.

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