NINA MONONEN

Polymorphisms in Genes Associated with Androgen Biosynthesis and Metabolism as Risk Factors for Human Prostate Cancer

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ACADEMIC DISSERTATION

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University of Tampere,
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Finland

Supervised by
Docent Johanna Schleutker
University of Tampere
Professor Olli-Pekka Kallioniemi
University of Turku

Reviewed by
Docent Virpi Launonen
University of Helsinki
Professor Pirkko Vihko
University of Helsinki

Distribution
Bookshop TAJU
P.O. Box 617
33014 University of Tampere
Finland

Tel. +358 3 3551 6055
Fax +358 3 3551 7685
taju@uta.fi
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“Go not to the elves for counsel, for they will say both yes and no.”
J. R. R. Tolkien

To My Family

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LIST OF ORIGINAL COMMUNICATIONS

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

I. Mononen N, Syrjäkoski K, Matikainen M, Tammela TL, Schleutker J, Kallioniemi OP, Trapman J, Koivisto PA. (2000) Two percent of Finnish prostate cancer patients have a germ-line mutation in the hormone-binding domain of the androgen receptor gene. Cancer Res. 60(22):6479-81.

II. Mononen N, Ikonen T, Syrjäkoski K, Matikainen M, Schleutker J, Tammela TL, Koivisto PA, Kallioniemi OP (2001) A missense substitution A49T in the steroid 5-alpha-reductase gene (SRD5A2) is not associated with prostate cancer in Finland. Br J Cancer. 84(10):1344-7.

III. Mononen N, Ikonen T, Autio V, Rökman A, Matikainen MP, Tammela TL, Kallioniemi OP, Koivisto PA, Schleutker J. (2002) Androgen receptor CAG polymorphism and prostate cancer risk. Hum Genet. 111(2):166-71

IV. Mononen N.*, Seppälä E.H.*, Duggal P., Autio V., Ikonen T., Ellonen P., Saharinen J., Saarela J., Tammela T.L.J., Kallioniemi O., Bailey-Wilson J.E., Schleutker J. Profiling genetic variation along the androgen biosynthesis and metabolism pathways implicates several SNPs and their combinations as prostate cancer risk factors. Submitted
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ABBREVIATIONS

1,25-D	$1,25(OH)_2D_3$
3α-diol	androstanediol
3β-diol	androstanediol
^{32}P	phosphate-32
A	alanine
AKR1C2	3-alpha-hydroxysteroid dehydrogenase type 3 gene
AKR1C3	3-alpha hydroxysteroid dehydrogenase type II/17beta-hydroxysteroid dehydrogenase gene
aOR	age adjusted odds ratio
APC	adenomatosis polyposis coli gene
AR	androgen receptor
AR	androgen receptor gene
ARE	androgen responsive element
ASO	allele specific oligo
bcl2	apoptosis regulator Bcl-2 protein gene
bp	base pair
BPH	benign prostate hyperplasia
BRCA1/2	breast cancer 1/2, early onset gene
C	cysteine
CAPB	prostate and brain cancer gene locus at 1p36
CDK2	cyclin-dependent kinase 2
CDKN1A	cyclin-dependent kinase inhibitor p21 gene
CDKN2A	cyclin-dependent kinase inhibitor-2A (p16) gene
CHEK2	CHK2 checkpoint *S. pombe* homolog gene
CI	confidence interval
CYP17A1	cytochrome P450, subfamily 17A, polypeptide 1 gene
CYP19A1	aromatase
D	aspartic acid
dATP	deoxyadenosine triphosphate
dCTP	deoxycytidine triphosphate
ddATP	dideoxyadenosine triphosphate
ddGTP	dideoxyguanosine triphosphate
del	deletion
dGTP	deoxyguanosine triphosphate
DHEA	dihydroepiandrosterone
DHT	dihydrotestosterone
DNA	deoxyribonucleic acid

dTTP	dideoxytymidine
dUTP	deoxyuridine triphosphate
E	glutamic acid
EGFR	epidermal growth factor receptor gene
ELAC2	elaC homolog 2 (*E. coli*) gene
ER	estrogen receptor
G	glysine
GnRH	gonadotropin-releasing hormone
H	histidine
HPC	hereditary prostate cancer
HPC1/2/20	hereditary prostate cancer gene 1/2/20
HPCX	hereditary prostate cancer gene locus at Xq27-q28
HSD	hydroxysteroid dehydrogenase
HSD17B2	hydroxysteroid (17-beta) dehydrogenase type 2 gene
HSD17B3	hydroxysteroid (17-beta) dehydrogenase type 3 gene
HSD3B1	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta isomerase type 1 gene
HSD3B2	hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta isomerase type 2 gene
I	isoleucine
IGFPB5	insulin-like growth factor binding protein 5
KLK3	kallikrein 3, prostate-specific antigen gene
L	leucine
LBD	ligand binding domain
LH	luteinizing hormone
LHB	luteinizing hormone beta polypeptide gene
M	methionine
MLH1	mutL homolog 1, colon cancer, nonpolyposis type 2 (*E. coli*) gene
mRNA	messenger ribonucleic acid
MSH2	mutS homolog 2, colon cancer, nonpolyposis type 1 (*E. coli*) gene
MSR1	macrophage scavenger receptor 1 gene
N	asparagines
N-terminal	amino terminal
OR	odds ratio
p	short arm of the chromosome
P	proline
PCAP	hereditary prostate cancer gene locus at 1q42.2-q43
PCR	polymerase chain reaction
PSA	prostate specific antigen
q	long arm of the chromosome
Q	glutamine
R	arginine
RNA	ribonucleic acid

RNASEL	ribonuclease L (2',5'-oligoisoadenylate synthetase dependent) gene
RR	relative risk
RT	room temperature
S	serine
SNP	single nucleotide polymorphism
SRD5A2	5alpha-reductase gene
SSCP	single strand conformational polymorphism
T	threonine
TAUH	Tampere University Hospital
TGFβ	transforming growth factor beta gene
U	enzyme unit
UGT	UDP glucuronosyltransferase gene family
UGT	UDP glucuronosyltransferase enzyme
UGT2B7/15/17/28	UDP glucuronosyltransferase 2 family, polypeptide B7/15/28 gene
UTR	untranslated region
V	valine
W	tryptophan

ABSTRACT

Prostate cancer is the most common malignancy among men in Finland and in most other industrialized countries. In most cancer cases, environmental factors probably play an important part in cancer causation. However, a recent large twin study indicated that 42% of the overall prostate cancer risk could be explained by heritable factors. As androgens play an important part not only in the normal growth and function of the prostate, but are also known to be essential for the development of prostate cancer, the genes acting along the androgen pathway represent ideal candidates for prostate cancer susceptibility genes. The purpose of this study was to identify genes responsible for prostate cancer predisposition in Finland.

We carried out an association analysis of 18 different variants in 10 genes (*SRD5A2, HSD3B1, HSD17B2, HSD17B3, AKR1C3, CYP19A1, CYP17A, KLK3, AR* and *LHB*) acting in the biosynthesis and metabolism of androgens. We made use of a large collection of population controls (n=923), unselected prostate cancers (n=847), and familial prostate cancers (n=121). This analysis revealed a novel alteration T201M in the *CYP19A1* gene. This variant as well as the length polymorphism of the CAG repeat in the androgen receptor gene *AR* were found to be associated with prostate cancer (OR=2.04, 95%CI 1.03-4.03 for T201M and OR = 1.47, 95%CI 1.00-2.16 for CAG). A stratified analysis revealed that individuals with early-stage prostate cancer were more than 5 times more likely to carry the *CYP19A1* T201M T allele ($p < 0.0001$) than population based controls. In our initial report, we also found a significant association between the *AR* mutation R726 and prostate cancer, but this did not retain statistical significance in a later, larger study. We did not find any prostate cancer risk association with the *SRD5A2*, which has previously been suggested as a risk factor. Overall, the risk ratios that we observed were small, and some of the variants were quite rare at the population level, making it difficult to achieve statistical significance despite the very large sample sets available to us. This suggests that many low to moderate penetrance alleles may contribute to genetic susceptibility to prostate cancer at the population level, and that comprehensive screens of the androgen axis and other pathways need to be carried out.

INTRODUCTION

The prostate is a hormone-dependent organ that participates in the production of seminal fluid in men. Prostate cancer is the most common cancer in males and the second leading contributor to male cancer mortality in most western countries. Most prostate cancer cases are sporadic; about 5-10% are familial, which is seen as clustering of excess prostate cancer cases in families.

Prostate cancer is a heterogeneous disease with multiple risk factors. The three most important risk factors for prostate cancer are age, ethnicity and family history. In addition, factors like hormones, diet, physical activity, occupation and virus infections have been suggested to affect the risk. Several studies have also shown that there is a definitive genetic component in prostate cancer causation. In the search for the genetic factors, rare high-penetrance susceptibility genes have been located in family-based linkage studies. In addition, there most likely are several common polymorphisms and low-risk alteration, which can be found by association studies. They cause a small relative risk of disease but because of the high population frequency of the risk alleles, they may make a considerable contribution to prostate cancer incidence at the population level.

Androgens, male sex hormones, produced by the testis and the adrenal glands play a pivotal role in male reproductive and sexual function. Androgens are also needed for muscle formation, body composition, bone mineralisation, fat metabolism and cognitive functions. For prostate development, function and maintenance, androgens are necessary. Interestingly, prostate cancer is also heavily dependent on testosterone and androgen metabolism and therefore it is reasonable to hypothesize that genetic variation in genes acting along the androgen pathway could have an impact on disease risk. In addition to androgens, estrogens, the female sex hormones, play an essential role in the male reproductive tract via their specific estrogen receptors.

The purpose of this study was to identify genetic variation in the genes involved in androgen metabolism and study the impact of such variation on prostate cancer risks.

REVIEW OF THE LITERATURE

1. Epidemiology of prostate cancer

1.1 Trends in incidence and mortality

After lung cancer, prostatic adenocarcinoma (prostate cancer) is the second leading cause of male cancer deaths and the most common cancer in men in Finland (Finnish Cancer Registry 2003). In 2003, 4225 new prostate cancer cases were diagnosed in Finland. In the USA too, prostate cancer is the most common male cancer (Ries et al. 1999), and the second most common in the entire European Union (Ferlay et al. 1999).

The incidence of prostate cancer has increased dramatically in the past decades (Merrill et al. 1996; Finnish Cancer Registry 2003, Sim and Cheng 2005). In Finland the age-adjusted incidence per 100,000 was 39.1 at the beginning of the 1990's. An especially rapid increase has been observed in the past 10 years - in 2003 the incidence was 95.1 (Finnish Cancer Registry 2003). The increasing incidence has resulted largely from early detection with the use of serum prostate-specific antigen (PSA) testing. In the USA, the incidence of prostate cancer was at its peak at the beginning of the 1990's, (179 per 100 000 in whites in 1992; 250 per 100,000 in 1993)(Hankey et al. 1999; Ries et al. 2000). Since then there has been a decline in the incidence, which can be explained by the detection of early-stage incident cases in the male population (Hankey et al. 1999). In many Asian countries there has been a dramatical increase in the incidence of prostate cancer – during the period 1978-1997 the incidence rose from 6.3 to 12.7 per 100,000 in the Miyagi area in Japan and from 6.6 to 14.4 in Sanghai, China (Sim et Cheng 2005). It has been suggested that with gradual Westernisation many Asian countries may be losing their cultural protective factors such as diet and acquire high risk ones.

In Finland, the age-adjusted mortality has been quite steady in the past decades. In 2003, mortality rate per 100,000 was 15.4 (Finnish Cancer Registry 2003). In the USA, the mortality started to decline slightly at the beginning of the 1990's and in 1995 the mortality per 100,000 was 24.9 (Ries et al. 1999), which has been attributed to the successful use of PSA screening, although definitive scientific proof for this claim is still missing. In contrast, in many Asian countries the mortality rates are rising along with the incrising incidence rates of prostate cancer. However, the mortality rates are still lowest in Asian

countries (5.2 in Singarpore, China, 4.8 in Japan and 3.6 in Hong Kong, China)(Sim and Chen 2005).

Before the age of 40, prostate cancer is rare. The rate increases with aging, faster than for any other cancer in men. In 2003, 74% of all prostate cancer cases in Finland were diagnosed in men over the age of 64 and only 140 cases (3,8%) were younger than 50 years of age at the time of diagnosis (Finnish Cancer Registry 2003).

Serum levels of the prostate specific antigen are elevated in conditions that increase the prostate size or lead to tissue destruction. Therefore serum PSA levels are used to detect and monitor the progression of prostate cancer (Aziz and Barathur 1993). PSA testing was introduced in the United States of America in the late 1980s with Federal Drug Administration approval for prostate cancer surveillance (Hankey et al. 1999). In the USA the use of PSA testing for early detection of prostate cancer dramatically increased from late 80's onwards (Potosky et al. 1995). In Finland PSA testing became more widely used after the beginning of the 1990's (Auvinen et al. 1996). As testing has become more common it has resulted in a significant change in the clinical presentation of the disease. Cancer cases where elevated PSA level led to further tests and to the finding of the cancer tend to be younger than those cases with clinical symptoms and this is the plausible reason behind the fall in the average age of diagnosis (Hoffman et al. 2005). The dramatic increase in the incidence, especially in the younger age group, most likely reflects PSA testing becoming common routine (Potosky et al. 1995; Hemminki et al. 2005). Testing has also reduced the number of clinically advanced cancers (Hankey et al. 1999). PSA testing has also raised concern – it is possible that clinically insignificant cancers are also detected and thus unnecessary treatments become more likely (Saksela 1998).

1.2 Environmental risk factors

A wide variety of different environmental factors has been suggested to affect the development of prostate cancer: diet, alcohol consumption, smoking, occupation, sexual behavior, vasectomy and physical activity. Although the results have been controversial in many cases, in general, dietary factors, especially fat intake, are suggested to influence the prostate cancer risk (Schulman et al. 2001).

1.2.1 Diet

Numerous studies have reported a positive association between the total fat intake, saturated fats, animal fats, red meat, polyunsaturated fatty acids, consumption of fatty foods or dairy products and prostate cancer risk (Bostwick et al. 2004). Dietary fat consumption, especially animal fat from red meat, has also been shown to be associated with an elevated risk of advanced prostate

cancer (Giovannucci et al. 1993). It has been suggested that dietary fats could alter hormonal profiles or increase the oxidative stress (Bishop et al. 1988; Ho and Baxter 1997). However, the role of dietary fat in prostate cancer etiology is not yet clearly defined as there are also studies where no association has been observed (Hirayama 1979; Severson et al. 1989; Hsing et al. 1990). In addition, there is some evidence that the type of dietary fat is important. A reduced risk has been reported in men with high consumption of fatty fish (Norrish et al. 1999; Terry et al. 2001).

Conversely to fat intake, high fruit and vegetable intake have been reported to decrease the risk of prostate cancer (Jain et al. 1999; Kolonel et al. 2000), particularly consumption of cruciferous vegetables (Cohen et al. 2000). In a study by Giovannucci et al. (1998) the protective role of fruit consumption was accounted for by fructose intake. Likewise, several vitamins have also been suggested to have a role in prostate cancer risk. Carotenoids (vitamin A and beta-carotenoids) have been widely studied but the results have not been easy to interpret (Bostwick et al. 2004). An association with cancer in some studies (Talamini et al. 1992; Giovannucci et al. 1995) and a protective role in other studies has been reported (Ohno et al. 1988; Mettlin et al. 1989). The hormonal form of vitamin D, $1,25(OH)_2D_3$ (1,25-D) inhibits invasiveness of prostate cancer cells *in vitro* (Schwartz et al. 1998) and has anti-proliferative and prodifferentiation effects on Dunning rat model (Getzenberg et al. 1997). An association of low level of 1,25-D with prostate cancer was found in men above the median age of 57 years but not in younger men and the risk was similar in black and white men (Corder et al. 1993). However, Gann et al. (1996) failed to confirm these results. Similarly, vitamin E has been shown to inhibit prostate cancer cell growth *in vitro* through apoptosis (Sigounas et al. 1997). The Alpha-Tocopherol Beta-Carotene Cancer Prevention study found that daily consumption of α-tocopherol reduced the incidence of prostate cancer and mortality (Heinonen et al. 1998). Hartman et al. (2001) also showed that long-term alpha-tocopherol supplementation decreases serum androgen concentrations, and could contribute to the observed reduction in incidence of and mortality from prostate cancer. In contrast, Andersson et al. (1996) observed no affect of α-tocopherol on prostate cancer risk.

Several trace elements have also been studied in prostate cancer causation. The prostate has the highest zinc concentrations of all human organs (Bedwal and Bahuguna 1994). Zinc is secreted by the prostate into the seminal fluid, where it is thought to extend the functional life span of the ejaculated sperm (Bedwal and Bahuguna 1994). Total androgen uptake by the prostate has been observed to increase significantly by the addition of zinc (Leake et al. 1984). Also, Colvard and Wilson (1984) reported that zinc enhances the binding of androgen receptor to cell nuclei. The role of zinc in prostate cancer remains uncertain. Some studies have reported a positive association between increasing zinc consumption and prostate cancer risk (Kolonel et al. 1988; Leitzmann et al. 2003). Yet zinc levels have been reported to be lower in older men than in younger men (Tvedt et al. 1989). On the other hand, it has been shown that zinc

inhibits human prostatic carcinoma cell growth, possibly due to induction of cell cycle arrest and apoptosis (Liang et al. 1999). Higher levels of another trace element, selenium, have been shown to be associated with a reduced risk of prostate cancer (Yoshizawa et al. 1998). Supplemental selenium has also been suggested to reduce prostate cancer risk. Because plasma selenium decreases as patients age, supplementation may be particularly beneficial to older men (Brooks et al. 2001). However, another recent study failed to confirm these results (Goodman et al. 2001).

Many studies have reported a positive association between milk consumption and prostate cancer risk (Bostwick et al. 2004). It has been suggested that calcium along with the fat in dairy products may contribute to increased risk. Giovannucci (1998) even reported an increased risk of prostate cancer among men with high calcium intake. One possible mechanism behind the effect could be that high intakes of calcium lowers circulating 1,25-D levels and thus increases the risk for cancer (Giovannucci et al. 1998).

1.2.2 Other environmental risk factors

Alcohol consumption has been reported to increase the metabolic clearance rate of testosterone (Gordon et al. 1976). However, several studies have observed no affect of alcohol intake in prostate cancer risk (Hsing et al. 1990; Adami et al. 1992; Le Marchand et al. 1994). Cigarette smoking has also been widely studied in relation to prostate cancer risk. So far the results have been inconsistent (Hickey et al. 2001).

Some studies have also tried to identify potential associations between workplace exposures and prostate cancer risk (Sharma-Wagner et al. 2000; Weston et al. 2000; Zeegers et al. 2004a). A modest association has been observed between farming and prostate cancer risk (Keller-Byrne et al. 1997). The most plausible explanation is the exposure of farmers to hormonally active agricultural chemicals. However, atrazine, which is a commonly used agricultural pesticide in crop production was reported to cause decreased testosterone production in male rats (Trentacoste et al. 2001). Also, cadmium expose in industry has been linked to prostate cancer is some epidemilogic studies, but not in all (Sahmoun et al. 2005).

Increased risk of prostate cancer has been observed in men with a large number of sexual partners. One possible explanation could be sexually transmitted infections, suggesting that infections may represent one mechanism through which prostate cancer develops (Dennis and Dawson 2002). On the other hand Roman Catholic priest do not have a significantly lower risk for prostate cancer, arguing against an important role for sexual activity (Ross et al. 1981).

The results from studies on vasectomy and prostate cancer risk have been contradictory. Several studies have reported a significantly elevated risk, but the reported risk has usually been small (OR<1.8) (Bostwick et al. 2004). In

addition, Hayes et al. (1993) found no overall effect for vasectomy, but noted an increased risk among men who had undergone vasectomy at age <35 years. Nevertheless, there are also several studies that have observed no association between vasectomy and prostate cancer risk or any variation in the risk by age at vasectomy (Bostwick et al. 2004).

For prostate cancer, the data are inconsistent regarding whether physical activity plays any role in the prevention. There have been reports that physical activity either reduces or increases the prostate cancer risk, or that the risk is similar compared to that of inactive men (Lee 2003).

1.3 Hormonal risk factors

1.3.1 Androgens

Endogenous androgens have long been considered to be risk factors for prostate cancer. In an early work, Noble (1977) demonstrated that androgens can induce prostate carcinoma in an experimental rat model. Similar results were reported by Bosland et al. (1990). In addition, there are case reports of prostate cancer in men who used androgenic steroids as anabolic agents, suggestive of a causal relationship (Roberts and Essenhigh 1986; Jackson et al. 1989; Ebling et al. 1997). Moreover, prostate cancer does not develop in men who are castrated at an early age or who have mutations that impair androgen production (Smith et al. 1991; Wu and Gu 1991).

Several studies have also established a possible connection between circulating levels of androgens, such as testosterone and dihydrotestosterone (DHT), and the variation in prostate cancer risk in different ethnic groups. An early work by Ahluwalia et al. (1981) revealed a small but significant increase in the plasma level of testosterone among African-American men compared to black Nigerians, whereas levels of DHT were not different. In another study on 50 healthy young African-American and 50 young European-American males, mean testosterone levels in blacks were reported to be 19% higher and free testosterone levels 21% higher in African-American than in whites (Ross et al. 1986). Yet, Ross et al. (1992) found no significant differences in serum testosterone concentrations in young adult Japanese men compared with those of young adult whites and blacks from U.S.A. However, white and black men had significantly higher values of 3α,17β-androstanediol glucuronide (31% and 25% higher, respectively) and androsterone glucuronide (50% and 41% higher, respectively) than Japanese men, suggesting that the low-risk Japanese population had a lower testosterone metabolism. Similar results have been reported by Lookingbill et al. (1991). In a study on 50-79 year old Dutch and Japanese men de Jong et al. (1991) reported significantly lower plasma levels of testosterone and estradiol in Japanese men when compared with those in Dutch men. On the other hand, the levels of total and bioavailable testosterone were

reported to be highest in Asian-Americans, intermediate in African-Americans, and lowest in whites by Wu et al. (1995). The DHT:testosterone ratio was highest in African-Americans, intermediate in whites, and lowest in Asian-Americans, corresponding to the respective incidence rates in these ethnic groups and providing indirect evidence for ethnic differences in the activity of one of the main enzymes in the androgen metabolism, 5α-reductase (Wu et al. 1995).

There are also prospective studies with prolonged follow-up where serums from men who either did or did not develop prostate cancer were collected. Nomura et al. (1988) found that serum DHT levels were lower and the testosterone/DHT ratios were higher in the prostate cancer cases compared with their controls. However, none of these associations was significant. In another study Barrett-Connor et al. (1990) observed no relation between total testosterone level and prostate cancer risk. However, Hsing and Comstock (1993) reported a small but statistically insignificant increase in the testosterone:DHT ratio in prostate cancer patients. It is noteworthy that the storage of serum samples for a long period (12-14 years) may have affected the samples and thus the results of these studies. Also, as androgen levels change with age, it is difficult to predict the androgen exposure level in men during different periods of life. There are also studies where the endocrine status in healthy men and prostate cancer patients have been measured (Carlström and Stege 1997; Signorello et al. 1997). However, these studies are probably not very informative as the malignancy may itself change the hormonal status.

1.3.2 Estrogens and estrogen receptors

Estrogens have been found to stimulate DNA synthesis and induce metaplastic epithelial morphology in human and rat prostate (Nevalainen et al. 1991; Nevalainen et al. 1993). The development of prostate seems to be especially sensitive to estrogens. Neonatal estrogen exposure results in inhibition of prostatic growth and function in mice (Naslund and Coffey 1986; Pylkkänen et al. 1991). When the mice age, early estrogen exposure seems to promote development of epithelial hyperplasia and dysplasia and inflammation in the mouse prostate (Pylkkänen et al. 1991). There are two estrogen receptor genes that code for two functionally distinct receptor proteins, ERα and ERβ. The presence of these receptors in the prostate suggests that estrogen may act directly in the prostate. In healthy prostate the ER expression levels are somewhat lower compared to mammary tissue (Linja et al. 2003). In cancerous prostate tissue the levels are clearly reduced and the lowest levels have been observed in hormone refractory prostate cancer (Horvath et al. 2001; Leav et al. 2001).

Estrogens have also been considered as hormonal risk factors for prostate cancer. The balance between the levels of androgens and estrogens is altered during aging. Plasma androgen levels decline where as estrogen levels remain relatively constant (Vermeulen et al. 2002). The altered ratio has been suggested to represent an additional risk for prostate cancer. There is experimental

evidence that estrogens may facilitate the development of malignancy in the prostate (Ho 2004). However, high serum levels of estrogen with low testosterone level in aromatase overexpressing mice or in estrogen-treated hypogonadal mice deficient in sex steroid production did not induce malignancy in the prostate tissue (Li et al. 2001; Bianco et al. 2002). In addition, phytoestrogens have been suggested to have a protective effect against prostate cancer (Morrissey and Watson 2003).

In a recent study Imamov et al. (2004) reported that in mice lacking ERβ receptor the proliferation in the epithelium was higher and apoptosis lower in prostate and that a larger fraction of the epithelial cells retained the capacity to proliferate. It has been suggested that the major biological function of DHT is not that it is a most potent androgen but that it acts as a precursor to 3βdiol, a ligand to ERβ (Weihua et al, 2002; Koehler et al, 2005). Thus both ERβ and 3βdiol would be part of a pathway important for the regulation of the prostatic growth.

1.4 Genetic risk factors

1.4.1 Race

The marked ethnic differences in prostate cancer incidence have been well documented, especially in the USA. The highest incidences in the world so far have been reported among the African-American men. The incidence in this racial group has been reported to be ~50-70% higher compared with Caucasians in the USA and 50 to 60 fold higher than Asian men who have the lowest rates in the world (Hsing et al. 2000b). Also, black men in the US are more likely to present with advanced stage cancers than white men (Pienta et al. 1995). Some of the racial differences may reflect access to care or differences in decision-making regarding whether seek medical help. However, PSA screening is more common among white American men than among the African-American men, which should favour higher incidences among white men, yet the opposite has been found (Bostwick et al. 2004). Interestingly, Japanese- and Chinese-American men have rates higher than men in their respective homelands. This may reflect differences in lifestyle factors such as diet (Whittemore et al. 1995). However, their rates still remain much lower than US Caucasians indicating that ethnic variation in polymorphic alleles of genes associated with prostate cancer risk could explain a proportion of the ethnic difference (Shibata and Whittemore 1997).

1.4.2 Positive family history

Case-control and cohort studies

Along with age and race, positive family history is one of the strongest risk factors for prostate cancer. There are several studies evaluating the risk of family history to the male relatives of a prostate cancer patient and the results have been fairly constant and favour a presence of genetic components in prostate cancer etiology. However, the results from case-control studies may be biased because those individuals with a prostate cancer case in a family may be more aware of the disease among family members than controls (Schaid 2004). Also, cases with prostate cancer can interpret any prostatic disease as cancer.

A meta-analysis of 11 case-control and two cohort studies reported a pooled OR of 2.5 among the first degree relatives (Johns and Houlston 2003). In the majority of the studies, the risk has been shown to be greater if the individual affected is a brother than if it was a father (OR=3.4 and 2.5, respectively). The risk of positive family history also seems to be influenced by age at diagnosis, number of those affected in the family and ethnic background. If the affected was diagnosed <65 years old the OR was 4.3 and, if older then the risk was 2.4 (Johns and Houlston 2003). If there was more than one affected relative then the estimated OR was 3.5. Steinberg et al. (1990) estimated that the OR increased from 2.2 to 4.9 and 10.9, respectively, if there were one, two or three or more affected first degree relatives. Similar results were obtained by Whittemore et al. (1995); if two or more first-degree relatives were affected the OR among African-Americans was highest, 9.7, while among Caucasians the OR was 3.9 and only 1.6 among Asian-Americans. However, there are studies where no ethnic/racial risk variability have been observed. Monroe et al. (1995) found a similar risk for African-Americans, Hispanics and Whites and Japanese (RR ranged 2.8-2.5). Similar results were observed by Whittemore et al. (1995), Cunningham et al. (2003), and Stone et al. (2003), supporting a possibility of a common genetic basis for the disease among different racial and ethnic groups (Schaid 2004).

The results concerning the effect of positive family history obtained from cohort studies have been similar to those of case-control studies. Isaacs et al. (1995) estimated a relative risk (RR) of 1.76 for first-degree relatives and Cerhan et al. (1999) calculated an RR of 3.2. If a brother had been affected then the RR was again greater (RR=4.5) than if the father was affected (RR=2.3). However, the close male relatives of prostate cancer patients may be more eager to seek medical advice, which may bias the risk for an affected brother to be greater than that for affected fathers. Also, Staples et al. (2003) stratified the research data according to the year of diagnosis of the relative. If the diagnosis had been made prior to 1992, the observed OR was 3.1 if the brother was affected and 2.8 if the father had prostate cancer. Had the diagnosis been later than 1991 the ORs were 3.9 and 2.5 respectively. The authors suggest that the introduction of PSA testing may have inflated the risk associated with an affected brother.

Further evidence that PSA testing may influence the risk estimates comes from PSA screening studies. A Finnish study of a randomized prostate trial of the efficacy of PSA screening where the relative risk of screen-detected prostate cancer was 1.3, comparing men with a positive versus negative family history (Mäkinen et al. 2002). Also, Narod et al. (1995) reported an RR of 1.7 for men with an affected first-degree relative (RR=2.6 if a brother was affected). Based on this evidence Schaid (2004) suggests that genetic etiology plays a less important role in the cancers detected by PSA testing solely compared to a clinical disease.

Family-based segregation studies and twin studies

Three family-based segregation studies have suggested a genetic model of prostate cancer, where a rare autosomal dominant susceptibility allele with a frequency ranging from 0.003-0.006 leads to a high lifetime penetrance (range 88-97%)(Carter et al. 1992; Schaid et al. 1998; Verhage et al. 2001). The studies by Schaid et al. (1998) and Valeri et al. (2003) suggest that there may be an additional genetic or shared environmental factors that the simple autosomal dominant model cannot explain. The studies above were all based on small pedigrees where only the first degree relatives of the probands were included in the analysis and thus provide only limited information about the modes of inheritance. Cui et al. (2001) also included paternal and maternal uncles in the analysis and evaluated genetic models that allowed for either one or two loci. The best fit model based on their analysis was a rare autosomal dominant allele (frequency 0.017) that has a larger risk at younger ages, and a more common allele (frequency 0.084) that is either autosomal recessive or X-linked, and has a great effect at older ages. Gong et al. (2002) suggest that a multifactorial model, which allowed multiple genes each having a low penetrance may be the best choice as a genetic model. However, segregation models have a limited potential in estimating the heritability patterns of complex diseases, and they are mostly used to obtain parameters for linkage analysis.

Twin studies can also provide useful information about genetic and environmental factors in prostate cancer etiology. The results from a large twin study with 44,788 pairs of twins from Sweden, Finland and Denmark conducted by Lichtenstein et al. (2000), reported that 42% of the prostate cancer risk could be explained by heritable factors. The authors suggest that single-gene mutations can only explain a fraction of the genetic effects found and other genes that are relative common and provide a moderate risk are involved in the causation of cancer. Risch (2001) also suggested, based on their interpretations of the twin study by Lichtenstein et al. (2000) that prostate cancer may not be explained by independent rare autosomal dominant genes, but possibly by recessive and/or multiple interacting genes. Similar result has also been reported by Page et al. (1997) and Schaid et al. (1998).

1.4.3 High penetrance susceptibility genes

Linkage analysis is a method that can be used in the analysis of cancer syndrome families to localize high-penetrance genes. During the last two decades, several such genes have been localized and the corresponding mutations identified in several cancers, including *BRCA1* and *BRCA2* in breast cancer, *APC* in familial adenomatous polyposis and *MSH2* and *MLH1* in hereditary-non polyposis colon cancer (Bodmer et al. 1987; Hall et al. 1990; Lindblom et al. 1993; Peltomäki et al. 1993; Wooster et al. 1994; Rapley et al. 2000). Yet these high-penetrant genes are rare and account for only small proportion of the excess risk.

In order to identify genes predisposing to prostate cancer, several genome-wide screens with linkage analysis have been performed on familial prostate cancer cases. This has led to the mapping of several predisposing loci: *HPC1* at 1q24 (Smith et al. 1996), *HPC2* at 17p11 (Tavtigian et al. 2001), *PCAP* at 1q42.2-q43 (Berthon et al. 1998), *HPCX* at Xq27-q28 (Xu et al. 1998), *CAPB* at 1p36 (Gibbs et al. 1999), and *HPC20* at 20q13 (Berry et al. 2000) and loci at chromosomes 8p22 (Xu et al. 2001a), 3p25-p26 (Schleutker et al. 2003), 16p13 (Suarez et al. 2000), 22q12 (Xu et al. 2005) and 9p (Gibbs et al. 2000). The large number of loci seen probably reflects the very heterogeneous nature of the disease. So far, despite tremendous efforts, only three candidate genes have been identified from the linkage regions: *ELAC2* (Tavtigian et al. 2001), *RNASEL* (Carpten et al. 2002), and *MSR1* (Xu et al. 2002).

Tavtigian et al. (2001) identified in the *HPC2* region a candidate prostate cancer susceptibility gene, *ELAC2* coding for a binuclear zinc phosphodiesterase. Two common missense mutations found in *ELAC2* were shown to be associated with prostate cancer (Rebbeck et al. 2000; Tavtigian et al. 2001). In contrast, Rökman et al. (2001) reported that these variants carried no significantly elevated risk for hereditary prostate cancer (HPC) or non-hereditary prostate cancer in Finnish subjects. However, a previously undescribed Glu622Val variant had a significantly higher frequency in prostate cancer cases (OR=2.94, 95%CI 1.05-8.23). A number of contradictory association studies have been reported since. A meta-analysis of six studies indicates evidence for the role of *ELAC2* in prostate cancer, suggests moderate familial risk, and estimates that risk genotypes in *ELAC2* may cause 2% of prostate cancer in the general population (Camp and Tavtigian 2002).

RNASEL/*HPC1* encodes a widely expressed endoribonuclease that participates in an interferon-inducible RNA-decay pathway that is thought to degrade viral and cellular RNA (Floyd-Smith et al. 1981). A nonsense mutation inactivating the *RNASEL* and a mutation in an initiator methionine codon of *RNASEL* were reported to segregate independently in two *HPC1*-linked families (Carpten et al. 2002). Results from two other studies also suggest that mutations in *RNASEL* gene may play a role in prostate cancer etiology (Casey et al. 2002; Rennert et al. 2002). In Finnish prostate cancer families, *RNASEL* mutations do not show segregation, but some of the variants are enriched in families with HPC

that include more than two affected members and may also be associated with the age of disease onset (Rökman et al. 2002).

Rare germline mutations of macrophage scavenger receptor 1 (*MSR1*) gene located on 8p22 have been reported to be associated with prostate cancer risk in families with HPC and in patients with non-HPC (Xu et al. 2002). Miller et al. (2003) also reported that MSR1 mutations are associated with increased prostate cancer susceptibility among African-American men. In Finland *MSR1* gene does not have a major role in the causation of hereditary or non-hereditary prostate cancer but studies suggest a possible modifying role in cancer predisposition (Seppälä et al. 2003a). Also, two studies have failed to observe any association between *MSR1* sequence variants and prostate cancer (Wang et al. 2003; Lindmark et al. 2004).

1.4.4 Low penetrance susceptibility genes

Predisposition caused by a combination of genetic variants that each show a small relative risk may be of much greater significance to the prostate cancer burden in the population than the marked individual risk ratios seen in inherited cancer syndromes. The search for low penetrance alleles has centred on association studies and several candidate alleles have been identified for various cancers (Houlston and Peto 2004). A large number of studies have also focused on identifying common genetic alterations that affect the risk of prostate cancer. Genetic alterations along the androgen pathway are perhaps the most natural candidates for conferring genetic susceptibility for prostate cancer. Several studies have also evaluated genes involved in the metabolism of environmental carcinogens, in DNA repair, in cell-cell interactions, as well as genes involved in such as angiogenesis, in methylation, in immune response to infection and in cell cycle regulation as possible low-penetrant susceptibility genes (Table 1). However, it is likely that environmental factors also play an important role in prostate cancer etiology and they together with common genetic alterations affect the prostate cancer risk.

2. Prostate gland and androgens

2.1 Development and maintenance

The normal development and function of prostate is dependent on androgens. Prostate development starts from the urogenital sinus at about 10-12 weeks of gestation in a process that is both initiated and dependent on circulating androgens produced in the fetal testes (Kellokumpu-Lehtinen 1985). The initiation of prostate development is also dependent on a functional androgen

Table 1. Examples of low penetrant susceptibility candidate genes studied in prostate cancer.

CYP2D6	cytochrome P450 2D6	Coughlin and Hall (2002)
CYP2C19	cytochrome P450 2C19	"
GSTM1	glutathione S-transferase M1	"
GSTP1	glutathione S-transferase p1	"
GSTT	glutathione S-transferase theta	"
NAT1 and *NAT2*	arylamine N-acetyltransferase 1 and 2	"
VDR	vitamin D receptor	"
BRCA1 and *BRCA2*	breast cancer 1 and 2	Sinclair et al. (2000)
CHEK2	CHK2 checkpoint *S. pombe* homolog	Seppälä et al. (2003b)
hOGG1	8-oxoguanine DNA glycosylase	Weiss et al. (2005)
ATM	phosphatidylinositol-3 kinase	Angele et al. (2004)
CDH1	E-cadherin, calcium ion-dependent cell adhesion molecule	Ikonen et al. (2001)
IGF-1	insulin-like growth factor 1	Friedrichsen et al. (2005)
IGFBP3	insulin-like growth factor binding protein 3	Friedrichsen et al. (2005)
COL18A1	collagen, type XVIII, alpha 1	Iughetti et al. (2001)
LEP	leptin	Ribeiro et al. (2004)
IL-8, IL-10, IL-1β	cytokines	McCarron et al. (2002)
ACE	angiotensin converting enzyme	Medeiros et al. (2004)
KLF6	kruppel-like factor 6	Narla et al. (2005)
KLK2	kallikrein 2	Chiang et al. (2005)
KLK10	kallikrein 10	Bharaj et al. (2002)
MTHFR	5,10-methylenetetrahydrofolate reductase	Singal et al. (2004)
FGFR-4	fibroblast growth factor receptor 4	Wang et al. (2004)
ERBB2	avian erythroblastic leukaemia viral oncogene	Yokomizo et al. (2005)

	homolog 2	
TGFB	transforming growth factor beta	Ewart-Toland et al. (2004)
TLR4	toll-like receptor 4	Zheng et al. (2004)
TMPRSS2	transmembrane protease, serine 2	Lubieniecka et al. (2004)
CDKN1B (p27)	cyclin-dependent kinase inhibitor 1B	Chang et al. (2004)
ETV6	ets variant gene 6	Kibel et al. (2002)

receptor. Prostate is absent in individuals with complete androgen insensitivity due to an inactivating mutation of androgen receptor AR (Quigley et al. 1995). The normal development of prostate is also heavily dependent on DHT. Congenital syndromes of androgen resistance, such as absence of functional 5α-reductase enzyme or defects in androgen receptor function, result in various forms of male pseudohermaphrodite syndrome, in which prostatic development and male external virilization are inhibited despite the presence of testosterone (Imperato-McGinley et al. 1985; Yeh et al. 2002). During puberty, serum testosterone level rises and prostatic wet weight and DNA content increase (Donjacour and Cunha 1988). In an adult male, androgens continue to promote the normal function of the prostate by maintaining the balance of cell proliferation and apoptosis. In the normal prostate, the rate of both cell death and proliferation is <0.20%/day (Berges et al. 1995). Studies on rats have shown that castration results in the loss of 70% of the prostate epithelial cells due to the apoptosis (English et al. 1989). Thus androgens seem to be especially important in maintaining the epithelia, which is the primary cell type of prostatic carcinoma (De Marzo et al. 1998).

2.2 Androgen pathway

The biosynthesis of the steroid hormones starts with the cleavage of the 6-carbon chain of cholesterol to yield the 21-carbon precursor pregnenolone (Figure 1a). There are two alternative pathways for testosterone synthesis. Pregnenolone may be converted either into progesterone, which branches into glucocorticoid and androgen/estrogen pathways (Δ^4path), or into 17α-hydroxypregnenologe, which is another route (Δ^5path) for the formation of androgens and estrogens. The presence of 17β-hydroxysteroid dehydrogenase enzymes in the Leydig cells in the testis ensures the formation of testosterone, which is the principal male sex hormone secreted by the testis into the circulation. In the blood about 98% of the testosterone is bound to sex-hormone binding globulin, albumin and other

proteins (Figure 1). Only free testosterone is able to enter the cells in the target tissues.

Most active sex steroids are synthesized locally in the peripheral target tissues. This provides autonomous control to target tissues to adjust the metabolism of steroids according to local requirements. In the prostate testosterone is irreversibly metabolized to DHT, which is the most potent androgen and has a five times higher binding affinity to androgen receptor (AR) than that of testosterone. Androgen receptor, which is expressed both in the stromal and epithelial cells, is the major key transcription factor in the prostate. There are regulatory mechanisms that control activity of AR. Mutation constructs show that specific regions in the N-terminal domain of the AR play a role in the regulation (Palvimo et al. 1993). Receptors lacking the ligand-binding domain yielded a constitutively active AR protein, indicating that in the absence of hormone this domain displays an inhibitory function (Jenster et al. 1991). Also, the phosphorylation status of the receptor seems to regulate the activation (Blok et al. 1998).

When DHT binds to the androgen receptor, the complex formed translocates to the cell nucleus and binds to specific DNA sequences called androgen responsive elements (ARE) located within the promoters of the genes sensitive to androgens (Figure 1b) (Claessens et al. 2001). There are also cofactors that take part in the complex and regulate the transcriptional activity and a whole panel of these factors has so far been identified, such as steroid receptor coactivators family (SRC family) and the AR-associated proteins (ARAs)(McKenna et al. 1999). Androgen-regulated genes include a large variety of proteins involved in a variety of functions such as cell proliferation, signal transduction, cellular protein trafficking, cellular energy metabolism (Xu et al. 2001b; Eder et al. 2003). The regulation may either enhance the gene activity (for example *EGFR*, *CDKN1A*, *KLK3*, *IGFPB5* and *CDK2*) or the genes may be negatively regulated (*CDKN2A*, *bcl2* and *TGFβ*).

It is widely accepted that the main site for peripheral steroid inactivation and catabolism is the liver. However, high levels of C19 steroid glucuronides in the prostate suggest that DHT and other androgens are irreversibly inactivated by glucuronidation, also in prostate tissue (Belanger et al. 1990).

In the prostate androgens are the major regulators of proliferation and cell death (Buttyan et al. 1999). The synthesis and secretion of testosterone is stimulated by luteinizing hormone (LH) derived from the anterior pituitary. LH is released to the circulation in pulsatile fashion under the influence of the hypothalamic gonadotropin-releasing hormone (GnRH). In addition, adrenal androgens, such as androstenedione, dehydroepiandrosterone (DHEA) and its sulphate, are secreted by adrenal cortex into the circulation (Rainey et al. 2002). The levels of serum testosterone and adrenal precursors are not constant but peak in early adulthood in men and fall progressively with age (Belanger et al. 1994; Swerdloff and Wang 2004). Also, there is notable fluctuation in the testosterone levels during early and mid puberty (Ankarberg-Lindgren and Norjavaara 2004).

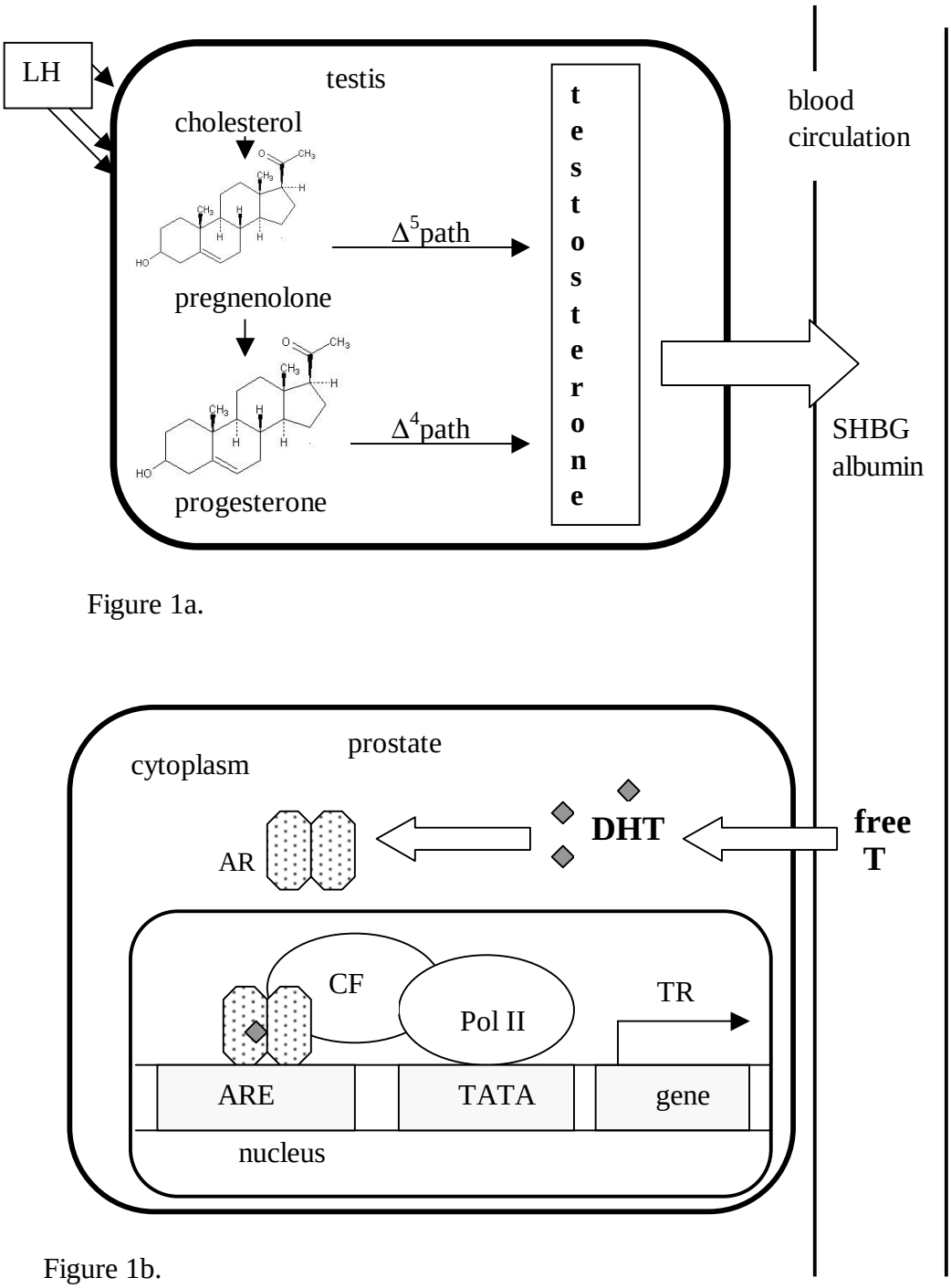

Figure 1a.

Figure 1b.

Figure 1. Biosynthesis of androgens (1a) and androgen signalling in the prostate (1b). AR, androgen receptor; CF, cofactors; Pol II, RNA polymerase II complex; T, testosterone; TATA, RNA polymerase II binding site; SHBG, sex hormone binding globulin; TR transcription.

2.2.1 Androgen–regulated *KLK3* gene

The *KLK3* gene codes for the prostate specific antigen, a widely-used serum tumor marker, whose expression is regulated by androgen signalling. PSA is a serine protease that has also been suggested to facilitate prostate cancer growth and invasion. Two polymorphisms have been identified in the androgen responsive element 2 (ARE2) of the *KLK3* gene promoter, -205_-206insA and -252A>G (Yang et al. 2001). In the Japanese study population -252 A was linked to the presence of -205 AA (allele with insertion), and the -252 G was always linked to the presence of -205 A (allele with no insertion). Therefore, only A-AA and G-A (-252--205) alleles were present. Compared to G-A allele homozygotes, prostate cancer patients carrying at least 1 A-AA alleles tended to exhibit high serum PSA levels (p=0.0002), poor differentiation (p=0.0149) and advanced clinical stage (p=0.0077). These results suggest that the polymorphisms in the *KLK3* gene promoter may affect the transcriptional activity of the gene, and an excess of PSA production may enhance rapid progression of prostate cancer. However, in a recent study several SNPs (-4643G/A, -5412C/T and -5429T/G) in the promoter region of the *KLK3* gene were reported to be associated with increased serum PSA levels in men without prostatic disease (Cramer et al. 2003).

Another polymorphism –158G>A is located in the androgen respontive element 1 (ARE1) of the *KLK3* gene. Subjects with the *KLK3* G/G genotype were at significantly increased risk for advanced, but not for localized, prostate cancer (OR=2.90; 95%CI 1.24-6.78)(Xue et al. 2000). In another study a significantly higher *KLK3* G/G distribution in prostate cancer (30%) than in population controls (16%; p=0.025) was observed. Morover, the G/G distribution within cases was even greater in younger men (<65 years; 42%; P = 0.012)(Binnie et al. 2005).

2.3 Androgens in prostate cancer

During its initial stages prostate cancer growth is still dependent on androgens. Thus one of the main treatments in prostate cancer is androgen ablation, which aims to block androgen activity. About 70-90% of prostate cancers initially respond to this treatment (Grayhack et al. 1987). Androgen blockage usually decreases the volume of the primary and metastatic lesions (Kyprianou et al. 1991). Androgen ablation was initially accomplished by surgical castration, but today efficient blockage can be achieved by using GnRH agonists and non-steroidal antiandrogens. Unfortunately, after the initial response to therapy the tumor reoccurs in an androgen-independent form and does not respond to androgen withdrawal (Oh and Kantoff 1998). The mechanism behind this transformation is still unclear (Grossmann et al. 2001; Visakorpi 2003). Androgen receptor may participate in tumor progression. One mechanism could be the upregulation of the receptor activity caused by amplification of the *AR*

gene (Visakorpi et al. 1995). Point mutations may also change the ligand-binding specificity and thus promote receptor activity (Veldscholte et al. 1990). There are morover studies reporting that androgen receptor may be activated through ligand-independent activation (Nazareth and Weigel 1996; Culig et al. 2000). Dysregulated cofactors may also contribute to the escape of tumors from endocrine therapy (Miyamoto et al. 1998).

3. Genes regulating androgen biosynthesis and metabolism as risk factors for prostate cancer

3.1 LHB

Luteinizing hormone (LH) secreted by the anterior pituitary stimulates the production of androgens in the testis (Figure 1a). LH is an α:β heterodimer, in which the α-unit is common to glycoprotein hormones, and the unique β subunit determines biological specificity. The LHβ subunit is coded by *LHB* gene on chromosome 19q13. The gene has two linked alterations leading to amino acid changes in the subunit: W8R and I15T (Furui et al. 1994). The variant LHβ-V (R8 and T15) has higher bioactivity and shorter half-life than the wild type protein (Haavisto et al. 1995; Suganuma et al. 1996)**.** A weak positive association between the LHβ-V genotype and risk of familial prostate cancer has been reported (OR=1.29, 95%CI 0.96-1.75)(Elkins et al. 2003).

3.2 CYP19A1

The biosynthesis of C_{18} estrogens from C_{19} androgens is catalyzed by an enzyme aromatase, which belongs to a large family of cytochrome P450 oxidases (CytocromeP450HomePage, http://drnelson.utmem.edu/CytochromeP450.html; Figure 2). Aromatase is coded by the *CYP19A1* gene on chromosome 15q21.1. It is widely expressed in a number of tissues throughout the body, such as ovary, placenta, testis, liver and brain (McNatty et al. 1979; Bulun et al. 1993)**.** Aromatase is also expressed in the stromal tissue of the prostate gland (Ellem et al. 2004). Interestingly, in malignant prostate tissue aromatase is active and also expressed in the epithelial cells and in LNCaP, PC3 and DU145 cell lines (Ellem et al. 2004).

A substitution polymorphism R264C (C>T) in the *CYP19A1* showed a weak tendency to increase prostate carcinoma risk among Caucasian men (Modugno et al. 2001). In another study of 101 Japanese familial prostate cancer cases the presence of at least one T allele was associated significantly with prostate carcinoma risk (OR=1.77, 95%CI 1.02-3.09) and especially with high-grade carcinoma (OR=2.565, 95%CI 1.47-4.46)(Suzuki et al. 2003a). However,

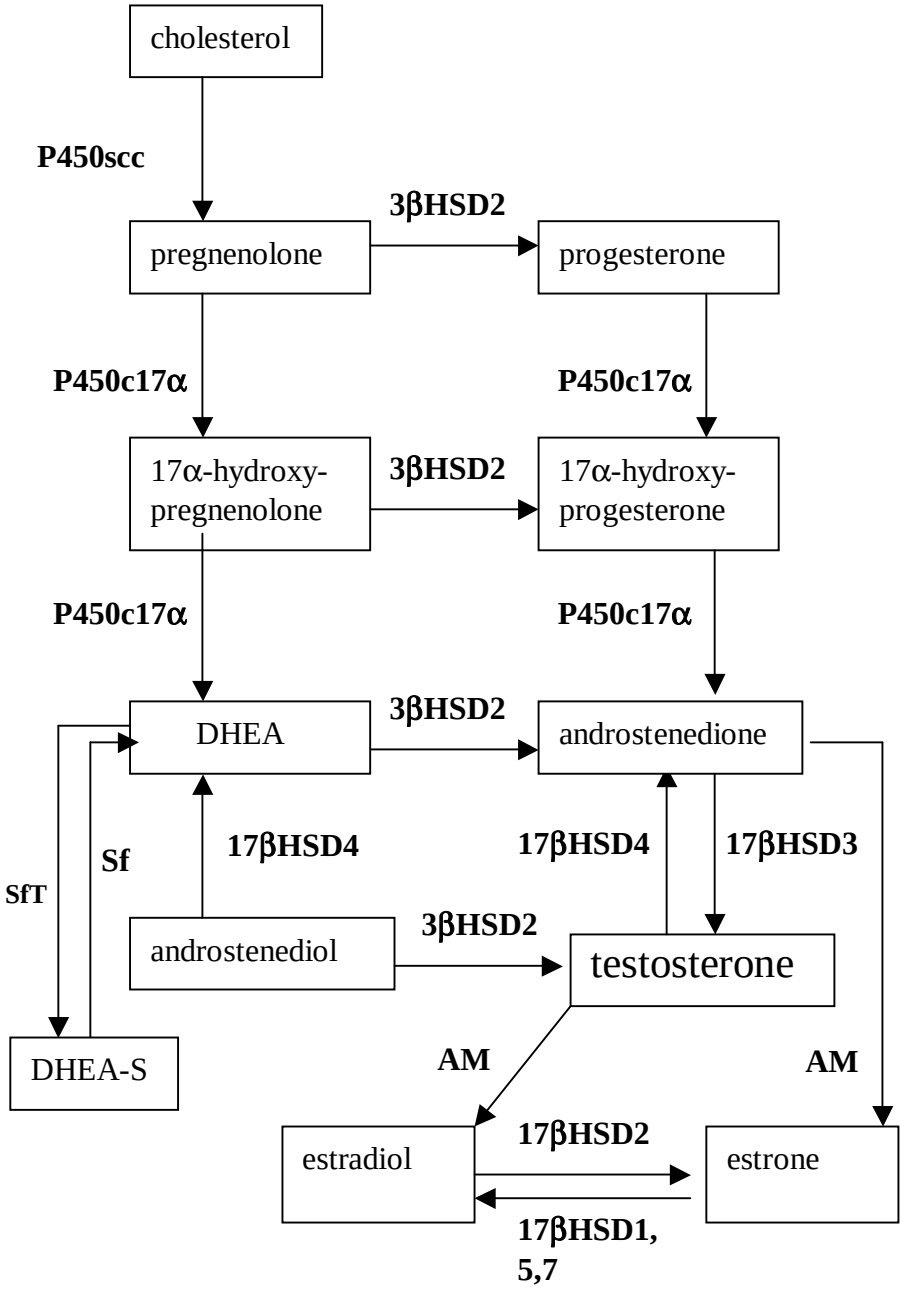

Figure 2. Biosynthesis of testosterone. AM, aromatase; DHEA-S, DHEA-sulfatate; P450scc, cytochrome P450scc; Sf, sulfatase; SfT, sulfyltransferase.

another Japanese group found no association between R264C polymorphism and prostate cancer risk (Fukatsu et al. 2004). In addition, Watanabe et al. (1997) reported that aromatase activity was not affected by the R264C alteration. However, Zmuda et al. (1999) reported that the level of bioavailable estrogen was 18.5% lower in the R264C carriers compared to noncarriers.

Exon 4 of the *CYP19A1* gene contains a tetranucleotide repeat $(TTTA)_n$. (Latil et al. 2001) found five different alleles among the 382 subjects of white French origin: 167 bp, 171 bp, 175 bp, 183 bp and 187 bp and reported an association between the alleles 171 bp and 187 bp and prostate cancer risk (OR=1.585, 95%CI 1.00-2.513, and OR=1.585, 95%CI 1.00-2.513, respectively). Among Japanese familial prostate cancer cases and controls; shorter repeats (>9 repeats, range from 7 to 13) significantly increased prostate cancer risk in comparison with longer genotypes (OR=1.8, 95%CI 1.04-3.11) (Suzuki et al. 2003b). No association between the pathological grade or stage of the cancer (Latil et al. 2001; Suzuki et al. 2003b), age of onset or preoperative PSA (Latil et al. 2001) and $(TTTA)_n$ repeat polymorphism was observed.

3.3 CYP17A1

CYP17A1 gene located on chromosome 10q24.3 encodes a cytochrome P450 subfamily 17A enzyme (P450c17α) that is involved in the biosynthesis of androgens (Figure 2). This enzyme mediates both steroid 17α-hydroxylase and 17,20 lyase activities at the key points in testosterone synthesis in gonads and adrenals. Carey et al. (1994) reported a single base pair change (T>C) in the 5'UTR that was positively associated with polycystic ovaries and male pattern baldness. This substitution in *CYP17A1* was hypothesized to create an additional binding site (CCACT to CCACC) for the transcription factor Sp-1, that may lead to increased transcription of the enzyme and enhanced steroid hormone production. However, in an *in vitro* assay Nedelcheva Kristensen et al. (1999) did not observe Sp-1 binding at this polymorphic site. However, they suggest, that there may be interactions between the *CYP17A1* polymorphism and transcription factors other than Sp-1, such as tissue specific transcription factors (Nedelcheva Kristensen et al. 1999).

Lunn et al. (1999) reported an association between the prostate cancer risk and the C allele in Caucasians in the USA (OR=1.7, 95%CI 1.0-3.0). Since then contradictory results concerning the potential role of this alteration in prostate cancer risk have been published. Some studies report an elevated risk of prostate cancer among the C allele carriers (OR=1.23-2.8) (Gsur et al. 2000; Yamada et al. 2001). Also, Stanford et al. (2002) reported a greatly elevated risk among white American the C allele carriers with an affected first-degree relative, (OR= 19.2, 95%CI= 2.2-157.4). Other studies claim that the T allele may increase the risk (OR=1.63-2.57) (Wadelius et al. 1999; Habuchi et al. 2000) and several studies have reported a negative association (Allen et al. 2001; Chang et al. 2001; Lin et al. 2003; Madigan et al. 2003; Nam et al. 2003; Cicek et al. 2004).

3.4 HSD3B family

The human *HSD3B* gene family has eight members of which *HSD3B1* and *HSD3B2* express 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD) types 1 and 2. The genes *HSD3B1* and *HSD3B2* are located respectively on chromosomes 1p13-p11 and 1p13.1 and are expressed in a tissue specific manner. *HSD3B1* is expressed in placenta and peripheral tissues, such as prostate, breast and skin (Labrie et al. 1992; Gingras et al. 1999; Gingras and Simard 1999). Type 2 is almost exclusively expressed in classical steroidogenic tissues, namely adrenals, testis and ovary (Rheaume et al. 1991; Lachance et al. 1992). 3β-HSDs are required for the biosynthesis of androgens (Figure 2). In addition, they are critical components in androgen metabolism because they catalyze the conversion of active DHT into inactive metabolites in steroid target tissues such as prostate (Figure 3). Alterations in the *HSD3B2* have been found to cause congenital adrenal hyperplasia and male pseudohermaphroditism (Simard et al. 2000; Simard et al. 2002).

Chang et al. (2002a) identified a total of five SNPs (single nucleotide polymorphism) in *HSD3B1* gene and six SNPs in *HSD3B2* in the screening panel of 96 subjects of Caucasian and African-American sporadic and hereditary prostate cancer cases and nonprostate cancer controls. They reported that the N367T variant found in *HSD3B1* increased the risk of prostate cancer among the Caucasians (RR=1.50, 95%CI 1.01-2.24). Further evidence for association was found when they studied the joint effect of *HSD3B1* N367T and another variant c7519g found on 3'UTR of *HSD3B2*. Men with the variant genotypes at either one of the tested alterations had a significantly higher risk of developing prostate cancer (RR=2.17, 95%CI 1.29-3.65, HPC *versus* controls; RR=1.61, 95%CI 1.07-2.42, sporadic *versus* controls). Among older men (age at diagnosis ≥60 years) the risk was even greater (RR=3.14, 95%CI 1.52-6.49, HPC *versus* controls; RR=2.62, 95%CI=1.34-5.14, sporadic *versus* controls). They also found that a subset of HPC probands, whose families provided evidence for linkage at 1p13, predominantly contributed to the observed association suggesting that the evidence for linkage at 1p13 may at least be partially be explained by the variants of *HSD3B* genes. They also studied the effect of the carrier status on clinical behaviour, but no statistically significant differences were found between the groups with low (≤6) or high (≥7) Gleason score or between the groups with disease confined to the prostate *versus* nonlocalized disease (Chang et al. 2002a).

3.5 AKR1C family

3α-hydroxysteroid dehydrogenases (3αHSDs) are members of the large aldo-keto reductase superfamily (the Aldo-Keto Reductase (AKR) Superfamily homepage http://www.med.upenn.edu/akr/). 3αHSDs regulate the occupancy of the androgen receptor in the prostate tissue (Figure 3). They also play a critical

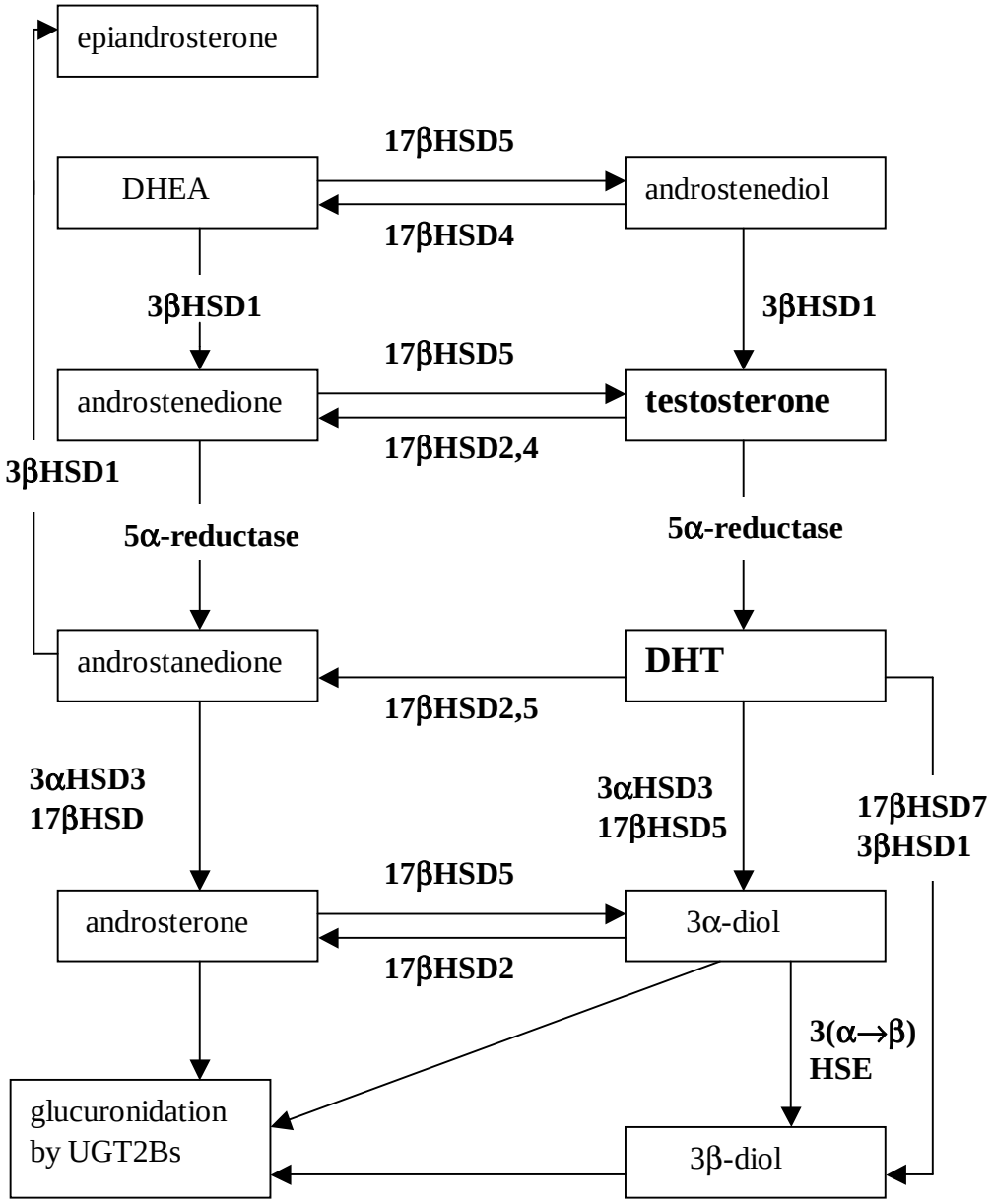

Figure 3. Metabolism and inactivation of testosterone and DHT in the prostate. 3(α→β)HSE, 3(α→β)hydroxysteroid epimerase;3β-diol, 5α-androstane-3β17β-diol.

role in the hepatic clearance of the steroid hormones and thus serve to protect against circulating steroid hormone excess (Pirog and Collins 1999). *AKR1C3* (alias *HSD17B5*) located on 10p15-p14 codes for a 3-alpha hydroxysteroid dehydrogenase type II/17β-hydroxysteroid dehydrogenase (17βHSD5), which has been shown to have both 3α- and 17β-HSD activities. 17βHSD5 is expressed in the liver, endometrium, ovary, prostate and mammary gland (Luu-The et al. 2001; Penning et al. 2001). The enzyme acts as a 17-ketosteroid reductase and converts androstenedione into testosterone. However, in the prostate the enzyme seems to prefer DHT and androstanedione as substrates and thus favours the inactivation of highly active DHT (Penning et al. 2001). *AKR1C2* gene on chromosome 10p15 codes for the 3α-hydroxysteroid dehydrogenase type 3 (3αHSD3), which is primarily expressed in the lung and liver, and also in the prostate, testis, mammary gland, uterus and brain (Penning et al. 2000; Dufort et al. 2001). This enzyme acts as a ketosteroid reductase in the prostate cells by catalyzing a reaction, which converts DHT into weaker androgen, 3α-diol (Penning et al. 2004). Its function is regulated by DHT (Jin and Penning 2001).

AKR1C4 codes for the 3α-hydroxysteroid dehydrogenase type 1 (3αHSD1). The gene is located on chromosome 10p15-10p14. It is expressed almost exclusively in the liver and is believed to be involved in the catabolism of circulating steroid hormones (Penning et al. 2000; Dufort et al. 2001).

3.6 HSD17B family

The *HSD17B* gene family encodes for the 17β-hydroxysteroid dehydrogenases (17βHSDs) that play a pivotal role in the production of steroid hormones. Some 17βHSDs are involved in the reduction of 17-ketosteroids leading to the biosynthesis of active androgens. The oxidative 17βHSDs catalyze the conversion of 17β-hydroxysteroids into less active androgens (Figures 2 and 3).
17β-hydroxysteroid dehydrogenase type 2 (17ßHSD2) is coded by *HSD17B2* gene located on chromosome 16q24. 17ßHSD2 enzyme is widely expressed in different tissues such as placenta, breast, uterus, testis, liver and prostate (Moghrabi et al. 1997; Peltoketo et al. 1999b). It is also expressed in BPH and prostate cancer and higher expression of *HSD17B2* has been observed in BPH compared with prostate cancer (Elo et al. 1996). Because the reductive 17ßHSD2 is involved in the conversion of active androgens into their less active forms, it may protect prostate tissue from excessive sex hormone action (Figure 3). An association between the loss of heterozygosity at chromosomal region 16q24.1-16q24.2, which includes the *HS17B2* gene, and the risk for clinically aggressive prostate cancer has been reported (Elo et al. 1997). Also, during the cellular transformation of LNCaP prostate cancer cells from the androgen-dependent stage to the androgen-independent stage the relative expression of HSD17B2 decreases markedly (Härkönen et al. 2003).

The *HSD17B3* gene on chromosome 9q22 codes for the 17β-hydroxysteroid dehydrogenase type 3 (17ßHSD3). 17ßHSD3 is almost exclusively expressed in

the testis, where it converts androstenedione into testosterone (Figure 2) (Geissler et al. 1994). Mutations in *HSD17B3* are responsoble for male pseudohermaphrotidism from 17ßHSD deficiency. In a recent study a missense substitution G289S in *HSD17B3* gene was reported to confer a statistically significant increase in risk for prostate cancer in the Italian study population (Margiotti et al. 2002). The 17βHSD type 4 coded by *HSD17B4* at 5q2 is ubiquitously expressed in a wide variety of tissues. This enzyme catalyses oxidative reactions such as conversion of testosterone into androstenedione (Penning 2003).

Several other members of the 17βHSDs family could be involved in androgen biosynthesis and degradation. Even though 17βHSD type 7 efficiently catalyzes the transformation of estrone into estradiol, it has been shown to also possess a 3-keto-reductase activity that inactivates DHT (Nokelainen et al. 1998; Luu-The 2001; Vihko et al. 2004). Also, the mRNA of this enzyme has been detected in the ovary, breast, placenta, liver and also in the prostate and testis (Luu-The 2001). Type 8 17βHSD is also involved in estrogen metabolism, but is also capable of catalyzing oxidative reactions of androgens (Peltoketo et al. 1999a). The 17βHSD type 10 expressed in human gonads as well as brain, liver and placenta can also catalyze the conversion of androstanediol into DHT (He et al. 1999; He et al. 2001). 17βHSD type 11 has been shown to efficiently catalyze the conversion of androstanediol into androsterone (Brereton et al. 2001). In mice 17βHSD type 10 and 11 have been shown to localize in epithelial cells of the prostate (Pelletier et al. 2004).

3.7 SRD5A2

Steroid 5α-reductase type II irreversibly converts testosterone into the most potent androgen DHT (Figure 3). The enzyme is coded by the gene *SRD5A2* located on chromosome 2p23 and is expressed in different tissues such as fetal genital skin, liver, epididymis and the prostate (Thigpen et al. 1993; Silver et al. 1994). In the prostate the *SRD5A2* seems to be expressed in the basal epithelial and stromal, but not in the luminal epithelial cells (Silver et al. 1994).

A missense substitution in *SRD5A2* gene, which results in the replacement of alanine residue at codon 49 with threonine (A49T) has been reported to increase the activity of the 5α-reductase (measured as V_{max}) substantially (Makridakis et al. 1999; Makridakis et al. 2000). However, men who possessed at least one copy of the variant T allele were shown to have a significantly lower androstanediol glucuronide concentration than men who were homozygous for the wild-type allele (p=0.0003), suggesting lower 5α-reductase activity *in vivo* (Allen et al. 2003). Makridakis et al. (1999) reported a significant association of this alteration and prostate cancer risk among American-African and Hispanic men. The African-American carriers had a 7.2-fold risk for clinically advanced prostate cancer. Accordingly, Jaffe et al. (2000) reported that the presence of the A49T variant was associated with a greater frequency of extracapsular disease

(OR=3.16, 95%CI 1.03-9.68) and a higher pathological tumor-lymph node metastasis (pTNM) stage (OR=3.11, 95% CI, 1.01-9.65). In addition, the A49T variant was overrepresented in two poor prognostic groups. Söderström et al. (2002) observed no connection between the A49T alteration and prostate cancer risk but reported that prostate cancer cases heterozygous for A49T alteration were significantly younger than cases that carried the AA genotype (mean age 66 years *vs* 71, P=0.038). However, several studies have failed to confirm these results (Margiotti et al. 2000; Latil et al. 2001; Chang et al. 2003; Lamharzi et al. 2003; Cicek et al. 2004).

Another substitution mutation V89L in the *SRD5A2* gene is most common in Chinese and Japanese populations, who have the lowest risk for prostate cancer among the different racial groups (Makridakis et al. 1997). Recent studies show that the L89 variant confers a 42% reduction in the 5α-reductase enzymatic activity *in vitro* (Makridakis et al. 2000). Also, in Asians the alteration has been shown to be associated with low serum concentrations of 3α-androstanediol glucuronide, a major metabolite of DHT and thought to reflect the activity of the 5α-reductase *in vivo* (Makridakis et al. 1997; Hsing et al. 2001). Similarly, a small but nonsignificant reduction of 3α-androstanediol glucuronide serum levels was also reported in British men with the L/L genotype (Allen et al. 2001). No association was found between the V89L polymorphism and 3α-androstanediol glucuronide (Febbo et al. 1999). Men carrying V89 allele were reported to have 2.5 fold increase in the risk of prostate cancer development and a 3.3-fold increase in the risk of progression compared with men with the L/L genotype (Nam et al. 2001). Japanese men with the V/V or V/L genotype were at significantly increased risk for prostate cancer compared with those with the L/L genotype (OR=1.69, 95%CI 1.07-2.65) (Li et al. 2003). In contrast, the findings suggest that the SRD5A2 V89L variant may influence the risk of developing prostate cancer, especially among men with a younger age at diagnosis or more aggressive disease (Cicek et al. 2004). Contrary to these, there are several studies where this polymorphism has not shown an association with the risk for prostate cancer. No significant association with prostate cancer risk has been observed among Caucasian men (Febbo et al. 1999; Lunn et al. 1999; Latil et al. 2001; Margiotti et al. 2002; Söderström et al. 2002), in Chinese (Hsing et al. 2001) or in Japanese (Yamada et al. 2001). Similarly, no effect was seen in a multiethnic study (Pearce et al. 2002) or in HPC families (Chang et al. 2003). Jaffe et al. (2000) also reported that V89L polymorphism has no significant impact on the pathological or clinical features of prostate cancer. However, Söderström et al. (2002) found an association with *SRD5A2* V89L L/L genotype and metastases at the time of diagnosis. Also, Shibata et al. (2002) observed poorer prognosis among men with the L/L genotype at codon 89 of the *SRD5A2* gene.

The polymorphic $(TA)_n$ dinucleotide repeat is located on the 3'UTR region in the *SRD5A2* gene (Davis and Russell 1993). The most common allele is $(TA)_0$ *i.e.* no (TA) repeat, which comprises over 96% of alleles and the two variants are $(TA)_9$ and $(TA)_{18}$. Some other rare alleles have also been reported (Reichardt et al. 1995). Because the $(TA)_n$ variation is located in the 3'UTR area, its functional

consequences are thought to be due to the instability of mRNA transcripts with UA-rich 3'UTRs (Zubiaga et al. 1995; Makridakis et al. 1999). However, no association of the $(TA)_n$ variation with serum 3α-androstanediol glucuronide level was observed (Hsing et al. 2001). Longer alleles have been associated with a modest non-significant reduction in the prostate cancer risk (Hsing et al. 2001; Lamharzi et al. 2003). However, there are other studies where no association has been seen (Kantoff et al. 1997; Latil et al. 2001; Allen et al. 2003).

The Q variant of the R227Q polymorphism also substantially reduces 5α-reductase activity *in vitro* (Makridakis et al. 2000) and subjects with Q/Q genotype are male pseudohermaphrodites (Sinnecker et al. 1996). Hsing et al. (2001) studied the possible effect of this variant on prostate cancer risk in Chinese subjects, but no association was seen.

3.8 AR

The biological effect of androgens is mediated through their binding to intracellular androgen receptors, which in turn regulate the transcription of target genes (Figure 1b). Androgen receptor is coded by the *AR* gene on chromosome Xq11.2-q12. Androgen receptor consists of three domains: a conserved ligand binding domain, a DNA binding domain and an N-terminal transactivation domain. The transactivation domain contains polyglutamine and polyglycine tracts that are encoded by two polymorphic trinucleotide repeat segments, $(CAG)_n$ and $(GGN)_n$ tracts respectively**.** The deletion of the polymorphic trinucleotide repeat GGN has been shown to reduce the transcriptional activity of AR by about 30% in transient transfection assays (Gao et al. 1996). Also, AR protein levels were inversely affected by glycine repeat length (Ding et al. 2004). An inverse correlation between the length of the other repeat polymorphism CAG and the transactivation activities of AR has been demonstrated by *in vitro* assays (Mhatre et al. 1993; Chamberlain et al. 1994; Beilin et al. 2000). Long CAG repeat length has also been reported to reduce the expression of *AR* mRNA and protein (Choong et al. 1996). In a recent study Irvine et al. (2000) also reported that increased CAG length inhibited androgen receptor transactivation in cultured prostate epithelial cells. Therefore, a decreased number of CAG repeats may make prostate tissue more vulnerable to long-time androgen stimulation leading to increased proliferative activity, which in turn may increase the rate of somatic mutations (Nelson and Witte 2002). Men with exceptionally long CAG repeat length in the *AR* gene have symptoms of clinical androgen insensitivity (Igarashi et al. 1992). Also, expansion of the CAG repeat (to 40-52 repeats) in the androgen receptor is found in patients with the X-linked neuromuscular disorder spinal and bulbar muscular atrophy (La Spada et al. 1991). In addition, the CAG repeat polymorphism has been associated with other androgen-related clinical conditions. A large number of repeats seem to adversely influence fertility and spermatogenesis, bone density and shorter

repeat areas have been associated with increased risk of baldness (Sawaya and Shalita 1998; Yoshida et al. 1999; Zitzmann et al. 2001).

In prostate cancer patients, somatic and germline mutations in *AR* gene are rare (Androgen Receptor Mutation database, www.androgendb.mcgill.ca). Six germline alterations have been reported so far: R726L in exon 5 (Elo et al. 1995), G2T and C214A in the 5'UTR region (Crocitto et al. 1997), Q798E in exon 6 (Evans et al. 1996), Q211Q in exon 1 (Koivisto et al. 2004) and E211 G>A, which was reported to be associated with a lower risk of metastatic prostate cancer (Hayes et al. 2005).

The R726L mutation originally described by Elo et al. (1995) is located in the ligand binding domain of androgen receptor. In the same study the mutated AR showed significantly more functional activity in the presence of 10 and 100 nmol/L estradiol than the wild type receptor ($p<0,005$). In a similar experiment no transactivation of the mutated or wild type receptor by estradiol was observed when using physiological level of estradiol (10^{-10} M)(Shi et al. 2002). However, the combination of progesterone and estradiol at physiological concentrations (10^{-9} and 10^{-10} M respectively) weakly activated the receptor. Thompson et al. (2001) reported that the substitution of arginine with leucine in the ligand binding area did not affect the stability of the receptor-DNA complex or the amino/carboxyl interaction, which coordinates the molecular events of a functioning receptor. However, the transactivation efficiency of R726L mutant receptor was slightly reduced (Thompson et al. 2001).

To date, there have been several studies analyzing the CAG repeat number and its effect on prostate cancer risk, some with positive association for shorter CAG repeat and increased prostate cancer risk (Giovannucci et al. 1997; Hakimi et al. 1997; Ingles et al. 1997; Hsing et al. 2000a), and several with negative association (Irvine et al. 1995; Stanford et al. 1997; Edwards et al. 1999; Beilin et al. 2001; Latil et al. 2001; Chen et al. 2002; Gsur et al. 2002b; Huang et al. 2003; Santos et al. 2003; Cicek et al. 2004; Gilligan et al. 2004; Freedman et al. 2005). In some studies the shorter length of the repeat has been reported to increase the aggressive nature of prostate cancer (Giovannucci et al. 1997; Bratt et al. 1999; Modugno et al. 2001; Balic et al. 2002). However, not all studies have been able to confirm these results (Stanford et al. 1997; Correa-Cerro et al. 1999; Edwards et al. 1999; Hsing et al. 2000a; Lange et al. 2000; Mir et al. 2002; Huang et al. 2003; Gilligan et al. 2004). Also, shorter CAG repeat lengths may be associated with the development of prostate cancer in men at a younger age (Hardy et al. 1996; Bratt et al. 1999; Beilin et al. 2001; Santos et al. 2003). In some studies, no correlation between the age of onset and the length of CAG repeat has been observed (Hsing et al. 2000a; Mir et al. 2002; Huang et al. 2003). In a recent study Forrest et al. (2005) reported that short *AR* (CAG)(n) repeats were associated with a significantly reduced risk of early onset prostate cancer. In another recent study, Powell et al. (2005) reported that men with prostate carcinoma who had >18 CAG repeats had an estimated 52% increased risk of disease recurrence. In some earlier studies no such effect was observed (Hardy et al. 1996; Edwards et al. 1999; Nam et al. 2000).

Irvine et al. (1995) reported that the distribution of GGC repeats, another length polymorphism in androgen receptor, was significantly different among African-Americans, white and Asian men and suggested a possible association between GGC microsatellite of the androgen receptor gene and prostate cancer development. Stanford et al. (1997) published a study where they claimed that the risk of prostate cancer was higher in men with short GGN repeat (≤16, OR=1.6, 95%CI 1.07-2.41). Since then similar results have been obtained by some studies (Hakimi et al. 1997; Chang et al. 2002b). There are also several studies where no association has been observed (Correa-Cerro et al. 1999; Edwards et al. 1999; Hsing et al. 2000a; Miller et al. 2001; Chen et al. 2002; Cicek et al. 2004; Forrest et al. 2005). Also, (Edwards et al. 1999) reported that non-significant trends with stage and grade were found in the proportion of short GGC alleles.

3.9 UGT2B family

Conjugation of steroids by glucuronidation is catalyzed by UDP-glucuronosyl transferase enzymes. The glucuronide products resulting from transfer of the glucuronosyl group from UDP-glucuronic acid to small hydrophobic molecules are generally water soluble and more easily excreted from the body than are the parent compounds. The *UGT* gene superfamily encoding UDP-glucuronosyl transferase enzymes is divided into two families, *UGT1*, which have preferential recognition for estrogens, and *UGT2*, members of which are further categorized into two subfamilies, *UGT2A* and *UGT2B* (Guillemette et al. 2004).

The seven human *UGT2B* genes isolated to date are clustered on chromosome 4q13-q21.1 and are expressed in the liver as well as in a large number of extrahepatic steroid target tissues such as skin, breast and prostate (Radominska-Pandya et al. 1999). Four members of this family, *UGT2B7*, *UGT2B15*, *UGT2B17* and *UGT2B28* are involved in the conjugation of steroid hormones (Turgeon et al. 2000)

UGT2B28 is only expressed in the liver and mammary glands and conjugates estradiol and 3α-diol (Levesque et al. 2001). Transcripts of *UGT2B7* have been detected in the intestine, liver, kidney, skin, brain, uterus and mammary glands (Turgeon et al. 2001). UGT2B7 conjugates 3α-diol and androsterone and thus may take part in the elimination of androgens in the liver. The UGT2B15 isoform has a high capacity for 3α-diol and moderate for DHT (Turgeon et al. 2001). In contrast to UGT2B15, which specifically conjugates the 17-hydroxy position, UGT2B17 conjugates both the 3-hydroxy and the 17-hydroxy position (Beaulieu et al. 1996). This isoform has the highest capacity for the androsterone and DHT, but also conjugates 3α-diol. Both *UGT2B15* and *UGT2B17* are expressed in a wide variety of tissues such as liver, kidney, testis, breast, and also in the prostate (Beaulieu et al. 1996; Levesque et al. 1997). In the prostate *UGT2B17* is detected in the basal cells, whereas *UGT2B15* is only found in the luminal cells (Barbier et al. 2000). It is likely that UGT2B17 is responsible for

glucuronidation of 3α-diol and androsterone formed in the basal cells, whereas UGT2B15 would conjugate DHT in the luminal cells.

In *UGT2B7* gene an alteration H268Y results in a nonconservative amino acid change in the region of substrate binding and may affect the enzyme's substrate specificity (Mackenzie 1990; Jin et al. 1993). Lampe et al. (2000) reported that Asians have a greater than 2-fold higher prevalence of the *UGT2B7* H268 homozygous genotype compared with Caucasians.

In the *UGT2B15* gene a single base-pair change results in an amino acid change at residue 85 from aspartate (D) to tyrosine (Y). The *UGT2B15* isoform with Y85 is twice as efficient as D85 in conjugating 3α-diol and DHT (Levesque et al. 1997). It was postulated that individuals homozygous for D85 would have a higher androgen exposure. A study with 64 prostate cancer patients and controls demonstrated that the D85 allele was significantly more prevalent in the prostate cancer patients and the risk of developing prostate cancer was threefold higher among the subjects homozygous for D85 allele (OR=3.0, 95%CI=1.3-6.5) (MacLeod et al. 2000). Accordingly, Park et al. (2004) reported a significantly increased risk of prostate cancer in subjects with the homozygous *UGT2B15* D85 (OR=2.7, 95%CI=1.1-6.6) genotype. However, in a study of 190 Austrian prostate cancer patients and 190 patients with benign prostate cancer patients as controls, no association was seen (Gsur et al. 2002a). In a recent study, Hajdinjak and Zagradisnik (2004) reported a correlation between the polymorphism D85Y and differentiation of prostate cancer; individuals homozygous for D85 allele had a higher risk for more aggressive prostate cancer (p=0.047).

AIMS OF THE STUDY

The overall purpose of this study was to identify low to moderate risk prostate cancer predisposition genes that participate in the biosynthesis and metabolism of androgens. The specific aims were:

1. to study the variation in some of the major genes acting along the androgen pathway in Finnish subjects

2. to study the roles of two androgen receptor gene *AR* variants, R726L and CAG repeat polymorphism, in prostate cancer

3. to investigate the role of polymorphisms in the 5α-reductase gene *SRD5A2* in prostate cancer risk in the Finnish population

4. to investigate possible interactions of genes carrying alterations in relation to prostate cancer risk

MATERIALS AND METHODS

1. Subjects

To study germline alterations blood samples were collected from unselected prostate cancer cases, from affected and healthy members of prostate cancer families and from patients with benign prostate hyperplasia (BPH) by the study group Genetic Predisposition to Prostate Cancer in the Laboratory of Cancer Genetics at the University of Tampere and Tampere University Hospital (TAUH). All the unselected prostate cancer and BPH cases were diagnosed in the Pirkanmaa Hospital District with a population of around 450,000. Samples from prostate cancer families were collected from the whole of Finland.

1.1 Unselected prostate cancer patients and patients with benign prostate hyperplasia

Samples from unselected prostate cancer and BPH cases have been collected since 1996. Systematic collection started in 1999 and at the moment there are 2805 DNA samples from prostate cancer patients and 460 from BPH patients. The diagnoses of all patients were confirmed through medical records.

1.2 Familial prostate cancer cases

Identification of families was accomplished through nation-wide registry based searches, referrals to physicians and newspaper, television and radio advertisements. Families with at least two affected first or second degree relatives were counted as familial prostate cancer cases. Samples were also collected from unaffected relatives of the prostate cancer patients. At the moment we have altogether 1397 DNA samples from the affected and unaffected members of prostate cancer families. The patients´ family histories for malignancies were initially obtained from family questionnaires and were confirmed from the Finnish Cancer Registry.

1.3 Controls

Samples from healthy anonymous blood donors (age range 16-65) were used as population-level controls. The samples were obtained from the Blood Center of the Finnish Red Cross in Tampere (500 samples), Kuopio (260 samples) and Turku (200 samples). These studies are therefore based on a population-based sampling of healthy blood donors and do not attempt to serve as a classical case-control study.

1.4 Samples used in Studies I-IV

The samples used in Studies I-IV are presented in Table 2.

Study I

During the sample collection in 1996-1999, 559 new prostate carcinomas were diagnosed at TAUH. Twenty-five per cent of the patients were excluded because of positive family history for prostate cancer or refusal to participate in the study. A sample from one randomly chosen affected case from each prostate cancer family was screened for the R726L mutation. The controls of this study included samples from men (656) and women (122) who donated blood in Tampere.

Study II

Samples from familial prostate cancer cases were randomly chosen from 94 Finnish prostate cancer families. Autopsy samples were obtained from males aged > 65 years who were determined to be cancer-free at autopsy in TAUH .

Study III

The mean age of the unselected prostate cancer patients was 68.1 years with a median of 67 (SD 8.3, range 48-94). The median serum PSA value at diagnosis was 14.9, with a range from 1.2 to 30.000 ng/ml. 14.1% of the unselected prostate cancer patients had a positive family history of prostate cancer according to their responses in the questionnaire. Familial prostate cancer cases were randomly selected from 99 Finnish prostate cancer families.

Study IV

Samples in single strand conformational polymorphism

The initial screening by single strand conformational polymorphism (SSCP) of genetic variation in 10 genes was performed among 32 men with familial prostate cancer and 32 men with unselected prostate cancer. The mean number of

Table 2. Sample sets used in Studies I-IV.

	Study I	Study II	Study III	Study IV/ genotyping
unselected prostate cancer cases	418*	449	461	847
familial PC cases	106	111	105	121
BPH cases	-	223	223	-
autopsy samples	-	72	-	-
controls (blood donors)	778	516	574	923
T-stage (unselected only)	-	92%	92%	100%
M-stage (unselected only)	-	81%	82%	51%
WHO grade (unselected only)	-	95%	94%	97%
Gleason score (unselected only)	-	67%	66%	92%

* patients with positive family history were excluded

affected family members in these 32 families was 3.6 (range 2-7) and the mean age at diagnosis was 64.8 years (range 50-76). The mean age at diagnosis of the 32 men with unselected prostate cancer was 54.9 years (range 49-58). The patients were diagnosed in the period of 1990-1999.

Samples used in large-scale genotyping

The youngest affected member with available sample was genotyped from each family. The mean age at diagnosis of the prostate cancer cases was 68.9 years with a range of 45-93 years. There was a total of 998 diagnoses of prostate cancer in Pirkanmaa during the three-year period, indicating that we obtained DNA samples and clinical data from 85% of all cancers diagnosed in the area during these years. All the 32 samples with familial prostate cancer and nine out of 32 samples from patients with unselected prostate cancer used in the SSCP screening were also included in the sample set (n=1891) used to determine the genotype frequencies of the variants found in the SSCP analysis.

1.5 Ethical considerations

Permission for the collection of the families throughout Finland as well as the use of the Finnish Cancer Registry data was granted 20.7.1995 by the Ministry of Social Affairs and Health (licence 859/08/95). Permission to collect and use blood samples, tissue samples as well as clinical data from the prostate cancer patients in the Pirkanmaa Hospital District was granted 8.5.2001 (latest extension 30.12.2003) by the Institutional Review Board of Tampere University Hospital (assurance numbers 95062 and 99228, valid to 31.12.2010). The use blood samples, tissue samples as well as clinical data from the prostate cancer patients treated in Hatanpää Hospital was granted 1.7.1996 (latest extension 30.1.2001) by the Institutional Review Board of the City of Tampere. Written informed consent for the use of their samples and medical records was obtained from all living individuals participating in the study.

2. Methods

2.1 DNA extraction (studies I-IV)

Genomic DNA was extracted from blood lymphocytes using Puregene kit (Gentra Systems, Inc., USA) according to the manufacturer's instructions.

2.2 Allele-Specific Oligonucleotide (ASO) Hybridization (Studies I and II)

In Study I the genotyping of the *AR* R726L alteration was done by ASO hybridization. First, genomic DNA was amplified using primers 5'-CCCAACAGGGAGTCAGACTTA-3' and 5'-CCTGGAGTTGACATTGGTGA-3'. Amplification reactions and conditions were as follows: 100 ng of DNA, 200 nM of both primers, 200 μM of each deoxy-NTP, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.75 mM $MgCl_2$, 0.001% (w/v) gelatin and 2.5 U AmpliTaqGold™DNA Polymerase (Applied Biosystems, Foster City, CA, USA) in a final volume of 50 μl; at 95°C for 10 min; at 95°C for 10 min, followed by 35 cycles of 95°C 30 sec, 57°C for 1 min, and 72°C for 1 min, with a 5 min extension at 72°C after the last cycle.

In Study II for genotyping the *SRD5A2* A49T, genomic DNA was amplified using primers 5'-ACTGGCCTTGTACGTCGC-3' and 5'-AGGGCAGTGCGCTGCACT-3'. Amplification reactions and conditions were as follows: 100 ng of DNA, 200 nM of both primers, 200 μM of each deoxy-NTP, 1.75 mM $MgCl_2$, and 1.5 U AmpliTaqGold™DNA Polymerase (Applied Biosystems, Foster City, CA, USA) in a final volume of 50 μl; at 95°C for 10

min, followed by 35 cycles of 95°C 1 min, 58°C for 1 min, and 72°C for 1 min, with a 5 min extension at 72°C after the last cycle.

Mutation detection was done using ASO hybridization as described by Friedman et al. (1995) with these exceptions: filters were prewetted and wells washed with 0.4 M Tris-HCl pH 7.5, probes were end-labelled with ^{32}P at 37°C for 3 h by Terminal Deoxynucleotidyl Transferase (Amersham Life Science Inc., Cleveland, OH, USA), and hybridizations were performed at 54°C. A mutation positive control as well as a negative control of the PCR reaction was included in each ASO hybridization.

In Study I ASOs used in hybridizations were: 5'-AGGCTTCCGCAACTTACA-3' (wild type) and 5'-AGGCTTCCTCAACTTACA-3' (mutation). All samples with R726L mutation as well as 42 randomly chosen samples negative for mutation were sequenced using the same primers as in PCR amplification. In Study II the ASOs used were: 5'-GCCTGCCAGCCCGCGCCG-3' (wild type) and 5'-GCCTGCCAACCCGCGCCG-3' (mutation). Randomly chosen samples were sequenced to verify the genotype result.

2.3 Fragment analysis (Studies I and III)

To determine the CAG repeat area length, the fragment containing the repeat sequence was amplified by PCR using previously published primers (Irvine et al. 1995), with the exception that one of the inner primers was labelled with 5'-6-FAM. Electrophoresis was performed with ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) according to manufacturer's instructions. During each run, the same two samples of previously determined sizes were included as controls to exclude the possibility of variation in different runs. Run results were analyzed with Genescan 2.1 and Genotyper 2.0 (Applied Biosystems, Foster City, CA, USA) computer programs.

2.4 Minisequencing (Studies II and IV)

The *SRD5A2* A49T genotypes in Study II and the genotypes for the *CYP19A1* T201M alteration in Study IV were determined by minisequencing. First the DNA amplification was performed as follows: 100 ng of DNA, 200 nM of both primers, 200 µM of each deoxy-NTP, 2.5 mM $MgCl_2$ (1.5 mM in Study IV), and 2.5 U AmpliTaqGold™DNA Polymerase (Applied Biosystems, Foster City, CA, USA) in a final volume of 75µl (Study II) or 50µl (Study IV). In the Study II the amplification cycles were: 95°C for 10 min, followed by 35 cycles of 95°C 1 min, 71°C for 1 min, and 72°C for 1 min, with a 5 min extension at 72°C after the last cycle, and in the Study IV: at 95°C for 10 min, followed by 35 cycles of 95°C 30s, 62°C for 30s, and 72°C for 45s, with a 5 min extension at 72°C after the last cycle.

PCR primers for A49T were: 5'biotin-GCGAAGCCCTCCGGCTACGGGA-3' and 5'- CGCGGGCACCCGCGAAGGAAGGC-3', and for the *CYP19A1* T201M 5'- AATCGGGCTATGTGGACGTG -3' and 5'biotin-GATGGTCAAGATGTGAGAGTG -3'. In minisequencing the detection oligo for the A49T alteration was 5'-CAGGAACCAGGCGGCGCGGG-3' and for the T201M was 5'- ATGCTGGACACCTCTAACA -3'. Minisequencing was performed as described by Syvänen (1998). The results were confirmed by sequencing with ABI PRISM 310 Genetic Analyser (Applied Biosystems, Foster City, CA, USA). The primers used in sequencing where the same as those used in PCR.

2.5 Single stranded conformational polymorphism (Study IV)

SSCP analysis of the entire coding sequence of the genes *SRD5A2, HSD17B2, HSD17B3, HSD3B1, HSD3B2, CYP11A, CYP17A1, CYP19A1, KLK3* and *AKR1C3* was performed using primer sequences that were designed to include all intron-exon boundaries. All primers are available at http://www.uta.fi/imt/sgy/schleutker/indexb.html. The 15 µl reaction mixture contained 1.5 mM $MgCl_2$; 20 µM each of dATP, dCTP, dGTP, and dTTP; 0.5 µCi of α(^{33}P)-dCTP (Amersham Pharmacia, Uppsala, Sweden); 0.6 µM of each primer; 1.0 U Ampli*Taq*Gold; the reaction buffer provided by the supplier (Applied Biosystems, Foster City, CA); and 25 ng of the genomic DNA. Radiolabelled PCR products were mixed with 95% formamide dye were denatured at 95°C for 5 min, and chilled on ice. The (^{33}P)-labelled PCR products were electrophoresed at 800 V for 12 h at room temperature, in 0.5×MDE (mutation-detection–enhancement) gel (FMC BioProducts, Rockland, ME) with 1% glycerol in 0.5× Tris-borate EDTA. After electrophoresis, ^{the} gels were dried and exposed to Kodak BioMax MR (maximum-resolution) films for 6-18 h. All samples in which variant bands were detected as well as two to three normal bands per exon were analyzed by sequencing using an automated ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

2.6 Allele-specific primer extension on microarrays (Study IV)

The following variants were genotyped using allele-specific primer extension assay: *LHB* I15T, *SRD5A2* A49T, *SRD5A2* V89L, *HSD3B1* N367T, *HSD3B1* R71I, *HSD17B2* A111T, *HSD17B3* G289S, *HSD17B3* 729_735 delGATAACC, *AKR1C3* Q5H, *AKR1C3* P180S, *CYP19A1* R264C, *CYP17A1* -34 T>C, *AR* R726L, *KLK3* D102N, *KLK3* L132I. Some modifications were made to the method described earlier by Pastinen et al. (2000). The ASOs contained a 5' NH_2 group, a spacer sequence of 9 T residues 5' of the actual gene specific sequence of 18-21 nucleotides in length and a 3' nucleotide complementary either to the normal or mutant nucleotide. One PCR primer of each pair contained a 5' RNA

polymerase promoter sequence (TAATACGACTCACTATAGGGAGA) and the other primer for each pair had a 5' tail of random sequence (GCG GTC CCA AAA GGG TCA GT). The polarity of the latter primer was the same as that for corresponding detection primer sequence. All primer pairs are available at http://www.uta.fi/imt/sgy/schleutker/indexb.html.

Aminosilane microarray slides were manufactured as described by Guo et al. (1994). The ASOs were printed onto aminosilane coated microarray slides from 20μM solution containing 0.4 M Na-carbonate (pH 9.0) forming a covalent bonding with the coated slide surface. Printing was carried out with Telechem SMP5 contact printing pins (ArrayIt) using OmniGrid arraying instrument (GeneMachines/A1 Biotech). Each microarray slide consisted of 80 identical subarrays containing ASOs for all multiplexed SNPs. Each subarray consisted of two copies of each ASO spot to ensure quality of printing.

The PCR primer pairs were grouped into multiplex PCR reactions with 4, 4, 4 and 3 primer pairs per reaction for 15 variants. The amplifications were carried out using 50 ng of DNA, 200 μM dNTPs, and 1.0 U of Ampli*Taq*Gold DNA polymerase (Applied Biosystems, Foster City, CA) in 25 μl of DNA polymerase buffer supplied with the enzyme. The primer concentration varied from 0.12 μM to 0.68 μM and had been adjusted to give similar signal intensities in the reactions on the arrays. After initial activation of the polymerase at 95°C for 10 minutes, the thermocycling parameters were as follows: 95°C for 30 seconds, 59°C for 30 seconds, and 72°C for 30 seconds for 30 cycles; and finally 72°C for 5 min. Two of the PCR products were then pooled so that in the next step there were 4, 4 and 7 variants in the same reaction. The PCR products carrying a 5' T7-RNA polymerase promoter sequence were transcribed to RNA using the T7 AmpliScribe Kit (Epicentre Technologies, Madison, WI). The 4 μl reaction contained 0.86 × T7 reaction buffer, 6.17 mM NTPs, 8.64 mM dithiothreitol, 0.35 μl AmpliScribe T7 enzyme solution and 2.0 μl of the PCR product. The reaction was carried out in 37°C for two hours. After transcription, the DNA templates were degraded using 0.1 MBU of DNase I enzyme (provided in the T7 AmpliScribe Kit) in 37°C for 15 min. Finally, the DNase I was denaturated in 65°C for 5 min.

Before hybridization, 2 μl of 5 M NaCl was added to 5 μl of DNase I treated RNA. Then RNA was allowed to anneal to ASOs on the arrays at 42°C for 20 minutes, followed by a brief rinse in washing buffer containing 5 mM Tris-HCl (pH 8.0), 0.5 mM EDTA, 0.3 M NaCl and 0.1% Triton X-100 at RT. Immediately after hybridization the allele-specific extension reaction was carried out using 0.04 U MMLV RT enzyme (Epicentre Technologies, Madison, WI), 10 mM dithiothreitol, 0.5 μM dATP, dGTP, ddATP and ddGTP, 1.0 μM Cy5-dCTP and Cy5-dUTP, 0.5 M trehalose and 8% glycerol. The reaction temperature was 52°C for 20 min. The slides were washed with washing buffer, briefly dipped in 50 mM NaOH and once again rinsed with washing buffer. Finally, the slides were rinsed with water and dried under compressed air.

The microscope glass slides were scanned using the confocal ScanArray 4000 (GSI Lumonics, Watertown, MA, USA), with excitation at 630 nm and

emission at 670 nm. Ten µm resolution 16-bit TIFF images were analysed using the QuantArray software (GSI Lumonics, Watertown, MA, USA). Accurate allele calling and genotyping were produced by SNPSnapper 3.88b software developed by Juha Saharinen, National Public Health Institute, Helsinki, Finland (http://www.bioinfo.helsinki.fi/SNPSnapper/).

2.7 5'nuclease assay (Study IV)

Two *KLK3* alterations, -252A>G and I179T, were genotyped using the TaqMan SNP Genotyping Assays (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions in 96-well format. The *KLK3* -252A>G genotypes were determined using the TaqMan®Pre-Designed Assay, product number C_1531052_10. For *KLK3* I179T genotyping a Custom TaqMan®SNP Genotyping assay was ordered. Briefly, the DNA was amplified for I179T analysis using the following *KLK3* specific primers: Forward 5'-CCCGTAGTCTTGACCCCAAAG -3' and Reverse 5'-CTTGCGCACACACGTCAT -3'. The *KLK3* I179T genotypes were determined using the following fluorogenic allele-specific probes with a conjugated minor groove binder (MGB) group: VIC-labelled 5'- CCTCCATGTTATTTCC - 3' for T-allele and FAM-labelled 5'- CCTCCATGTTACTTCC -3' for C-allele. The nucleotide sequences of the primers and probes used in the PCR were deduced from publicly available sequences deposited in the GeneBank database and were chosen and synthesized by Applied Biosystems (Foster City, CA, USA) using the Assay-by-Design service. DNA samples were genotyped by means of 5' nuclease assay for allelic discrimination using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Known control samples previously genotyped by sequencing were run in parallel with unknown samples. After PCR, end-point fluorescence was measured and genotype calling was carried out using the allelic discrimination analysis module.

2.8 Sequencing (Studies I-IV)

Sequencing was performed using the ABI PRISM ®BigDye™ Terminator Cycle Sequencing Ready Reaction Kit with the ABI 310 and ABI3100 sequencers (Applied Biosystems, Foster City, CA, USA). Variants were identified using Sequencher software version 1.0-3.0 (Gene Codes Corporation, Ann Arbor, MI).

2.9 Statistics (Studies I-IV)

Studies I and II

Statistical analyses were performed using GraphPad InStat version 2.04a (GraphPad Software, CA, USA). Categorical variables were compared with the Fisher's exact test and t^2 (chi square) test for independence, t^2 test and t^2 test for linear trend and continuous variables were analysed with Student's t test. In addition, odds ratio (OR) and its 95% confidence intervals (95% CI) were calculated. All the tests were two-tailed.

Study III

We used ≤18 as a cut-off point as it has been commonly applied in the earlier literature. To compare extreme ends of the CAG repeat distributions, we chose another cut-off of ≥25 CAGs so that the percentage of samples in the two extreme groups (≤18 and ≥25) was approximately the same. Logistic regression was used to estimate Odds Ratios (ORs) and corresponding 95% confidence intervals (95% CIs). The relation between the length of the CAG repeat area and tumor stage and grade was assessed with chi square test. Association with PSA value at diagnosis was assessed with regression analysis. The effect of the CAG repeat length on age at diagnosis was analysed with regression analysis and Kruskal-Wallis test. The possible influence of CAG repeat length on disease recurrence was examined by using Cox proportional hazard modelling. Potential confounding variables in the multivariate analysis included T stage, M stage, WHO grade, age at diagnosis and treatment. For this study, only patients with no evidence of metastatic cancer at diagnosis (stage M0) were selected. These 301 patients had a mean follow-up time of 22.6 months, median 20, range 4-98. The primary endpoint was biochemical disease recurrence defined as two consecutive serum PSA measurements greater than 0.5 ng/ml for patients who had undergone prostatectomy, 1.0 ng/ml for those who had been treated by hormonal therapy, and 4.0 ng/ml for those treated by radiation. During follow-up, 84 cases out of 301 prostate cancer cases experienced relapse. Mean relapse time was 23.2 months (median 17, range 4-91). All calculations were done by SPSS version 10.0.

Study IV

Odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs) were calculated using logistic regression to estimate prostate cancer risk. Categorical variables were compared with the Fisher's exact test and Pearson χ^2 test for independence. These analyses were performed with SPSS 11.0 statistical software package. The magnitude of the association between the *CYP19A1* and *CYP17A1* SNPs and the occurrence of prostate cancer and other related outcomes was measured with the OR using polytomous logistic regression. Outcome definitions included WHO grade (I-III), Gleason score (2-6, 7-10), PSA at diagnosis (<20ng/ml, >20ng/ml) and T stage (T1-T2, T3-T4). Polytomous regression analyses were performed using STATA v8.0.

2.10 Bioinformatics (Study IV)

The effect of the T201M mutation on the structure and function of aromatase was investigated with several bioinformatics methods and tools including PHD and PROF (Rost and Sander, 1993; 1994), Jpred (Cuff et al. 1998), SADM (Chen and Zhou 2005) and SIFT (Ng and Henikoff 2001; Saunders and Baker 2002). The effect of point mutation had to be evaluated sequence-based, because the known structures are for the core domain of P450.

RESULTS

1. Variation along the androgen pathway (study IV)

We identified 50 different variants by SSCP among the ten genes studied *SRD5A2, HSD3B1, HSD17B2, HSD17B3, AKR1C3, CYP19A1, CYP17A, KLK3, HSD3B2* and *CYP11A1*. Of the variants found 15 were located in the introns, 3 in the 3'UTR area, 5 in the 5'UTR area and 27 in the coding exons. Of the coding area variants, 14 affected the amino acid composition of the protein sequence. Almost all variation was due to a substitution of a single nucleotide. In addition, one 7 pb long deletion and three insertion mutations (1-3 bp) were detected.

2. R726L alteration in the androgen receptor gene *AR* (Studies I and IV)

Study I

Germline R726L *AR* mutation was found in eight out of 418 (1.91%) sporadic prostate cancer patients. Among the controls, the carrier frequency of the R726L was established as 3/900 (0.33%), which was significantly less than the frequency of the R726L mutation among the sporadic prostate cancer patients (OR=5.8, p=0.006). The R726L mutation was also found in two out of the 106 patients (1.89%) with a positive family history for prostate cancer (OR=5.8, p=0.09). Analysis of additional affected and unaffected cases from these two families indicated that the mutation was systematically present in all affected cases, whereas unaffected male individuals were not carriers. Since the R726L mutation was also found in eight prostate cancer patients who reported no family history of prostate cancer, we constructed extended pedigrees from their families based on family questionnaires sent to the patients. Two of these cases turned out to have a maternal relative with prostate cancer.

The clinical features of the R726L mutation-positive prostate cancers were compared with those in non-carriers. There were no significant differences in the T-stage, M-stage or the tumor grade between these groups. The average age at prostate cancer diagnosis was slightly lower in patients harbouring the R726L mutation (65.5 ± 7.0, range 59-79 years) as compared with the rest of the prostate cancer patients (68.4 ± 8.3, range 48-92 years), but this difference was not statistically significant (p=0.25).

In order to explore whether the R726L mutations shared the same origin we studied the distribution of the adjacent *AR* CAG repeat in the 13 mutation carriers. Strong evidence of linkage disequilibrium was observed between the two different loci in the same gene. Eleven cases (85%) had 26 CAG repeats, with the remaining two having 25 and 27 repeats. Compared to the distribution of the CAG repeat lengths in the general Finnish population, the CAG repeat length of 26 is rather long. In unselected Finnish population, the average CAG repeat length is 21.6 ± 2.6 (unpublished data). Only 2.7 % of men have 26 CAG repeats, a significant difference compared to R726L mutation carriers (OR=197.3, 95%CI 41.2-943.8, p<0.0001). The two mutation-positive cases with 25 and 27 CAG repeats could represent the inherent instability of the tri-nucleotide repeats, especially over many, perhaps dozens, of generations. This strong linkage disequilibrium between the two markers, as well as the homogeneous distribution of CAG repeat lengths in the cases suggests that the R726L mutation originates from a single ancestral event.

Study IV

The frequency of the R726L alteration was 1.3% (11/847) among the unselected prostate cancer cases, 2.5% (3/121) among familial prostate cancer patients and 0.9% (8/923) among controls. No association was seen between this variant and prostate cancer risk (unselected prostate cancer, OR=1.161, 95%CI 0.876-1.538; familial cases, OR=0.867, 95%CI 0.487-1.545) nor did carrier status affect the clinical characteristics of the cancer.

3. CAG repeat in the androgen receptor gene *AR* (Study III)

The mean and median CAG repeat lengths did not differ significantly between the unselected PC cases, familial PC cases, cases of BPH and controls. When we used 18 CAGs as a cut-off (comparing individuals with ≤18 repeats with those having >18 repeats), a tendency for an association with prostate cancer was found (OR=1.47 as compared to controls, p=0.05). Also, a similar result was obtained when extreme ends of the CAG allele distributions (≤18 versus ≥25) were compared (OR=1.62 as compared to controls, p=0.06). There was no significant difference between the number of CAG repeats between cases and controls when the analysis was restricted to patients with advanced disease only (data not shown). Interestingly, we found that a very short CAG repeat length was significantly less common among patients with benign prostate hyperplasia than in controls (OR=0.47, p=0.030).

CAG repeat length was not associated with disease stage, grade, age at diagnosis or serum PSA value at diagnosis among the unselected PC cases. Neither was the length of the CAG repear region a significant factor in predicting

the recurrence of the disease (patients devided according to CAG genotype in three groups (≤18 CAGs, 19-24 CAGs, and ≥25 CAGs, p=0.458,

4. *SRD5A2* alteration A49T (Studies II and IV)

Study II

The prevalence of A49T among the autopsy samples was 4.2% (3/72) and 6.0% (31/516) among the 516 blood donors. Because of the small number of autopsy samples, we combined data for all donors as controls in the statistical analysis.

The frequency of the A49T substitution was 6.0% in among prostate cancer patients and 5.8% among the controls (samples from blood donors and autopsies). Compared to sporadic prostate cancer, the frequency of the A49T was slightly lower among the 94 prostate cancer families (5.3%), whereas patients with benign prostatic hyperplasia had slightly higher frequency (6.3%). However, none of these differences was statistically significant and no risk for prostate cancer among the carriers was observed (unselected prostate cancer cases *versus* controls, OR=1.04, 95%CI=0.62-1.76, p=0.89).

There was a tendency for A49T substitution to be more common among the localised or lower grade cancers defined either by T1-T2 status, M0 status, Gleason score 2-6 or WHO grade I. However, none of these trends was statistically significant. The mean age at diagnosis did not differ between patients with A49T mutation (67.8 years, 27/449) compared to non-carrier patients (68.3 years, 422/449). The distribution for A49T genotype among population controls was in the Hardy-Weinberg equilibrium.

Study IV

The frequency of the A49T alteration was 5.3% (44/845) among the unselected prostate cancer cases, 8.3% (10/121) among familial prostate cancer patients and 4.9% (46/923) among controls. No association was observed between this variant and prostate cancer risk (unselected prostate cancer, OR=1.047, 95%CI=0.685-1.601; familial cases, OR=1.718, 95%CI=0.843-3.500). Variant did not affect the clinical characteristics of the prostate cancer.

5. Characterization of prostate cancer susceptibility gene *CYP19A1* (Study IV)

We identified a novel C to T polymorphism in exon 4 of the *CYP19A1* gene, which results in a non-conservative replacement of threonine for methionine at codon 201. The T allele of the variant T201M showed an association with

prostate cancer (unselected prostate cancer cases, OR =2.04, p=0.040, 95% CI 1.03-4.03). Unlike T201M, the other studies *CYP19A1* variant R264C was not associated with prostate cancer. We also wanted to study whether the carrier status of the T201M genotype affected the clinicopathological features (T stage, M stage, WHO grade, Gleason score) of the unselected prostate cancer cases. Interestingly, the frequency of *CYP19A1* T201M carriers was higher among patients with organ confined tumors (T stage T1-T2) compared to patients with extracapsular tumor (p=0.045). In addition, *CYP19A1* T201M carriers had well-differentiated tumors (WHO grade I) more often compared to non-carriers (p=0.03).

To determine the nature of disease association with *CYP19A1* T201M, we performed a polytomous logistic regression analysis. The T201M association was only seen in patients with organ-confined disease as well as in those with a low PSA value (<20ng/ml) at diagnosis (p=0.006 and p=0.031 respectively). In contrast, individuals with severe stage classification showed no association with the *CYP19A1* T allele. We obtained similar results for the histological classifications, WHO and Gleason, in which individuals with less aggressive prostate cancer defined by a low grade tumor (WHO grade I) were 4.5 times more likely to carry the *CYP19A1* T allele than population controls (OR= 4.5, 95% CI 1.94-10.5, p < 0.0001). More severe cases (WHO II and III or Gleason >7) did not show an over-representation of the *CYP19A1* T allele.

To further refine our risk categories, we created a risk score in which an individual had severe or aggressive prostate cancer (T3-T4 and WHO grade II-III), moderate cancer (T1-T2 and WHO grade II-III) or clinically less significant cancer (T1-T2 and WHO grade I). Individuals with clinically less significant prostate cancer were more than 5 times more likely to carry the *CYP19A1* T201M T allele (OR= 5.42, 95% CI 2.33-12.6, p <0.0001) than population based controls. This association was still significant after the conservative Bonferroni correction (8 independent genes overall, n=8).

We tested also a hypothesis of a joint impact of *CYP19*A1 T201M and five common polymorphisms (*LHB* I15T, *CYP17A1* -34 T>C, *KLK3* -252A>G, *AKR1C3* Q5H and *SRD5A2* V89L) on prostate cancer risk. Increased risk was noted in individuals carrying both *CYP19*A1 T201M and *KLK3* -252A>G (OR=2.87, 95% CI=1.10-7.49, p=0.03).

The effect of the T201M mutation on the structure and function of aromatase was investigated with numerous bioinformatics methods and tools. Position 201 is not highly conserved in aromatases and in P450 family. The residue most common in this position in the aromatase family is arginine. T201 is predicted to be close to the C-terminal end of a long α-helix by several methods including PHD, PROF and Jpred. The mutation was predicted not to increase disorder or aggregation tendency of the protein. Predictions of surface accessibility of T201 with programs PHD, PROF and SADM are somewhat contradictory. It is possible that the residue is on the buried surface of the helix. The SIFT program (sorting intolerant from tolerant) uses multiple sequence information to predict whether an amino acid substitution affects protein function. According to SIFT

prediction T201M mutation is not tolerated. SIFT predictions have been shown to be very accurate in detecting deleterious mutations.

6. Other risk gene candidates along the androgen pathway (studies IV)

Association studies showed no increased risk among the carriers of *SRD5A2* V89L, *HSD3B1* N367T, *HSD17B3* G289S, *AKR1C3* Q5H, *AKR1C3* P180S, *CYP17A1* -34 T>C, *KLK3* D102N, *KLK3* -252A>G, *KLK3* I179T and *LHB* I15T for prostate cancer. For the very rare mutations (*HSD3B1* R71I, *HSD17B2* A111T, *HSD17B3* 729_735 delGATAACC, *KLK3* L132I) no ORs were calculated because of a small number of carriers.

However, some variants affect the clinicopathological features (T stage, M stage, WHO grade, Gleason score) of the unselected prostate cancer cases. The frequency of *CYP17A1* –34T>C carriers was higher among patients with organ confined tumors (T stage T1-T2) compared to patients with extracapsular tumor (Pearson $\chi 2$ test; p=0.003). We used refined cancer categories in the further analysis of *CYP17A1* –34T>C. No association was seen with clinically less significant prostate cancer (OR=1.09, 95% CI = 0.75-1.59, p=0.65). This alteration increased the risk for moderate cancer (OR=1.42, 95% CI = 1.09-1.83, p=0.007) but for aggressive cancer the risk was decreased (OR=0.76, 95% CI = 0.58-1.00, p=0.05). This association of *CYP17A1* –34T>C with moderate cancer was marginally significant after conservative Bonferroni correction (n=8).

Likewise, *KLK3* -252 A>G carriers had a low Gleason score (2-6) more often than non-carriers (Pearson $\chi 2$ test p=0.045). *LHB* I15T showed a borderline association with organ confined tumor (Pearson $\chi 2$ test, p=0.074). In contrast, carriers of the *KLK3* I179T alteration were more likely to have metastases than non-carriers (Pearson $\chi 2$ test; p=0.009).

DISCUSSION

1. SSCP – methodological considerations

SSCP (single-strand conformation polymorphism) is widely used for mutation detection because of its simplicity and versatility. The pitfalls of SSCP analysis are well known. First, this method has been considered to have low sensitivity for the investigation of longer DNA fragments (> 250 bp). However, in a blind sensitivity trial Jordanova et al. (1997) reported an overall mutation detection rate of 85% running fragments up to 500 bp in length. The sensitivity was 90% for insertions/deletions and 82% for base substitutions. Second, the electrophoretic behaviour of the mutant strand is unpredictable. This can be dealt with by running the analyses under several different sets of conditions (Hayashi and Yandell 1993). In our study, we used 32 samples from familial prostate cancer patients and 32 samples from unselected prostate cancer patients with early age at onset (range 49-58). We were able to identify 50 variants in 10 genes acting in androgen biosynthesis and metabolism pathway by SSCP analysis under one set of running conditions. Using a diverse population in discovery efforts might lead to the detection of a large number of variants. On the other hand, using disease specific populations may reveal mutations not seen in a diversity set.

Most of the mutations we detected were either in the non-coding areas or in the areas where the alteration did not affect the amino acid composition of the protein. Therefore it was difficult to predict the possible significance of such variants and most of these alterations were excluded from further analysis. However, there is evidence that such variation may have functional effects (Steinberger et al. 1996; Enattah et al. 2002).

2. Contribution of two androgen receptor alterations

R726L mutation in the AR ligand binding domain LBD

The ligand binding pocket of androgen receptor LBD comprises helices 3,4,5,7,11 and 12 together with the β-sheet preceding helix (Matias et al. 2000). Amino acid R726 is located between helices 3 and 4 in the AR LBD and could thus play critical role in mediating the amino/carboxyl interactions which

coordinates the molecular events of a functioning AR (Thompson et al. 2001). An AR carrying a R726L mutation has been reported to have altered ligand binding and transactivation properties (Elo et al. 1995; Shi et al. 2002).

We originally reported an increased risk of almost 6-fold among the carriers of AR R726L mutation (OR=5.8, p=0.006). Since then, three other studies have screened for this variant in African-American, white, Asian and Hispanic prostate cancer patients from the United States, in Finnish male breast cancer patients and in patients with psychiatric disorders (Gruber et al. 2003; Syrjäkoski et al. 2003; Yan et al. 2004). Only one carrier was found – a patient suffering from alcoholism (Yan et al. 2004). In our recent study we found new carriers of this mutation but the statistically significant association between the alteration and the prostate cancer risk did not persist. In the previous study the amount of samples from patients whose cancer was detected by PSA screening alone was smaller, and thus the discrepancy in the result may reflect the different disease spectrum before and after the increased use of PSA testing in Pirkanmaa.

Also, in the first study this mutation was found in two families where it segregated with the disease. In Study IV we identified a new carrier family with two affected prostate cancer cases. Unfortunately we were unable to determine possible segregation in this family because no informative samples were available.

CAG length polymorphism of the AR

Several studies have tried to establish the role of the variation in CAG repeat length in prostate cancer risk, yet there has not been a clear and consistent picture of the possible affect of this polymorphism (Chen et al. 2002). In our study we observed a tendency for an association with prostate cancer (cut-off ≤18, OR=1.47, p=0.05). Also, a similar result was obtained when extreme ends of the CAG allele distributions (≤18 versus ≥25) were compared (OR=1.62, p=0.06). Similar results have been reported in some studies (Giovannucci et al. 1997; Hakimi et al. 1997; Ingles et al. 1997; Hsing et al. 2000a; Modugno et al. 2001). Balic et al. (2002) reported an approximately 3-fold increased risk of prostate cancer (OR=2.7, 95%CI 1.21 to 6.01, t test p=0.013, age adjusted OR=3.03, 95%CI 1.27 to 7.26) among the subjects of Hispanic origin using the ≤18 as a cut-off repeat length. However, there are also several studies where no association among populations of different origin/ethnicity has been observed (Chen et al. 2002). In one such study in Sweden, Bratt et al. (1999) reported that among the Swedish study population the frequency distribution of CAG repeat length was strikingly similar for cases and controls, and no significant correlation between CAG repeat length and prostate cancer risk was detected. However, for men with non-hereditary prostate cancer shorter CAG repeats correlated with younger age at diagnosis (p=0.03). We were unable to confirm this result although such an affect has also been reported by Hardy et al. (1996), Beilin et al. (2001) and Santos et al. (2003). Giovannucci et al. (1997) and Bratt et al. (1999) reported an association between fewer CAG repeats and a more

aggressive disease. Since then, most studies have failed to confirm these results (Study III, Stanford et al. 1997; Correa-Cerro et al. 1999; Mir et al. 2002; Huang et al. 2003). The reported risk per of one CAG repeat has usually been small (3% to 7%) (Stanford et al. 1997; Hsing et al. 2000a; Freedman et al. 2005). Such a slight effect may be difficult to detect and would require even larger sample sets, especially if the effect is seen only in a small subgroup of patients. In a meta-analysis of 19 studies including Caucasian, African-American and Asian subjects a 1.19 fold risk was observed in men with shorter (≤21) repeats (Zeegers et al. 2004b). In another large study with 2160 controls and 2036 cases of a mixed population no significant association was observed (Freedman 2005).

3. A novel alteration T201M in the aromatase gene *CYP19A1*

We identified a novel T201M alteration in the CYP19A1 gene, which showed a mild, statistically significant increase for prostate cancer among the unselected prostate cancer cases (OR=2.04, p=0.040). The observation that *CYP19A1* T201M variation was not associated with familial prostate cancer may be due to the fact that in prostate cancer families the possible effect may be masked by some other stronger genetic component. Interestingly, stratified analysis revealed a strong association with clinically insignificant cancer only. Siegal et al. (1995) estimated that although 42% of males have prostate carcinoma identified by postmortem examination, only 9.5% will have a clinical diagnosis in their lifetime, and only 2.9% actually will die of the disease. Due to the extensive use of PSA screening the clinical cancers detected are more often small organ-confined cancers. So far no diagnostic biomarkers indicating the disease aggressiveness and progression have been discovered. Hence, the development of prognostic tests is essential to identify those patients who would benefit from more vigilant surveillance and to avoid unnecessary treatments.

The *CYP19A1* gene codes for aromatase, which belongs to the large cytochrome P450 family. This enzyme is unusual in that the same cytochrome P450 enzyme catalyzes three consecutive oxygen activation steps, converting C19 androgens into aromatic C18 estrogenic steroids. The T201M mutation of this protein could, through altered enzyme function, result in lower levels of testosterone and have an effect on the progression of the disease. There are at least two possible mechanisms: The higher activity of the variant enzyme could result in lower levels of androgens in carriers as androgens are more efficiently converted into estrogens. Alternatively, the alteration could result in less active enzyme and thus the increased level of testosterone in blood could result in stronger negative feedback effect through the hypothalamus and thus in a lower level of androgens in carriers. Based on the bioinformatics analyses we can assume that T201 is in structurally important α-helix, most likely at least partly buried in the core of the protein. Substitution by methionine affects the packing

of the helix and consequently the local and global fold of the enzyme. The effect may also arise from loss of stabilizing polar interaction(s) formed by the hydroxyl group of threonine. Apparently, the change does not completely alter the function of the protein, which could lead to modified activity and phenotype.

Because prostate cancer is very likely a polygenic disease, we wanted to test the hypothesis of joint/modifier effects of T201M variant together with five common polymorphisms (*LHB* I15T, *CYP17A1* -34 T>C, *KLK3* -252A>G, *AKR1C3* Q5H and *SRD5A2* V89L). A significant association with prostate cancer was seen when the patient carried both *CYP19A1* T201M mutation and KLK3 -252 A>G variant. However, for gene-gene interaction analysis our cell numbers are getting too small, because *CYP19A1* T201M is relatively rare and there were only 41 individuals carrying at least one copy of the variant. Due to the small allele frequency we seem to have limited power for interaction analyses. Nonetheless, the results suggest a multigenic model of prostate cancer susceptibility.

4. Other low-penetrant susceptibility genes along the androgen pathway

CYP1A19 R264C

Another C to T alteration in the *CYP19A1* gene results in a non-conservative arginine to cysteine amino acid change at codon 264. In contrast to Modugno et al. (2001) and Suzuki et al. (2003a) we observed no association between the *CYP19A1* R264C alteration and prostate cancer risk. However, the T, allele which results in the nonconservative amino acid change, is relatively rare with an allele frequency of 4.4%.

Rare variants *HSD17B3* 729_735 delGATAACC, *HSD3B1* R71I, *HSD17B2* A111T and *KLK3* L132I

We identified another novel alteration, 729_735 delGATAACC, in the *HSD17B3* gene coding for the 17β-hydroxysteroid dehydrogenase type 2 protein. The deletion leads to a truncated protein with an altered carbosyl terminal compared to the wildtype protein, which consists of 310 amino acids. The first affected amino acid is at codon 201. This is followed by 10 novel amino acids and a stop codon. This mutation proved to be very rare, as only four heterozygous carriers were found among the 1,457 samples genotyped – two among the unselected and familial prostate cancer cases and two among the controls. Because of the small number of carriers no statistical evaluation was made. Accordingly, no association was calculated for the rare variants *HSD3B1* R71I, *HSD17B2* A111T and *KLK3* L132I. Even though these variants may have functional consequences and thus affect the disease risk in carriers it is very difficult to predict their

importance through association studies, because of the small frequencies of the alterations.

AKR1C3 Q5H, *AKR1C3* P180S, *KLK3* I179T, *KLK3* D102N, *LHB* I15T, *HSD3B1* N367T, *HSD17B3* G289S and *KLK3* -252A>G

No previous literature has been published on the variants *AKR1C3* Q5H, *AKR1C3* P180S, *KLK3* I179T and *KLK3* D102N, which in our study did not alter the prostate cancer risk. Single studies have reported a possible association between variants *LHB* I15T, *HSD3B1* N367T, *HSD17B3* G289S and *KLK3* -252A>G but we were unable to confirm these positive associations. However, we report that *KLK3* -252 A>G carriers had more often well-differentiated tumour compared to non-carriers. Likewise, *LHB* I15T showed a borderline association with organ confined tumor. In contrast, carriers of the *KLK3* I179T alteration were more likely to have metastases than non-carriers.

SRD5A2 A49T and V89L

In 1997, Makridakis et al. described an alteration A49T in the SRD5A2 gene coding for a central enzyme 5α-reductase in testosterone metabolism. It has been suggested that this mutation could increase the prostate cancer risk (Makridakis et al. 1999). In addition, variant T49 is completely absent in low-risk populations (Hsing et al. 2001; Yamada et al. 2001). Several studies have tried to establish the role of this variant in prostate cancer causation but with contradictory results (Hsing et al. 2001; Latil et al. 2001; Yamada et al. 2001; Söderström et al. 2002), Studies II and IV in this thesis). Ntais et al. (2003b) suggest, based on the results of a meta-analysis of seven studies, that A49T polymorphism may have a modest affect on prostate cancer susceptibility. Further evidence of a possible role comes from biochemical studies. However, Ntais et al. (2003b) estimate that even in subjects of European descent the attributable fraction of prostate cancer is probably less than 1% for A49T.

Another variant V89L in the SRD5A2 gene is associated with reduced 5α-reductase activity both *in vitro* and *in vivo* (Makridakis et al. 1997). While there have been some reports of a positive association between the V89L polymorphism and incidence of prostate cancer, most epidemiological studies do not support the hypothesis (Febbo et al. 1999; Lunn et al. 1999; Hsing et al. 2001; Nam et al. 2001; Yamada et al. 2001; Pearce et al. 2002; Ntais et al. 2003b). Accordingly, we observed no relationship between V89L polymorphism and prostate cancer risk.

A few studies have generated some data addressing whether these two alterations may have an affect on the clinical behaviour or other clinicopathological attribute of the cancer. Nam et al. (2001) claim a 3.3 fold increase risk in disease reoccurrence with the V/V or V/T genotypes at codon 89. Conversely, Söderström et al. (2002) reported a significant 5.7-fold increased risk of metastatic disease with the L/L genotype. In three other studies no

significant association between the clinicopathological and V89L variation was observed (Jaffe et al. 2000; Latil et al. 2001, and study IV). Jaffe et al. (2000) claim that T alteration in A49T polymorphism is associated with poor prognosis of the cancer and a younger age at diagnosis. A trend for more advanced stage was also reported by (Söderström et al. 2002). However, for A49T alteration no such associations were observed in Study IV or by Latil et al. (2001).

CYP17A1 –34T>C

Several studies have reported seemingly contradictory results concerning a potential role of this variation in *CYP17A1* in prostate cancer. Some studies have claimed that the C allele may be associated with an increased risk (Lunn et al. 1999; Gsur et al. 2000; Kittles et al. 2001; Yamada et al. 2001). However, other investigations have been inconclusive (Chang et al. 2001; Latil et al. 2001) or have even reported that the T allele increases the risk for prostate cancer (Wadelius et al. 1999; Habuchi et al. 2000). In a meta-analysis of subjects of European, Asian and African descent (including respectively 7, 2 and 3 comparisons) no overall effect of this variation was observed. Only in subjects of African descent did the C allele increase susceptibility to prostate caner (Ntais et al. 2003a). In our study, we found no association between –34T>C variation and prostate cancer risk. However, this alteration increased the risk for moderate cancer but for aggressive cancer the risk was decreased.

5. Genetic aspects

The cause of irreproducibility in any particular association of genotype and phenotype is difficult to identify and can be attributed to biological, statistical or technical reasons. Also, differences in study population characteristics may contribute to inconsistency in results between studies. In some studies cases are compared with BPH or other urology clinic patients or men being screened. In others comparison is made between cases and population controls or hospital controls without prostatic disease. Many human traits and their underlying polymorphic genes show independent patterns of racial/ethnic/geographical variation. For example, studies have shown that the CAG repeat length is shortest in African-Americans, intermediate in whites and longest in Asians, and that this may be related to higher, intermediate and lower prostate cancer risk (Irvine et al. 1995). Also, as the endogenous factor affecting the functional genome may be different, it is important to define the polymorphic spectrum of genes implicated in cancer causation in different racial/ethnic populations.

Morover, for several reported associations, it is still uncertain whether the variants studied play a causal role in prostate cancer or whether they are merely in linkage disequilibrium with other functional variants within or flanking the

genes under study. In addition, the definition and clinical characteristics of prostate cancer may have changed over time. The possible failure in replication could be due to a shift in the spectrum and characteristics of prostate cancer cases, as compared with prostate cancer cases detected prior to PSA testing. Nor can bias and chance findings cannot be excluded; even the genuine genetic effects of low-risk gene account for only a small proportion of prostate cancer risk in the population.

A complete understanding of the role of sex steroid hormones in prostate tumorigenesis requires evaluation of both the genes associated with these hormones as well as the extent to which hormone exposure modifies the associations of these genes with prostate cancer risk. This understanding can only emerge if research extends beyond single-gene studies to gene-gene and gene-environment interaction studies.

6. Future prospects

In this study, we selected candidate genes for study among those that act in androgen biosynthesis and metabolism and regulate the androgen signalling in the prostate tissue. However, many potential candidate genes were omitted from this study. For example, the *UGT2B* genes are involved in the irreversible inactivation of androgens in the prostate tissue. Another critical class of genes involves co-factors that regulate the activation of the androgen receptor and its down-stream consequences. The details of the androgen pathway are still unclear, and the role of this pathway in prostate cancer is under active investigation. Undoubtedly new factors operating in androgen biosynthesis, metabolism and inactivation will be identified and the upstream and down-stream signalling events will be better known at the tissue and cell level, in health and in disease. This will help to formulate a comprehensive picture of the role of androgen signalling in prostate carcinogenesis.

This study was carried out with low-throughput genetic methods, allowing one or a few SNPs to be analysed at a time from the large clinical materials available. The introduction of new genetic screening methods will significantly enhance these capabilities in the future. There are now important developments underway in this respect. Analysis of up to 100,000 SNPs at a time is emerging using microarray-based, bead-based and mass spectrometry-based methods. Furthermore, the linking of the SNPs to common haplotypes (human haplomap project) will enhance the capability to analyze their risk effects on disease. Finally, re-sequencing of the entire genome is already estimated to be possible within a decade or so. Obviously, the cost of these analyses will remain a significant issue for their implementation in large-scale research projects.

The enormous increase of screening power will then shift the emphasis from screening a few SNPs in candidate genes/pathway to carrying out genome-wide screening analyses in predisposed individuals as well as at the population level.

This will require significant efforts in developing matching informatics capabilities to analyse these effects at the genetic level and in concert with environmental effects. One of the key factors for success will remain the same: the research will in the future also be heavily dependent on the availability of large, high-quality sample sets, from epidemiologically and clinically meaningful, representative and informative patient and population cohorts. These capabilities in the Nordic countries are excellent, and the present study materials will gain in value in the future. The collection of samples and clinical data has continued and now a much larger sample set is available, making it possible to validate the positive results obtained in this study. Increased information on environmental risk profiles would be important, but its acquisition is still a major challenge.

Finally, introduction of genetic analyses as part of the clinical routine and public health policy requires further research evidence, which will hopefully emerge from the promising early results and their follow-up with next-generation research tools. In the next step, the clinical needs, cost-benefit analyses and ethical aspects of the introduction of genetic analyses into the prevention, risk assessment, genetic counselling, and management of prostate cancer patients and their families will need to be explored.

CONCLUSIONS

Low-penetrant susceptibility genes may have a major role in causing disease in a population if the risk alleles are common in that population. The aim of the study was to identify mutations and polymorphisms in genes along the androgen biosynthesis and metabolism pathway that could be responsible for the altered risk for prostate cancer and/or impact the clinical behaviour of the cancer.

Among the loci of genetic variation studied, two alterations were found to be associated with prostate cancer: the highly polymorphic CAG repeat area in the androgen receptor gene *AR* and the T201M alteration in the aromatase gene *CYP19A1*. Both these alterations produced only a small increase in the risk and thus represent low-penetrant variation. The T201M allele is quite rare in the population studied, suggesting that its impact on the disease is not very great.

Several other gene alterations, like the widely studied A49T polymorphisms on the 5α-reductase gene *SRD5A2* showed no association with prostate cancer in Finnish population. This may reflect differences in study populations.

Stratified analysis revealed that individuals with clinically less significant prostate cancer were more likely to carry the *CYP19A1* T201M T allele than patients with more aggressive cancer. Also, the frequency of the carriers of another candidate alteration, *CYP17A1* –34T>C, was higher among patients with organ confined tumors compared to patients with extracapsular tumors. Likewise, *LHB* I15T showed a borderline association with organ confined tumour. *KLK3* -252 A>G carriers had more often a histologically differentiated tumour than non-carriers. In contrast, carriers of the *KLK3* I179T alteration were more likely to have metastases.

In conclusion, our results indicate that genetic variants of the genes coding for proteins along the androgen pathway may have an impact on the risk of prostate cancer. These genes may have different contributions to distinct clinical subsets as well as combinatorial effects in others, illustrating that profiling and joint analysis of several genes along each pathway may be needed to understand genetic contributions to prostate cancer etiology.

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