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Prostate Cancer
Epidemiological studies of Risk Factors

By

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To my family

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Original publications

This thesis is based on the following studies:

I Thellenberg C, Malmer B, Tavelin B, Grönberg H
Second primary cancers in men with prostate cancer: an increased risk of male breast Cancer Journal of Urol 2003 Apr;169(4):1345-8

II Thellenberg-Karlsson C, Malmer B, Wiklund F, Grönberg H
Breast Cancer as a Second primary in Patients with Prostate Cancer- Estrogen Treatment or Family history of Cancer Journal of Urol 2006 Aug;176(2):538 543

III Thellenberg-Karlsson C, Lindstrom S, Malmer B, Wiklund F, Augustsson-Balter K, Adami HO, Stattin P, Nilsson M, Dahlman-Wright K, Gustafsson JA, Gronberg H.
Estrogen receptor beta polymorphism is associated with prostate cancer risk.
Clin Cancer Res. 2006 Mar 15;12(6):1936-41

IV Thellenberg-Karlsson C, Wiklund F, Grönberg H, Bergh A, Malmer B
A Cohort and nested Case-Control Study of Trans Urethral Resected BPH Patients and Risk of Prostate Cancer, Submitted manuscript

Abstract

In spite of the fact that prostate cancer is the most common male cancer in both Sweden and many other countries in the developed world, little is known of risk factors and predisposing conditions. The only well recognized risk factors are age, race and familial aggregation. More knowledge about risk factors could lead to better preventive measures together with better treatments.

One way to evaluate this is to study second primary cancers; the connection between two different cancers can give valuable insight in etiology or clues to shared risk factors. This thesis aims at evaluating risk factors for prostate cancer.

We constructed a cohort of 135,713 men diagnosed with prostate cancer and reported to the Swedish Cancer Registry 1958-1996. The cohort was followed for second primary cancers and a doubled risk of male breast cancer was found. We also noted increased risks for small intestine cancers and melanoma.

As a follow-up on the increased risk of male breast cancer, we performed a nested case – control study. Included cases were men with first prostate and then breast cancer (n = 41) matched to men with only prostate cancer (n =81). For these men, we collected medical records and extracted data regarding treatment. Furthermore, all men diagnosed with both prostate and breast cancer irrespective which came first (n = 83) were used as probands. To both these sets of cases with breast and prostate cancer, we identified first degree relatives and grandchildren from parish offices throughout Sweden. Linking to the Cancer Registry retrieved all cancer diagnoses amongst relatives. Results from this study show a relation between estrogen treatment of prostate cancer and the risk of developing breast cancer. We also found that a small part of the cases with both cancers appeared in families with inheritance patterns possibly attributed to BRCA2.

As estrogen treatment seemed involved in increased risk of breast cancer after prostate cancer, we wanted to investigate the newly discovered Estrogen receptor β and the relation to prostate cancer risk. Previous reports have shown that ERβ acts as a negative regulator of proliferation. ERβ expression occurs mainly in prostatic epithelial cells and the expression gradually diminishes when cancer develops and aggravates. We used a single nucleotide polymorphism (SNP) association study approach to evaluate genetic variation in ERβ as a risk factor for prostate cancer. One SNP, located in the promoter region associated with a small increased risk of prostate cancer whereas variation in the rest of the gene did not.

In the last paper, we investigated trans-urethral resection (TURP) of the prostate due to benign prostate hyperplasia (BPH) as a risk factor for later development of prostate cancer. Evidence has gathered that both BPH and prostate cancer are associated to inflammation. By comparing incidence and mortality in a cohort of 7,901 men with the general population there appeared to be an increased risk of prostate cancer but decreased mortality. Analyzing this increased risk further, we conducted a nested case - control study with men extracted from the cohort. Cases had a TURP and later developed prostate cancer and controls just had a TURP. We then evaluated the specimens from TURP regarding extent of inflammation, degree of androgen receptor down regulation and expression of p53, all factors previous associated with prostate cancer. None of these parameters differed between cases and controls and they can therefore not explain the increased risk. Decreased mortality but increased risk might be explained by surveillance bias, which means more medical attention to these patients, resulting in diagnosing clinically non-significant cancers.

In summary, our results show a doubled risk of male breast cancer following prostate cancer. A risk that can be attributed to the use of estrogen to treat prostate cancer or to some extent a possible mutation in BRCA2. We also propose that a SNP change in the ERβ promoter confer a small increased risk of prostate cancer. A small risk elevation of prostate cancer following TURP most probable could depend on surveillance bias.

Svensk populärvetenskaplig sammanfattning

Trots att prostatacancer är den vanligaste cancerformen hos män i Sverige och i västvärlden så är mycket lite känt om bakomliggande orsaker. Kända riskfaktorer är hög ålder, etnisk bakgrund och ärftlighet. Mer kunskap om riskfaktorer skulle kunna ge bättre möjligheter till prevention och också bättre målinriktad behandling.

Målet för den här avhandlingen har varit att undersöka riskfaktorer som är kopplade till prostatacancer.

I det första delarbetet har vi studerat sekundära tumörer efter prostatacancer. Sekundära tumörer är en ny cancer som uppkommer hos samma person men inte utgörs av metastaser eller dottertumörer ifrån den första tumören. Vi samlade in data på män med prostatatumörer 1958-1996 registrerade i det svenska cancerregistret. Sedan följde vi upp dem och noterade vilka nya tumörer de fick. Vi jämförde sedan med det förväntade antalet tumörer svenska män i samma åldersgrupp skulle ha fått. Vi fann då att det fanns en fördubblad risk för manlig bröstcancer samt en något mindre ökning för tunntarmscancer och maligna melanom efter en prostatacancerdiagnos.

I arbete två som bygger på det första ville vi gå vidare och ta reda på varför vi såg den här ökade risken för manlig bröstcancer. Vi samlade då in data på alla män med först prostatacancer och sedan bröstcancer och jämförde med män som bara fått prostatacancer. Vi jämförde vilken behandling man fått, hur tumören såg ut, var den ilsken eller mer godartad, fanns det metastaser vid upptäckt eller var den lokaliserad till prostatan. Vi fann då att män som fått bröstcancer i större utsträckning hade behandlats med kvinnligt könshormon, östrogen jämfört med dem som inte fått bröstcancer. Andra faktorer skilde sig inte åt mellan grupperna. Vi samlade även in data på släktingar till alla män med båda diagnoserna, även de som fått bröstcancer före prostatacancer och såg om det i släkten fanns en ökad förekomst av tumörsjukdomar. Det vi kunde konstatera var en fördubblad risk för förstagradssläktingar (söner, bröder, fäder) att få prostatacancer. Det var väntat eftersom ärftlighet är en av de få kända riskfaktorerna. I övrigt kunde vi inte se någon ökad risk för annan cancersjukdom hos släktingarna. Dock måste påpekas att studien var relativt liten och riskökningar mindre än en fördubbling kunde vi inte förväntas att hitta. I vissa av familjerna fanns en ansamlig av olika tumörer som gav misstanke om mutation i en gen som heter BRCA2. Det är en gen som orsakar bröst och äggstockscancer men man har också sett att den kan orsaka prostatacancer i viss utsträckning. Det var dock få familjer där detta kunde ses.

Sammanfattningsvis kunde vi konstatera att den ökade risken för manlig bröstcancer till största delen berodde på östrogenbehandling men att en liten andel av männen sannolikt hade mutationer i bröstcancergenen. Man skall dock komma ihåg att manlig bröstcancer är en ovanlig sjukdom så att inte ens en fördubblad förekomst gör den vanlig.

Tredje delarbetet handlar om huruvida normala genetiska variationer i östrogen beta receptorn (ERβ) har med risk för prostatacancer att göra. En receptor kan liknas vid en signalstation, östrogen, det kvinnliga könshormonet är signalen och när den har signalerat till stationen, receptorn så fördelar receptorn sedan vidare uppgifter i cellen om vad som skall göras. Just i prostatavävnad finns ERβ i stor utsträckning. Tidigare studier har visat att ERβ bromsar tillväxten i cellen, det verkar ha betydelse också för cancertillväxt eftersom det finns mer ERβ i normal prostatavävnad jämfört med i cancer där tillväxten sker ohämmat. Vi undersökte om små variationer i denna gens byggstenar skilde sig mellan män som fått prostatacancer och de som var friska. Studiepopulationen vi använde var en stor svensk populationsbaserad studie som kallas CAPS (CAncer Prostate in Sweden). Den inkluderade nydiagnosticerade män med prostatacancer och kontroller utan prostatacancer. Variationerna kallas för polymorfier och är enstaka mutationer i arvsmassan. Polymorfier utgör den stora merparten av genetisk variation mellan människor. En förändring, ett basparsutbyte i en del av genomet som reglerar uttrycket, (hur mycket eller lite utav ERβ det finns) skilde sig i frekvens mellan män med respektive utan prostatacancer. Detta skulle tala för att förändringen bidrar till ökad prostatacancerrisk. Dock har senare undersökningar av andra grupper inte kunnat verifiera fyndet och vårt resultat kan ha berott på slumpen.

I det sista delarbetet har vi undersökt en kohort av 7901 norrländska män som genomgått en hyvlingsoperation (TURP) av prostatan pga. vattenkastningsbesvär. Vi jämförde incidensen och mortaliteten hos dem med samma parametrar i befolkningen. Vi såg då en liten ökad risk för att insjukna i prostatacancer men däremot en minskad risk att avlida i prostatacancer.

Inflammation, en reaktion i vävnader har visat sig kunna orsaka cancer, till exempel magsäckscancer där den bakomliggande faktorn är magsårsbakterien Helicobacter pylori och levercancer där hepatitvirus leder till inflammation och cancerutveckling. Det är en relativt långsam process som tar flera år innan cancer har utvecklats. Det finns en del data som tyder på att även i prostata kan inflammation vara en faktor som ökar risken för cancer. Därför tittade vi närmare på 201 fall från kohorten, män som efteråt fått prostatacancer och som jämförelse 201 män som genomgått samma operation men inte

fått cancer. Vi ville se om det fanns ökad förekomst av inflammation hos dem som fått cancer vilket skulle styrka teorin om inflammation. Vi kunde inte påvisa någon sådan skillnad. Vi gick vidare för att se på två andra centrala funktioner i prostataceller. Först androgenreceptorn med liknande funktion som östrogenreceptorn samt ett protein som kallas p53 och är ett slags bromsprotein, det slår igång stoppmekanismer om något går snett i cellen för att fel inte skall fortplanta sig när cellen delar sig. Trots att vi såg oväntat hög förekomst av p53 och att vi såg att androgenreceptorns uttryck påverkades av inflammation så fanns det ingen skillnad mellan fallen och kontrollerna. Som en sammanfattning av delarbete fyra kan vi säga att vi påvisade en liten ökad risk för prostatacancer efter en TURP men att dödligheten i sjukdomen tvärtom var minskad. Det kunde inte förklaras med inflammation vid tiden för operationen och inte heller med förändringar av AR och p53 uttryck. Vi tolkade riskökningen som beroende på att dessa män med urinvägsbesvär oftare söker läkarvård och därför i större utsträckning får små tumörer upptäckta. Tumörer som kanske aldrig annars hade givit sig tillkänna.

Sammanfattningsvis har denna avhandling visat att manlig bröstcancer är ökad efter prostatacancer och att det till största delen beror på östrogenbehandling som givits pga. prostatacancern. Vidare har vi påvisat att en genförändring i ett regleområde till östrogenreceptor β skiljer sig i frekvens mellan män med och utan prostatacancer men det har sannolikt ingen större betydelse för uppkomsten av cancer. Till sist kan vi också konstatera att män har en liten ökad risk att diagnosticeras med prostatacancer efter en hyvlingsoperation men att det inte påverkar mortaliteten och att inflammation i prostatan vid tiden för operationen inte verkar påverka risken att få cancer.

Abbreviations

3'UTR	3' Un-Translated Region
AR	Androgen Receptor
BPH	Benign Prostate Hyperplasia
CAPS	CAncer Prostate in Sweden
CI	Confidence Interval
CRPC	Castration Resistant Prostate Cancer
DHT	DiHydroTestosterone
ERβ	Estrogen Receptor Beta
FDR	First Degree Relative
HGPIN	High Grade Prostatic Intraepithelial Neoplasia
htSNP	haplotype tagging Single Nucleotide Polymorphism
IARC	International Agency for Research on Cancer
LD	Linkage Disequilibrium
LUTS	Lower Urinary Tract Symptoms
PIA	Proliferative Inflammatory Atrophy
PSA	Prostate Specific Antigen
SDR	Second Degree Relative
SEER	Surveillance, Epidemiology and End Results
SIR	Standardized Incidence Ratio
SMR	Standardized Mortality Ratio
SNP	Single Nucleotide Polymorphism
TNM	Tumor Node Metastasis
TURP	Trans Urethral Resection Prostate

Introduction

Epidemiology

In Sweden, prostate cancer is by far the most common cancer diagnose, with approximately 10000 new cases per year [1]. The incidence has been rising continuously during the last 30 years with a marked surge in incidence in the early 90´s. This incidence peak corresponds to the introduction of PSA testing, which led to an increased number of diagnoses of asymptomatic men [2]. The latest available statistics show a modest decrease in number of new cases, indicating the peak is reached. This would mimic the development in the USA where the incidence peaked in the mid-nineties and then declined to a lower but still high level.

Prostate cancer mortality has stayed on near the same level during these years and 2,500 Swedish men succumb to this disease every year. Interesting though is the fact that less men than before are diagnosed with advanced, metastatic disease, implicating that more men are potentially curable [3].

Worldwide the highest incidence is seen in developed countries and in some African regions. In the year of 2002, 679.000 men were diagnosed with Prostate Cancer worldwide, corresponding to 11.7% of all male cancer, 221.000 died [4]. Explanations to the unequal distribution in frequency of prostate cancer diagnoses are probably the spread of risk factors for the disease. Lack of complete registration and less intensive and accurate work-up of diagnoses are other possible explanations [5].

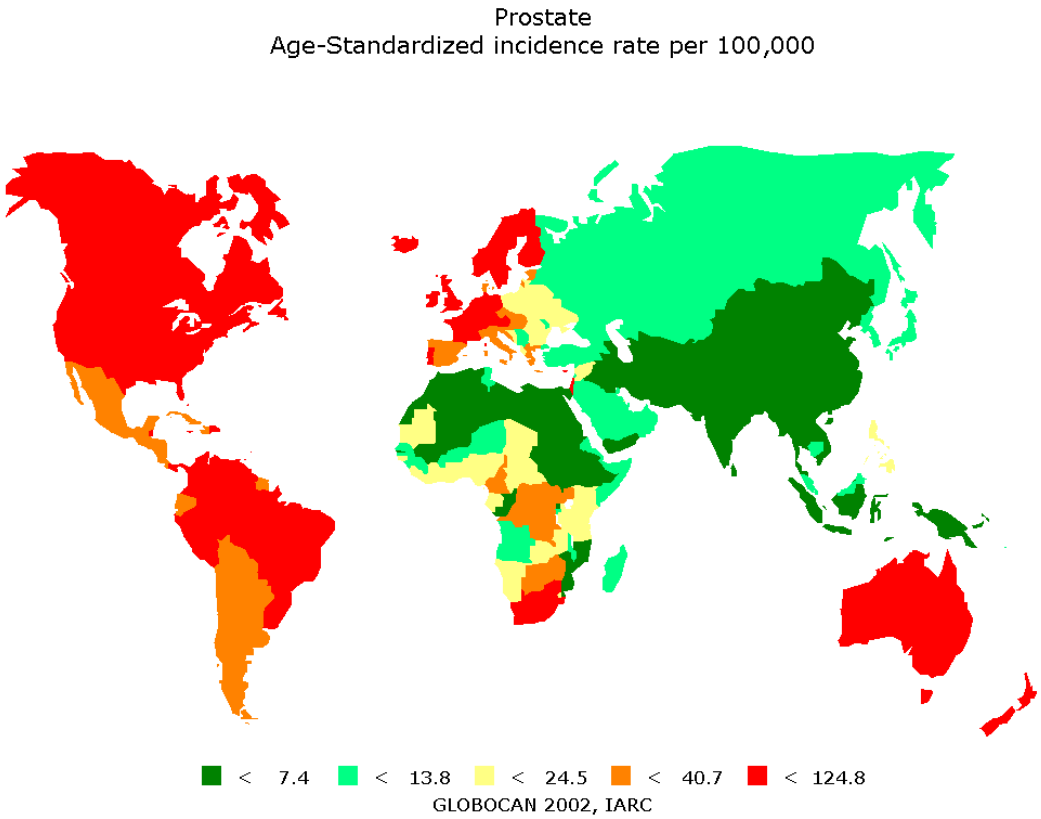

Fig 1. Distribution of prostate cancer incidence around the world. Globocan 2002, IARC [6]

Risk Factors

Age

Most prostate cancer occurs in the elderly population with a mean age at diagnose of 70 years. Very few, about 13% are diagnosed before the age of 60 according to the Swedish National Prostate Cancer Registry [3]. In autopsy studies the frequency of both High Grade Prostate Intraepithelial Neoplasia (HG-PIN) and manifest cancer increases with age [7, 8].

Ethnicity

Incidence of Prostate cancer varies around the world being highest in African-Americans shortly followed by Caucasian Americans. North and Western Europe are also high-risk areas whereas Southeast Asia displays a low frequency of prostate cancer. Migration of Asians to the US renders them a higher rate of cancer but not to the level of whites or blacks indicating a true difference in risk related to ethnicity and genetic susceptibility. Increase in rate might relate to lifestyle factors e.g. diet but also to more diagnostic procedures. [4, 5, 9]In Africa, for example, Nigeria the vast majority of diagnosed cases have advanced disease and an underestimation of the disease is likely [10].

Diet

Dietary factors involved in the development of prostate cancer have been studied over the years and many contradictory results have been published. Supporting of diet as a risk factor is the variable incidence worldwide with higher rates in Europe, US and Australia compared to low incidence areas in Asia. Evidence of a protective effect on prostate cancer is present for selenium and prospective prevention trials are ongoing (http://www.clinicaltrials.gov/). High intake of cooked tomatoes and fatty fish also seem to diminish risk of prostate cancer. [11] On the opposite, increased risks of prostate cancer have been associated with consumption of high amounts of red meat, dairy products and Zinc.

Heredity

A large proportion of prostate cancer patients report family history of the disease, about 9-15 % [12]. Several cases – control studies show a substantially increased risk of Prostate Cancer if there is a brother, a father or both diagnosed with the disease. Risk estimates around 2.5 are consistently reported. [13]; [14] Constant findings in all of the studies are higher risks of prostate cancer if a brother is affected than a father; younger age of the proband also confers higher risk. Caution in interpreting these studies is warranted because of the high incidence of prostate cancer. Not all cases in a family depend on hereditary factors; some will probably be due to other reasons such as shared environment or just chance. Cases also tend to remember relatives with cancer to a higher extent, recall bias that will inflate risk estimates. Using registries of cancer and family relations will overcome this problem and tend to lower risk estimates [15].

In a large twin-cohort study on several cancers in the Scandinavian countries, Lichtenstein et al found the heritability of prostate cancer to be as much as 42% [16]. This was much higher than in any of the other 28 cancer forms studied, for example, heritability for breast cancer was 27%.
A well accepted definition of hereditary prostate cancer is; a family consisting of either two First Degree Relatives (FDR) younger than 55 at prostate cancer diagnosis, three or more affected relatives irrespective of age in the nuclear family or prostate cancer in three successive generations on either the paternal or maternal line [17].

Genetic Epidemiology

Prostate Cancer and Genetic Studies

Since heredity is of such importance in prostate cancer there has been much effort put into the pursuit of the genes responsible. Different approaches have been used, segregation analysis, linkage analyses and more recent, association studies. Segregation studies have consistently shown evidence for autosomal dominant inheritance, even though there are reports on recessive or x-linked inheritance as reviewed by Schaid in 2004 [18]. Early on, there was much optimism around linkage and the finding of the first putative genetic locus for prostate cancer [19]. This locus was soon followed by more loci [20-23] that were suggestive of linkage. However, non-replication of the results subdued the initial enthusiasm. Proposed candidate genes could not explain heredity except for maybe a very small fraction [18]. To overcome the problem with many studies that includes too few families, an international cooperative group was formed, the International Consortium for Prostate Cancer Genetics (ICPCG). Creating the ICPCG, identification of prostate cancer susceptibility genes through combining the sets of families, would gain in statistical power and thereby help to find true linkage. A combined genome wide linkage scan of 1.233 families from 10 groups within the ICPCG provided only suggestive linkage to five different chromosomal regions. These regions were 5q12, 8p21, 15q11, 17q21, and 22q12 [24]. When selecting the families most probable to be informative, e.g. with many affected members, ≥ 5 and low age at onset, significant evidence for linkage at 22q12 was found in the families with many members. The difficulty to find linkage to a specific locus even in the combined effort with many

families clearly indicates a genetic heterogeneity of prostate cancer with many low penetrant genes that together contributes to increased risk.

SNPs

A single nucleotide polymorphism (SNP) is a variation in the genome that is common in the population. By definition, the SNP occurs in more 1% of the population, otherwise by definition it is a mutation. To date, near 12 million SNPs are known [25].

SNPs are distributed throughout the genome, in introns, promoters and exons. Being robust and easy to measure, combined with high throughput genotyping techniques they are very good to use in association studies. Association studies take advantage of the existence of LD, linkage disequilibrium, defined by the non-random association of one polymorphic allele at a single locus with another allele at a different locus. Alleles in LD that are inherited together constitute a haplotype. The degree of LD depends on recombination rate and number of generations since the first occurrence of the SNP in the population. Simply put, alleles longer apart and with longer time-span since the allele changed from the ancestral variant, the lower is LD. One SNP can be used as a surrogate marker for another allele that is in LD or the whole haplotype, and the SNP is then referred to as haplotype tagging SNP, htSNP. This is the ultimate use in association studies of complex diseases, where the polymorphism or the haplotype being tested either affects risk of disease directly or is a marker for some nearby genetic variant that affects risk of disease.

Haplotypes and Haplotype Blocks

Haplotypes are a specific combination of variant alleles in a population and they make up blocks. Within blocks there are different haplotypes, some very common to the population and some that is represented in a small fraction. The number of variant haplotypes that covers the majority, ≈ 90%, of variation within a block is usually few, often not more than 3-5 different haplotypes [26]. The number of SNPs that needs typing to cover the majority of variation in a haplotype is also much lower than one could predict. This is depending on that some SNPs occur on a haplotype together with another specific SNP, being in LD, therefore only one of them needs to be typed. As one SNP automatically gives the information of the other, these SNPs are named haplotype tagging SNPs (ht SNPs) [27]. Haplotype blocks are interrupted by boundaries were

recombination have occurred, and it seems that there are locations where recombination occur more frequent, so called “recombination hotspots”. A recombination occurs during meiosis. Chromosomes are copied and are afterwards located close to each other; they can then cross and exchange parts of DNA before they are drawn apart in the meiotic spindle to their respective cell. Some of the haplotype blocks reported are very long, indicating low frequency of recombination in this area and some are short, the size of blocks range between <1kb up to 173 kb [28]. Depending on the population typed, the size of blocks varies in length where European and Asian populations tend to have longer blocks compared to Sub-Saharan and African-American populations [28]. The longer block size in European populations might depend on the phenomenon of “bottleneck”. Bottlenecks arise due to a temporary reduction in the size of the population, which causes a reduction in genetic variation; an example would be the migration out of Africa of the European population [29]. Hotspots of recombination are not evenly distributed throughout the genome; instead, there are regions where recombinations take place more often. These regions concentrate around genes instead of within the transcription region, most recurrent are the 5´ region of transcription start [30].
From the Phase II of the HapMap project comes also evidence that recombination hotspots more often surround genes involved in cell surface and out-of-gene activities such as immunity and signaling [30].

Prostate Cancer Association Studies

In the beginning of the era of association studies, the main characteristic of the studies were small sample sizes and few polymorphisms studied in each gene as exemplified in a study by Chen et al [31]. Polymorphisms that were included mostly persisted of functional non-synonymous SNPs.
Evolving techniques together with increased knowledge of LD and mapping of the genome made studies with larger sample sizes using a haplotype tagging approach possible. An example of an enlarged study is the creation of CAPS (CAncer Prostate in Sweden) case – control study. This is a population-based study where accrual has been carried out in two steps and Lindmark et al [32 402] comprehensively described the study population. This study has been the base for several investigations of different pathways, for example sex hormone metabolism and inflammation response [32-35] [36]. However, confirmation of associations have been sparse [37] [38], a phenomenon

shared with studies of other diseases using the same methodology. A way of circumvent this is proposed by Colhoun et al, by using even larger sample sizes and carefully asses the probability of chance findings they suggest improvement of replication rates [39]. Replicating findings in another population with different ancestry will improve confidence in the finding because using different populations increases the genetic variance [40]. Non-replication in a different population does not necessary reject the association since associations can vary between populations. Using several populations can also reduce the region of interest by taking advantage of LD differences. Making use of this approach Amundadottir et al [41] managed to replicate their finding of association at 8q24. Starting with a genome wide linkage study of 871 prostate cancer patients belonging to 323 families, they found positive lod score at this position. They further refined the location with more markers in two case–control settings. After refinement, they undertook an association study with SNPs spanning a 600kb region combining the two case–control groups. Of 37 SNPs that were positive for association, rs1447295 showed the strongest association. In the same study, this SNP was further replicated in two populations of European ancestry and one of African-American descent. Other groups have also managed to replicate the finding of association at 8q24 [42] and also find more independent locus at 8q24 [43]. Earlier studies on hereditary prostate cancer have not revealed any specific clinical or pathological features other than young age at onset [44, 45]. However, a recent study investigating tumor characteristics in carriers and non-carriers of these susceptibility alleles, found association of high-grade disease, Gleason score ≥7, and these alleles [46]. In addition, familial history of prostate cancer together with one or more of these alleles further increased the association.

Prostate Cancer – The Clinical View

Normal Function

The prostate is a small, walnut-size gland located just below the urinary bladder and circumflexing the urethra. Containing clotting and proteolytic enzymes, the alkaline prostatic secretion facilitates the sperms movement towards the ovum during ejaculation [47].

Disease Symptoms

Mainly, symptoms emanating from the prostate relate to micturition. Benign Prostatic Hyperplasia (BPH) occurring mostly in the sixth decade and onwards causes hesitancy, decreased peak flow, and urinary retention [48]. Similar symptoms may emanate from cancerous lesions. Symptoms of disseminated disease include back pain as the most frequently occurring, related to skeletal metastases, but symptoms originating form other tissues, as lymph nodes, lung or liver might also occur.

Treatment

For localized disease, there is several treatment options; surgery by radical prostatectomy with or without robotic assistance, external radiotherapy, sometimes combined with high dose rate brachy-therapy or low-dose rate radioactive implants, seeds, as the most common variants. For low risk disease there is also the option of active surveillance, aiming at postpone treatment with potential harmful side effects [49]. Active surveillance can also be an option for selecting patients not in need of treatment at all.

If the disease relapses after primary treatment or is metastatic from the beginning, the main treatment option is androgen deprivation therapy. In the early forties, Huggins and Hodges experimented with estrogen treatment and found it to be effective in meliorating symptoms [50]. Palliating symptoms and prolonging survival can be achieved by androgen deprivation with castration, surgical or medical or by antiandrogen medication. Eventually this palliative treatment cease to be effective and the disease enters the castration resistant phase. During this phase, the androgen receptor (AR) continues to be expressed, even to a higher extent [51]and is possibly activated through other pathways, thereby escaping the negative regulation of androgen deprivation.

Second Primary Tumors

The Concept of Second Primary Tumors

Definition of a second primary tumor is a new tumor in a patient who earlier experienced a cancer diagnose. Sometimes a time limit of six months is applied where the tumor is called synchronous in the first six months and afterwards is named metachronous. The

reason to study second primary tumors is to find common etiologies and to find connections between treatments given for the first cancer and the development of a second one. A well established risk factor for Head and Neck tumors is smoking and investigating second primary cancers after Laryngeal cancer shows the connection with another well known smoke related cancer, Lung cancer [52]. A true relationship of shared etiology can be further established if studying tumors in reverse order show similar results. This is the case when following lung cancer patients for second primary cancers shows increased risk for tumors in the head and neck region [53].
To illustrate the connection with treatments given, the investigation by Travis et al of Testicular cancer and second primary cancers is a good example. They found excess risks of several solid tumors and leukemia following chemo and radiotherapy for both seminoma and non-seminoma testicular cancer. [54, 55]The Risk ratios of solid tumors after radiation therapy were higher "in-field" compared to regions where lower doses were distributed, indicative of a dose-response relationship. Risks of a second primary tended to be increased for as long time as 20 years or more.
This of course have implications in the follow-up on patients after successfully cured first cancers.

Prostate Cancer and Second Primary Tumors

Since prostate cancer is so common, several studies regarding risk of second primary tumors have been conducted. Increased risks are generally not noted [56-58] except for some tumors such as bladder, small intestine and melanoma [59, 60] Small intestine tumors consist mainly of four subtypes; Carcinoids, adenocarcinomas, sarcomas and lymphomas where the two first subtypes are most frequent. Diagnosing tumors of the small intestine is not easy given that they often have diffuse symptoms. They are therefore a common autopsy finding and as many as 18-25% can be found at autopsy [61]. Increased risk of small intestine tumors after prostate cancer is mainly confined to carcinoids [62, 63]. This augmented risk is often explained by detection bias, a possible explanation since small carcinoids often give few if any symptoms. The elevated risk of prostate cancer after small intestine carcinoids [61] as well as the other way around [62, 63]definitely indicates shared risk factors. This association is present both ways with similar magnitude and the association gains further strength when present in different populations.

Detection bias or close watch bias can be a problem in epidemiologic cancer studies, since cancer patients more often go for check-ups, detecting asymptomatic cancers. This can explain the huge increased risk for second bladder cancer often found in the first period following prostate cancer [59, 60, 64] Finding bladder cancer is common in the first work up around prostate cancer because investigating urinary tract symptoms often demands a cystoscopy. Risk persisting over the years cannot solely be referred to close watch over the patients, because treatment effects from the first cancer can also be responsible. A common treatment for localized prostate cancer is external radiation given in fractionated doses over seven to eight weeks. Ionizing radiation is a well-recognized carcinogen associated with several cancer types such as sarcomas, non-melanoma skin cancer and leukemia. Following radiation treatment of prostate cancer, Brenner et al found increased frequency of in-field sarcomas, bladder cancer and after more than 10 years follow-up time also rectal cancer [65]. Findings confirmed by Baxter et al and Moon et al, using SEER registries (Surveillance, Epidemiology and End Results) [66, 67]. In the registries, data regarding primary treatment, tumor characteristics and stage are documented, that enables comparison of irradiated and surgically treated patients. Kendal et al [68] produced contradictory results with non-elevated rectal cancer risk when they compared radiation treated patients with patients who received neither radiation nor surgery. A possible bias was that the untreated group had a higher mean age. Other possibilities such as co-morbidities or smoking that influence the risk of rectal cancer were higher in the non-surgical, non- radiated group. Therefore, this study must be interpreted with caution. There are several small single institution studies addressing the issue, none of them have found increased risks for subsequent cancers, one of the largest is presented by Chrouser [69] comprising of 1743 patients. The advantage single center studies have is very good control of treatment, patient characteristics and often complete follow up. That does not compensate for the far too small size to detect increased risks.

Typically, radiation induced tumors take place in previous irradiated fields and have a lag-time of at least five years. Absolute risk for prostate cancer survivors to develop a second primary tumor after radiotherapy is low, as reported in above mentioned studies, but with decreasing age at diagnose and increasing survival after therapy this might be increasingly important in patient follow-up.

Male Breast Cancer

Epidemiology

Male breast cancer as being one of the rarest types of tumors among males has attracted much interest over the years. In Sweden 2006, 37 new male breast cancers were detected, corresponding to 0.1% of all cancer in males [1]. In the U.S. male breast cancer incidence is about one in 150 female breast cancers with rising incidence over the last three decades but the reason for the increase is not known [70, 71]. Worldwide, the incidence of male breast cancer much mimics the prostate cancer rate and distribution with low rates in Asia and high in western countries [72]. Male breast cancers have similar clinical features as female breast cancer, save for higher diagnostic age, mean age about 10 years above female, and a tendency to present with a higher stage [70, 71]. Survival according to stage is comparable. Male breast carcinoma demonstrates a high degree of hormone receptor positivity, an expression that gives therapeutic opportunities [73]

Risk Factors

Due to the rarity of the disease, epidemiological studies on male breast cancer often consist of small series where few strong conclusions can be drawn, although hereditary factors are well recognized. A history of female breast cancer in relatives increases the risk for male breast cancer about 2-3 fold, higher risks when more than one relative has breast cancer or the relative is young [74].

The higher risk in families is much attributed to BRCA2 mutations; mutation frequency varies widely in different populations [75-77] [78]. BRCA2 is a regulating gene located on chromosome 13q12-13, and in some populations a founder mutation is present. In Iceland, as much as 40% of male breast cancer patients carry the 999del5 mutation, a mutation traced back to a common ancestor in the 16th century [77]. Likewise, in Ashkenazi Jews, a founder mutation is present, 6174delT. The risk for a man carrying a BRCA2 mutation to develop breast cancer is considered in a study by Thompson et al [79]and estimated to 6,92 % by age 80.

Another genetic abnormality associated with male breast cancer is Klinefelters syndrome, a condition characterized by XXY karyotype. This chromosomal anomaly gives the carrier a long, narrow-shouldered feature often accompanied by gynecomastia

and infertility [80]. In a large cohort study, Swerdlow et al [81] examined the cancer risk in cytogenetically diagnosed men with Klinefelters syndrome. They found significant increased risks for breast cancer, SIR 17.8 [95% CI 3.7-51.9], together with substantially higher SMR 28.8 [95% CI 3.5 to 104.0]. Drawbacks of that study was that only cytogenetically identified men were included, possibly creating a selection bias since the syndrome is supposed to be more common in the population than estimated in the study.
Using another approach, Hultborn et al [82] retrospectively analyzed karyotypes of 93 male breast cancer patients, finding a prevalence of 7.5%. This prevalence corresponds to a 50-fold risk increase in Klinefelter patients to develop breast cancer. Speculation regarding this increase in risk has mainly focused on higher estrogen levels, creating an estrogen – androgen ratio much larger than that of normal men.
Supporting this assumption, are other established risk factors such as; obesity, testicular injury, liver damage/cirrhosis, estrogen treatment, all conditions that alters the estrogen – androgen ratio [83-85]. Furthermore, as with other tumors, male breast cancer can be induced by radiation [86, 87] with elevated risks 20-30 years after exposure.

Prostate Cancer and Breast Cancer – the Relation

Sobin and Sherif examined relation of prostate cancer to male breast cancer by comparing incidence ratios retrieved from Cancer Incidence in Five Continents [88]. They found a direct relation between incidences; countries with high frequency of prostate cancer also had a high breast cancer rate. There is also common familial aggregation of both breast and prostate cancer, where early onset and familial disposition to PC increases breast cancer risk in first degree relatives (FDR) [89]. In breast cancer families, there is evidence that the occurrence of prostate cancer in the family increases the risk of breast cancer to FDR's more than if there is a male breast cancer present [90]. The simultaneous occurrence of breast and prostate cancer in families has been linked to BRCA2. In a study by the Breast Cancer Linkage Consortium, mutation carriers had a 4.65 times increased risk for prostate cancer [91]. Reporting from Iceland, Sigurdsson et al [92] found increased risk ratio for prostate cancer in FDR of Icelandic breast cancer probands. An increased risk not confirmed in a Swedish population based study where there was a tendency towards lower risk [93].

BRCA2 does not seem to contribute a lot to hereditary prostate cancer; low frequency of mutation is reported from family studies both of Ashkenazi and non-Ashkenazi origin [94, 95]. It does seem to play an important part in some families and may render carriers a more aggressive phenotype. Grönberg et al [96] described a family of a father and four sons, all of them with the same BRCA2 mutation, 6051delA. All of them had the same pathological pattern with Gleason score 10 tumors (personal communication). This more aggressive pathology has been observed in other studies [92, 97, 98].

Prostate Cancer and Estrogen

Ever since Huggins and Hodges did their experiment with castration and found effects on metastatic prostate cancer, hormonal treatment has been a cornerstone in the treatment of prostate cancer [50]. Castration with either orchiectomy or LHRH analogues (Luteinizing Hormone Releasing Hormone) is nowadays a regular treatment used in both metastatic and (neo) adjuvant settings. Estrogen, given as muscular injections or orally has also been used but is accompanied with side effects. The side effects are more pronounced with oral administration when there is a first-pass effect in the liver. The most common side effects are connected to the cardiovascular system with venous thrombosis and cardiac ischemic attacks, sometimes fatal, as the most severe outcomes [99]. A large Scandinavian study, SPCG-5, comparing total androgen blockage with either LHRH analogues or orchiectomy in combination with antiandrogen to parenteral estrogen could not demonstrate any difference regarding survival. Concerning safety, no difference was found for fatal cardiovascular events but increased cardiovascular morbidity was present in the patients treated with estrogen [100]. Long time follow up of this study was reported at the 26th meeting of the Scandinavian Association of Urology in 2007 and conclusions added were that there also was evidence of skeletal morbidity in the androgen deprivation group [101]. With increasing use of androgen deprivation therapy in the adjuvant setting as well as for locally advanced prostate cancer, osteoporosis and fractures will be more of a concern. Shorter time to cardiovascular death was recently found in a combined analysis of three studies [102] all of who investigated adjuvant androgen deprivation to radiotherapy of prostate cancer. Men in these studies treated with androgen deprivation had shorter time to fatal myocardial infarction compared to men not receiving adjuvant hormones but there did

not seem to be a dose-response relation since there was no difference between 3 months duration of treatment compared with 6-8 months.
Estrogen treatment might have a place in the arsenal of treatments since bone mineral loss does not seem to be a problem and cardiovascular events might be manageable. There are ongoing clinical studies investigating both transdermal administrations of estrogen and oral preparations in different situations (www.clinicaltrials.gov).
If estrogen as a treatment for prostate cancer faces a revival, reintroduction must be done with caution since there are case reports in the literature of male breast cancer following estrogen treatment for prostate cancer [85, 103]. Higher incidence of male breast cancers might be the result of increased use of androgen deprivation early in the disease course in combination with mammary gland radiotherapy to prevent gynecomastia
Evaluating this incidence is of need in future studies and long follow-up periods are needed.

Estrogen Receptor β

Structure and General Function

Estrogen receptor status have been recognized and used as a prognostic and predictive factor in breast cancer treatment for many years. In 1996, another estrogen receptor, estrogen receptor β (ERβ) was discovered in rat prostate tissue [104], human testis [105] and attained much interest. The gene encoding ERβ is located on chromosome 14q23.2 and consisting of approximately 61.2 kb. This corresponds to a protein of 530 amino acids, weighing 60kDa [106]. There are at least five isoforms of the protein, named ERβ1-5 [107, 108] and they differ in the C-terminal end downstream exon 7. They also differ in type of tissue where they are expressed [107, 108]. The ERβ belong to the nuclear receptor super family where the AR also is a member. To exert their function, receptors form dimers in order to activate transcription on responsive genes. These receptors consist of five distinct domains, A/B, C, D, E and F, all with specific functions [109]. The variable A/B domain is ligand independent and has transcription activating function. The DNA binding part of the receptor, the C domain, show high homology, 95% [105] between the ERα and the ERβ and it is also involved in receptor dimerization. The D-domain acts as a hinge, connecting the DNA binding domain to the ligand-binding

domain E. The ligand-binding domain located in the C-terminus in ERβ share some homology with ERα and contains a ligand dependent transactivation area.

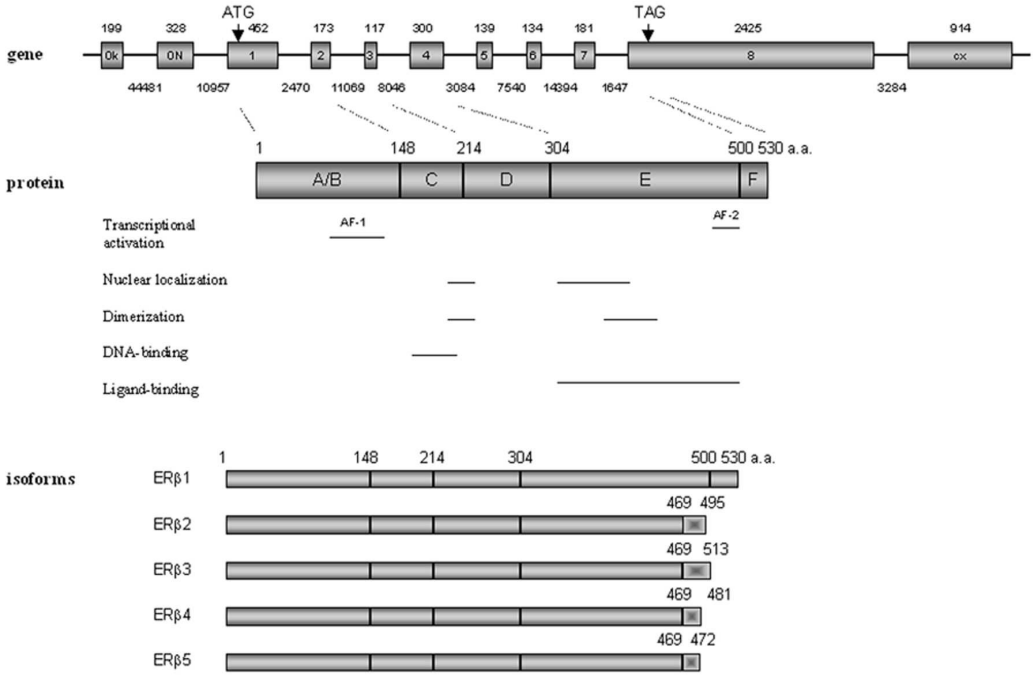

Figure 2 Structure of the human ERβ gene, protein and functional domains, and mRNA isoforms. From Zhao et al. Nuclear Receptor Signaling (2008) **6**, e003 [110]

ERβ expression occurs in many different tissues, prostate, ovary, lungs, mammary gland, bone, uterus, epididymis, kidney, bladder, intestine, central and peripheral nervous system [111-113].

ERβ in the Prostate

In the prostate, ERβ is mostly expressed in epithelial cells and the previous known ERα in stromal cells [114] During embryogenesis and in the early postnatal period, expression of ERβ occurs in both epithelial and stromal cells, whereas ERα staining is virtually absent in the fetal prostate. ERα appears in the postnatal period when it takes on a pattern similar to the adult prostate [115]. Patterns of expression vary in the different

compartments of the prostate, where ERβ is found in both the peripheral and transition zone and ERα seems restricted to the peripheral zone [116].

Expression of ERβ in normal prostate tissue is almost universal but regarding hyperplastic lesions or cancer there are divergent results. Most authors find decreasing expression during cancer aggravation, expression is lower in high grade compared to low grade cancer [117-120]. The opposite, with gradually increasing expression as the prostate tissue turns from normal to hyperplastic and further into cancer is reported [121]. There is also a study reporting association between Gleason score and ERβ [122]. In addition, this study suggests worse prognosis associated with high ERβ expression. Horvath et al [118] also found ERβ expression in prostate cancer to be a negative independent prognostic factor. Paradoxically, in metastases, reappearance of the receptor is reported [117, 120, 123]. Some of the divergent results can depend on the different methods used in the studies, immunohistochemistry, western blot and RT-PCR but also on the different antibodies used [124].

The pre-neoplastic lesion PIN (proliferative intraepithelial neoplasia) that is characterized by proliferation, exhibit lower levels of ERβ, [117, 123] indicating an anti-proliferative function of ERβ. This proposed anti-proliferate function of ERβ was first suggested in studies of ERβ knockout mice [125]. The mice, deficient of the ERβ gene have continuous proliferation in the ventral prostate as shown by Ki67 staining in almost all of the epithelial cells. They have also higher levels of AR compared to wild type mice, implying a regulatory effect of ERβ on AR. The resulting hyperplastic nodules show elevated levels of Bcl-2 together with increased BrdUrd labeling, representing low apoptosis and high proliferation [126]. Reintroduction of ERβ into cell-cultures deficient of the receptor diminishes proliferation and triggers apoptosis [127]. Furthermore, ERβ is capable of inhibition of invasion in a ligand-independent way; growth reduction was about the same with or without 17-β estradiol [127].

The promoter region of ERβ contains a CpG rich region and two transcription start sites [128], there is also two untranslated exons, exon 0K and exon 0N [129].

During cancer development and aggravation, the expression of ERβ changes, from general to more sparse. Methylation in the promoter region shows an inverse relation to expression of ERβ [120, 130, 131] suggesting a causal relationship. This suggestion was tested when prostate cancer cell lines found to be negative for ERβ expression, DU145 and ND1, restored the expression after treatment with a demethylation agent [130, 131].

This pattern also exists in other cancer types where demethylation treatment restores silenced ERβ [132].
In an experiment, using DU145 prostate cancer cells Pravettoni et al [133] saw decreased cell proliferation when stimulating ERβ with either E2 or a selective ERβ agonist. They also observed increased ERβ expression and proposed an auto-regulatory mechanism conducted through the ERE element in the ERβ promoter earlier described [128]. Reduced proliferation can be the result of either inhibition of growth/ cell division or enhanced cell death. In a recent study, Walton et al [134]showed synergistic effects of demethylation and inhibition of histone deacetylation on the expression of ERβ, resulting in decreased proliferation and increased apoptosis. They showed that two different epigenetic mechanisms could co-operate in the regulation of the suppressor gene ERβ. By using the same cell-line as in the Walton study, Guerini et al [135] could also demonstrate impaired migration of cells through stimulation of ERβ and further on E-cadherin, implying an ERβ mediated effect on the metastatic process.
Given the common expression of ERβ in prostate epithelium and the anti-proliferative effect it exerts, it is a potential therapeutic target in clinical use. There are promising trials in cell –lines and xenografts showing response and ongoing clinical phase II and III trials to evaluate the function [136, 137]

ERβ SNPs

Since there appears to be a distinct function of ERβ in prostate cancer development and progression, genetic variation and familial aggregation of genetic aberrations is of interest to investigate. One approach is to examine common genetic variation in the gene among cases and comparing with a control population without the disease. Genetic association studies have been performed and will be discussed in later sections.

Prostate Cancer and Inflammation

In humans, approximately 18 % of all cancer attribute to infectious agents causing inflammation. Well-established connections are liver cancer hepatitis B or C and stomach cancer Helicobacter Pylori. There is emerging evidence for a role of inflammation in the pathogenesis of prostate cancer. For example, where there is high incidence of prostate cancer, like in the U.S., there is also high incidence of prostatitis. In Asia, both conditions occur less frequent [138, 139]. This co-variation around the world

of prostatitis and prostate cancer supports the connection, as does the decreased risk of prostate cancer in relation to the use of anti-inflammatory drugs [140, 141]. Dennis et al have estimated the magnitude of prostatitis contribution to the risk of prostate cancer in a meta-analysis. They found an OR of 1.6 – 1.8 for prostate cancer in relation to prostatitis [142].

Prostatitis can develop due to several reasons, infectious agents, urine reflux, physical trauma or hormonal changes [143] and is a very common feature. In pathological specimens of different types, biopsies, trans-urethral resections and prostatectomies, the presence of inflammation varies from 44-100% [144]. Nickel et al. also demonstrated universal presence of inflammatory cells in trans-urethrally resected specimens but the area covered by inflammatory cells was very low, 1.1% of the specimen. They did not see any correlation between grade and extent of inflammation and catheterization or bacterial growth [145]. It seems that inflammation is more frequent in the region of benign glands, and scarce around malignant glands [144]. If malignant glands are involved with inflammation, it may be a negative prognostic sign. In a study of patients treated with curative intent, Irani et al found that patients with high-grade inflammation adjacent to malign glands in prostatectomy specimens were more prone to biochemical recurrence than were patients where inflammation only occurred in benign areas [146]. This would agree with the description by De Marzo et al of Proliferative Intraepithelial Atrophy (PIA) [147]. This condition is characterized by inflammatory mediated atrophy and proliferation in the glands. These glands highly express the proliferation marker Ki67 and BCL-2, an apoptosis regulating protein; therefore, PIA may be looked upon as a neoplastic precursor. Genes involved in inflammation and host response has been in focus and there are some positive associations reported but there are many contradictory results [148].

Benign Prostate Hyperplasia, BPH

Benign prostate hyperplasia is a condition that mostly affects men from their fifth decade and onwards. Although BPH share some features with prostate cancer, it is not regarded to be a precursor to prostate cancer. These conditions both depend on hormones for growth, have a similar age distribution and respond to hormonal therapy [149]. BPH is a progressive disease; symptoms like low peak flow, urgency, frequency and nocturia aggravates and eventually turn into acute urinary retention or a need for BPH related

surgery [48, 150]. Until the early 90-ies the gold standard and almost only treatment available was surgery, as either trans-vesical adenomectomy or the more common trans-urethral resection. At this time, introduction of medical treatments, 5α-reductase inhibitors and α-blockers, changed the treatment profoundly. As a result there was a reduction in the need of surgery and the number of TURP performed decreased [151]. Studies investigating the risk of prostate cancer in connection with BPH have also assessed prostate cancer mortality. A finding repeatedly reported is an increased risk of prostate cancer [152-154]. This increased risk of prostate cancer after a BPH diagnose has not been accompanied by increased prostate cancer mortality [153-157]. However, one early study did find increased mortality after BPH but this study was affected by selection bias. Selection was biased because half of the patients included did not have patological specimens taken at inclusion, thereby possibly including prostate cancer cases in the BPH group and thereby increase the risks [152].

p53

The p53 pathway responds to stress inflicted upon cells. Stress that initiate DNA damage can come from both the outside or from within the organism. Gamma or ultraviolet radiation, toxic compounds that depurinates or alkylates DNA are factors that triggers p53 pathways as does hypoxia or nitic oxide [158]. When induced, p53 stabilizes, the half-life in the cell increases from minutes to hours and cellular concentration of the protein rises. The ability for p53 to bind to response elements in the DNA also increases. Different stress factors lead to divergent responses in the cell, cell cycle arrest, apoptosis or senescence.

Mutations in p53 is very common in several human cancers, including prostate cancer. In prostate cancer, p53 expression detected by immunohistochemistry is found more often in high-grade cancers compared to low-grade [159] although mutations are not confined to a late event in cancer progression. Reviewing the literature on prostate cancer and p53, Downing et al found that in both benign conditions like BPH and PIN and early organ-confined cancer, mutations of the gene and expression in the tissue was relatively common [160]. There was a wide span between studies, ranging 0-100% (average 32%) for early cancers and 0-78% (average 27%) for BPH as measured by IHC and sequencing methods. Some mutations cause synonymous changes that does not alter expression and are therefore of doubtful importance.

Since p53 have such an important function in the cell, several investigations concerning the role of p53 as a prognostic factor have been done, both with biopsy and prostatectomy specimens. Stackhouse et al [161]examined expression of p53 as measured by immunohistochemistry, both in pre-treatment biposies and in radical prostatectomies. They found that p53 staining in the biopsies could not predict prostate cancer recurrence after prostatectomy. On the other hand, p53 expression in cancerous cells in the prostatectomy specimen was prognostic for recurrence. Another study, by Oxley et al [162] found p53 staining in biopsies to be prognostic in multivariat analysis, independent from PSA and Gleason score. Contradictory to the Stackhouse study, p53 in the prostatectomy specimens did not provide additional prognostic information . T1a tumors found accidentially at TURP, mostly of transition zone origin are often of lower Gleason score and have less proliferation compared to periferal zone cancers [163]. Some of these cancer eventually progress and in an attempt to find a prognostic tool Abaza et al [164]stained for p53 and Bcl-2 in 44 transurethral resection specimens. As many as 32% of the tumors stained positive for p53 but it could not predict progression. This might reflect the fact that transition zone cancers not only express less proliferation markers than peripheral zone cancers, they also have less Bcl-2 and p53 staining [163]. Therefore, these markers are perhaps not suitable for prognostication in this zone. Analyzing patients curatively treated with radiation therapy within the RTOG 9202, Che et al [165] suggests that it definitely can be used as a prognostic factor. In addition, long-term anti-androgen treatment seemed even more beneficial to those with high p53 compared to those with normal or low p53.

Androgen Receptor

Androgens are necessary for prostate development and growth and they function via the androgen receptor (AR). AR belongs like the ERβ to the nuclear steroid hormone receptor family. In the inactive state, it is located in the cytoplasm but upon ligand binding it dimerizes and translocates to the nucleus. There it binds to androgen responsive elements on the DNA together with co-activators, exerting transcriptional function [166], figure 3.

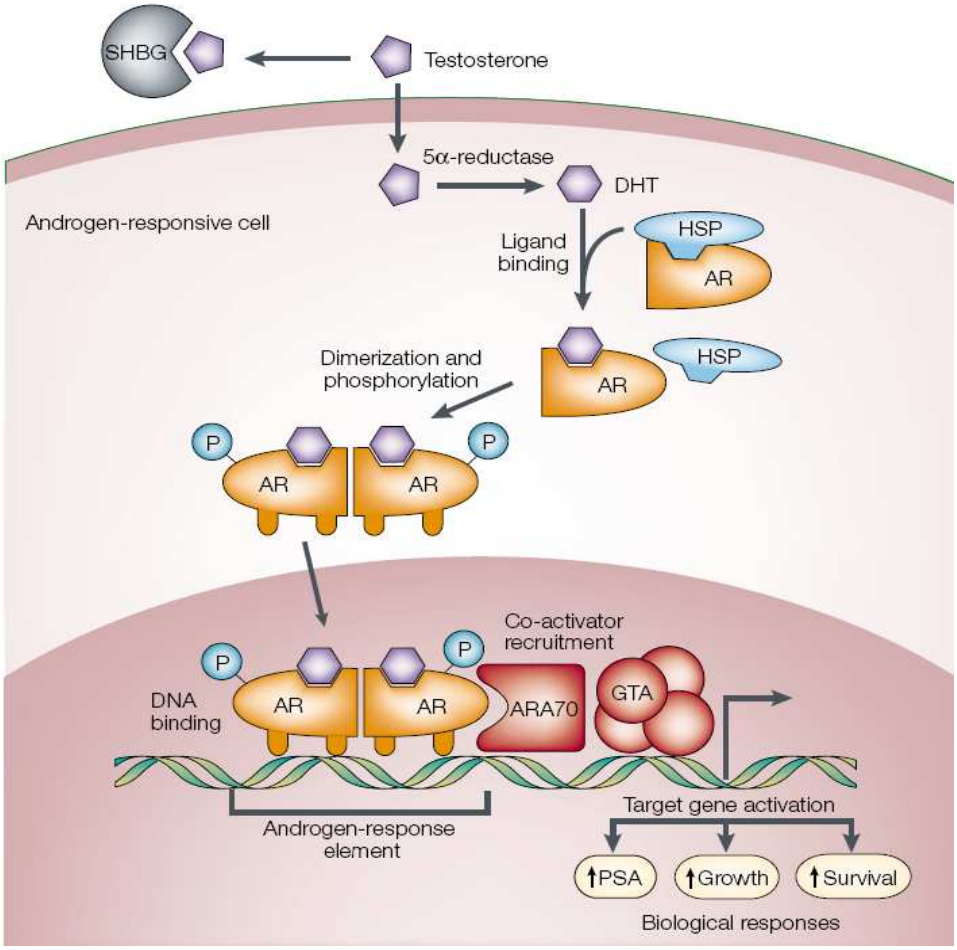

Fig 3. Adapted from Feldman and Feldman, Nature Reviews 2001 [166] Picture showing the action of AR upon binding of ligand. Note that one of the genes regulated by AR is PSA.

First line treatment for metastatic prostate cancer is to block the AR pathway, either by using Luteinizing hormone releasing hormone (LHRH), anti-androgens or a combination of both. This depletion of the natural ligand leads to impaired growth and regression of the tumor, often mirrored by a sharp decrease in the AR regulated protein PSA. Eventually, the cells stop responding to the treatment and resume growth [166, 167]. They then enter the "hormone independent" or "castration resistant" phase, where the

prostate cancer cells still depend on the AR signaling pathway but are independent of normal androgen levels. Recurrence of tumor and escape of therapy can occur due to several reasons, where amplification of the AR is one. Other mechanisms are hypersensitivity of the receptor, making it respond to very low levels of androgen and ligand independent activation [51, 166, 167]. Mutation in the AR, resulting in activation rather than inactivation by the anti-androgen flutamide is also reported [168]. This paradox seems to depend on a mutation at codon 877 and in the study by Taplin et al, treating the patients with bicalutamide, another antiandrogen, restored response to hormonal deprivation [168].

The AR is involved in several pathways, proliferation/differentiation and inflammation response are two [169] distinct pathways as shown by Asirvatham et al. When stimulating male Sprauge Dawley rats with an androgen agonist and examining the gene expression, they found several pathways to be influenced by androgens. These pathways, addressing growth, apoptosis and proliferation, are also involved in the transition of androgen dependent to androgen independent features [170]. AR expression is commonly downregulated in proliferative inflammatory atrophy lesions (PIA) [147, 171]) that lies in proximity to inflammation. Close to inflammation, Bcl-2 is found to be upregulated[172], seemingly as a response to the inflammation and Bcl-2 in turn downregulates the Androgen receptor [173]. In LNCaP cell lines p53 have been shown to regulate negatively the expression of androgen receptor [174]. Focusing not only on the AR, there have also been studies on circulating levels of sex hormones and the risk of prostate cancer. For example, the two most potent ligands, testosteron and dihydrotestosterone, different levels are not found to associate with prostate cancer risk in a recent metaanalysis [175]. The AR is definitely involved in many aspects of prostate growth and have regulative functions, but it is very complex and not yet fully understood.

Aims

The main aim of this thesis was to investigate risk factors associated with prostate cancer.

Paper I. To thoroughly identify increased risk for second primary cancers in a large prostate cancer cohort study and to generate hypotheses.

Paper II. To investigate the possible mechanisms underlying the increased risk of male breast cancer following prostate cancer as found in study 1.

Paper III. To do a comprehensive examination of genetic variation in the ERβ and eventual association to prostate cancer risk.

Paper IV. To analyze a cohort of men who have undergone TURP and their risk of prostate cancer and to se if inflammation in the prostate at the time of TURP confers increased risk of prostate cancer.

Material and Methods

Study Populations

Paper I

This study was made to generate hypotheses and to investigate risks of second primary cancer after prostate cancer. Selecting all prostate cancer cases diagnosed 1958-1996 and registered in the Swedish Cancer Registry made up a cohort of 135,713 men. For these men, calculations were made regarding the risk of any second primary cancer. Cancers diagnosed before the prostate cancer were excluded. To eliminate autopsy cases, and to exclude those cancers accidentally discovered in the diagnostic work-up, a six-month time cut-off from the date of prostate cancer was applied. This time cut off was used in order to collect only clinically important cancers.

Paper II

This study was set up as a follow-up study to study I and it was performed in two phases. In the first phase, a nested case – control study were set up by selecting cases with prostate cancer followed by male breast cancer and controls with only prostate cancer from the cohort in study I. This was done with addition of cases from two more years 1997-1998, the whole time period was then 1958-1998. Controls were matched by age, year of prostate cancer diagnose and region in Sweden from where diagnose was reported. Retrieving medical records for these men allowed for extraction of data regarding primary treatment and disease parameters such as stage and grade. Clinical data made comparisons between cases and controls possible. In the second phase, a family cohort study was constructed. All patients in the Swedish Cancer Registry with both prostate cancer and male breast cancer, irrespective, which came first, were considered as probands. First-degree relatives, parents, siblings or children and second-degree relatives, grandchildren to the prostate-breast cancer patients were identified through parish offices. All relatives were then linked back to the Cancer Registry to retrieve their cancer diagnoses and to calculate their risk of cancer. One patient had three cancer diagnoses, first a male breast cancer followed by prostate cancer and then another breast cancer.

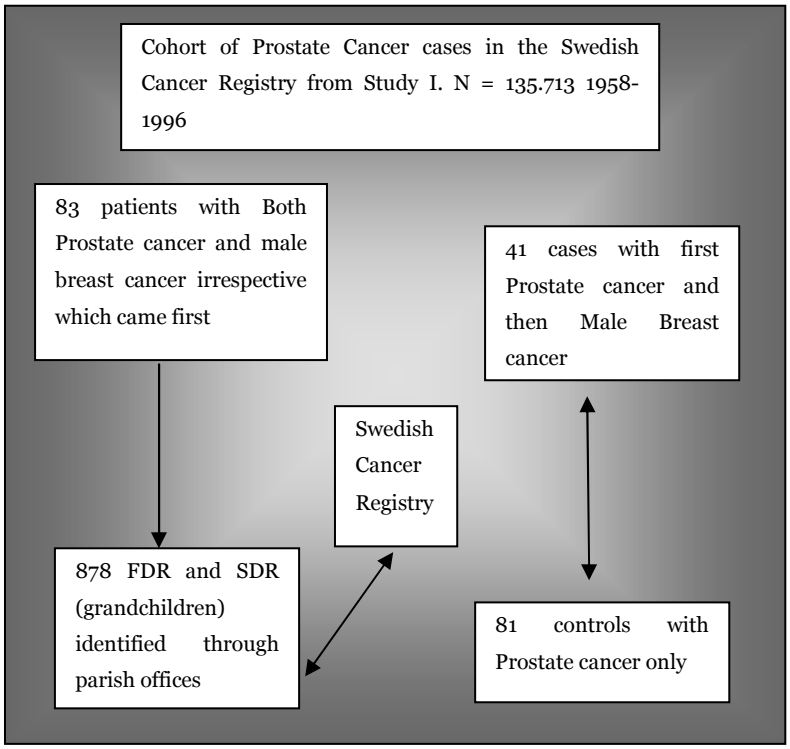

Figure 4. Flowchart over patients and relatives included in study I and II.

Paper III

The CAPS study (Cancer Prostate in Sweden), were used for the association analysis. This is a large-scale case control study consisting of incident prostate cancer cases from 4/6 regions in Sweden and population based controls. Patients included in this study were under the age of 65 and had a newly diagnosed prostate cancer that was verified pathologically or by cytology. Inclusion was done from March 2001 to September 2002. 1,915 prostate cancer patients were eligible and invited to participate, of them 1,470 (75%) agreed to join the study. They were mailed a questionnaire concerning risk factors and family history. Control subjects were randomly selected from the Swedish Population Registry, frequency matched according to gender, the expected age distribution of cases (groups of 5-year interval), and geographical

region (two regions, representing North and South of Sweden including Stockholm). Control subjects were recruited alongside subjects. 1,697 controls were invited to the study and 866 (51%) agreed to participate.

For patients, clinical data regarding stage, PSA, TNM classification, and Gleason sum were obtained through linkage to the National Prostate Cancer Registry. Included patients donated blood for DNA extraction and filled in a questionnaire about lifestyle and heredity. Analysis of genetic variation in the ERβ gene were analyzed in cases (n = 1415) and controls (n = 801). This was the number of cases and controls with available DNA samples at the time of this study.

Paper IV

All pathologic specimens derived from TURP surgery 1982-1997 were identified from the pathology department at University Hospital of Umeå. To the study, only unique individuals were considered so for patients with more than one operation of BPH only the first TURP were included in the study. These men were linked to the regional cancer registry and prostate cancer diagnoses were retrieved. By selection from the cohort, a nested case – control study was constructed. Selected cases developed prostate cancer subsequent to their TURP. They were ≤ 75 years at the time of TURP and had a time-span of at least 6 months between TURP and cancer diagnose. Controls were matched by age at TURP and year of TURP. Controls must also be alive when their matching case got cancer, to allow for same follow-up time. Pathological specimens from 201 cases and 201 matched controls were retrieved from the archives for evaluation of grade and extent of inflammation in relation to prostate cancer risk. From these, a smaller case control study consisting of 50 cases and controls were randomly selected for further study of p53 and the androgen receptor.

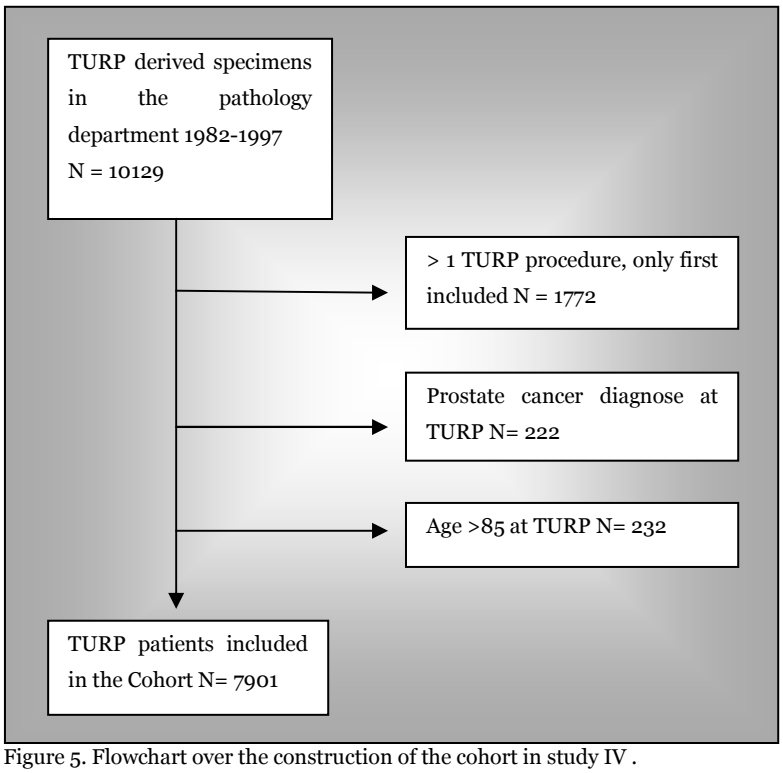

Figure 5. Flowchart over the construction of the cohort in study IV .

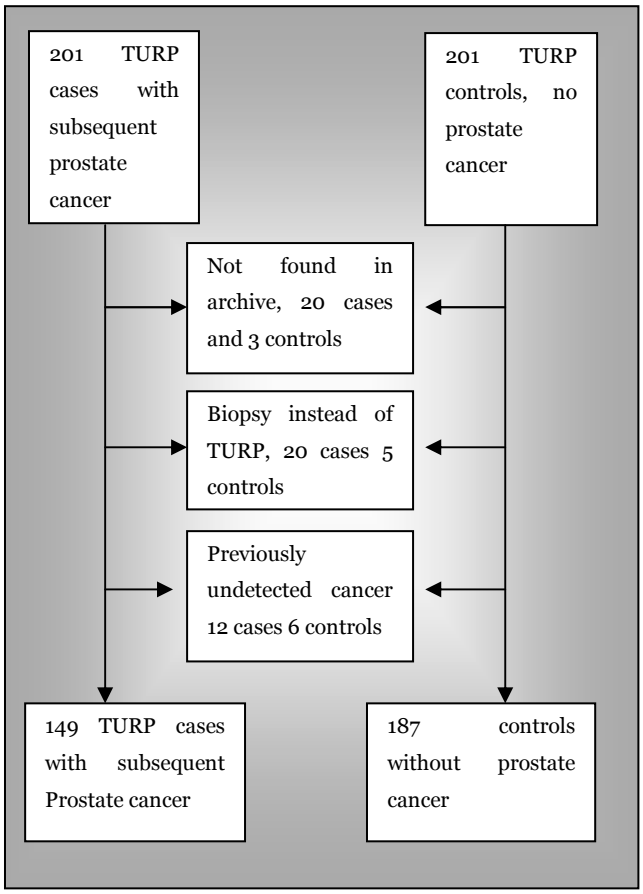

Figure 6. Schematic view of the nested case – control study part in study IV. Primarily included and excluded patients.

Methods

Cohort Analyses

In paper I and IV, person-years at risk in the cohort analyses were calculated from the date of prostate cancer and from the date of TURP until the predestined endpoints. Endpoints in study I were; date of second cancer, death, and loss to follow-up or December 31 1996 and in study IV endpoints were diagnosing of prostate cancer, death, age of 85, or February 12 2008. This was done by using the program PYRS, developed at IARC, Lyon, France. All newborns and immigrants in Sweden are granted a personal identification number used in all official registries, thereby making linkage between different registries feasible. Linking study patients to the Cancer Registry of Sweden, established in 1958, made it possible to retrieve all cancer diagnoses together with information on reporting pathological department and hospital. The observed number of cancer diagnoses was then compared with the expected number in the general population. SIR-values (Standardized Incidence Ratio) are ratios of risk and calculated by dividing observed no. of cancer by expected. Byars' formula was applied for calculating 95% Confidence intervals (CI) [176]. The family-cohort in paper II were analyzed in a similar manner with linkage to the Cancer Registry, the only difference was that the program S-PLUS® was used to calculate person-years at risk.
SMR (Standardized mortality ratio) used in paper IV is calculated in the same way as SIR by calculating person years and dividing observed prostate cancer deaths with expected.
Mortality rates for the population were obtained through the causes of death registry of northern Sweden and this was the base for expected number of prostate cancer deaths.

Medical Records Analysis

To understand if treatment administered for prostate cancer differed between those who later developed breast cancer and those who did not, medical records were collected from hospitals were diagnoses were made. Access to medical records was possible in the majority of patients, for cases, 38/41 (92.7%) records were available, corresponding number for controls were 69/81 (85.2%). Data was extracted in a systematical way with special emphasis on TNM stage, grade and treatment administered, including treatment

duration. Chi-square analysis was used to evaluate differences between cases and controls.

Association Study, SNP and Genotyping

SNPs in the ERβ gene were selected from publicly available databases, SNPer http://snpper.chip.org/bio/snpper-enter and NCBI dbSNP http://www.ncbi.nlm.nih.gov/sites/entrez . Selection criteria was minor allele frequency of ≥5% and an even spread all over the gene including promoter region, introns, exons and 3'UTR. As a mean, the distance between SNPs was 1800bp. In all, 37 SNPs were selected initially and genotyped in 94 randomly selected control subjects. For genotyping, a 5' nuclease TaqMan assay was used together with probes designed by using the Assay-on-demand and Assay-by design service from Applied Biosystems, Foster City, CA. For haplotype estimation, 22 SNPs were used since five were monomorphic in the control subjects and the assay did not work for 10 SNPs. Haplotype estimation was done by using the PHASE software (http://www.stats.ox.ac.uk/mathgen/software.html), a software that implements a Markov Chain Monte Carlo approach. 99.6% of the haplotype variation in the control subjects was captured by using four SNPs, rs2987983, rs1887994, rs1256040 and rs1256062. Selection of these SNPs as haplotype tagging SNPS were done with the htSNP2 package for the STATA software (http://www.gene.cimr.cam.ac.uk/clayton/software/stata. Genotyping of all four htSNPs were done in the same way as for the initial 37 SNPs. Position of htSNPs and LD blocks of ERβ are shown in figure 7.

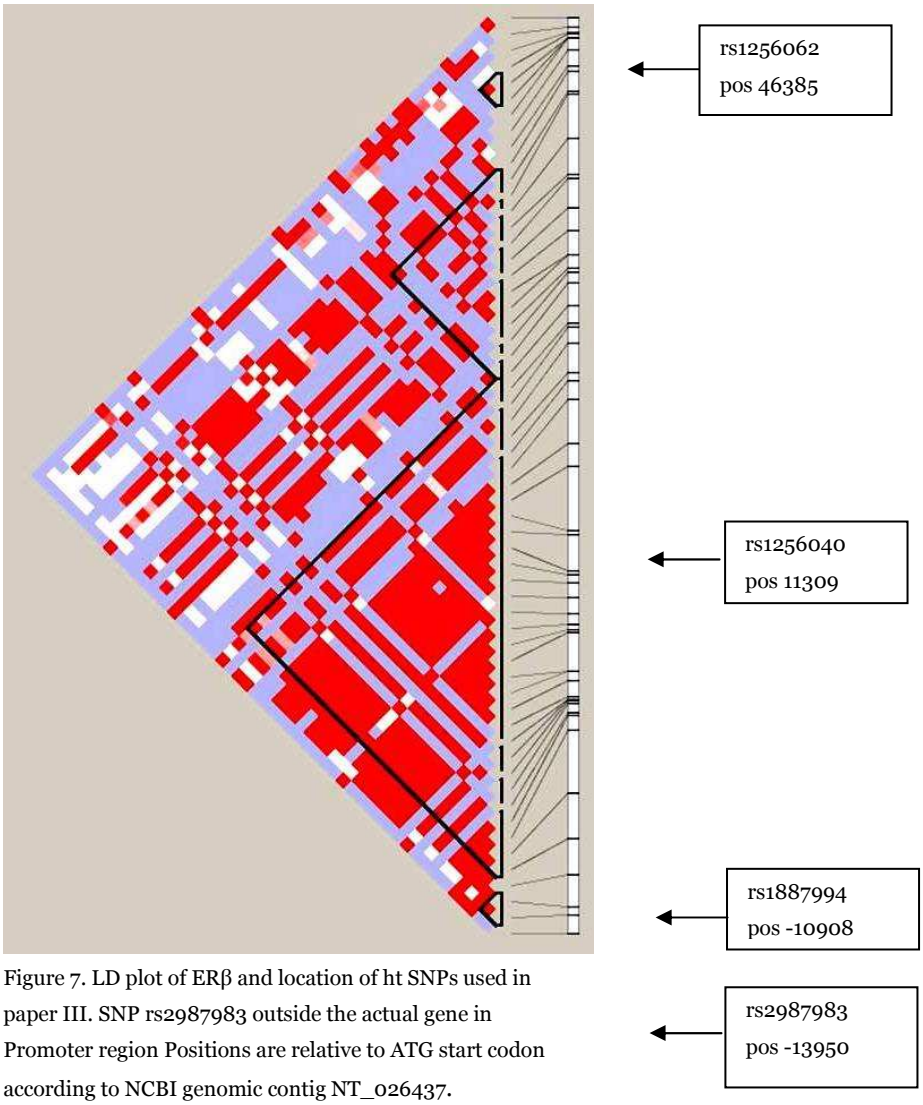

Figure 7. LD plot of ERβ and location of ht SNPs used in paper III. SNP rs2987983 outside the actual gene in Promoter region Positions are relative to ATG start codon according to NCBI genomic contig NT_026437.

Testing each sequence variant for Hardy-Weinberg Equilibrium and pair-wise LD was performed by using a replication method and for each test, 10,000 permutations were done.

Associations of genotypes to prostate cancer risk were estimated in an unconditional logistic regression model assuming dominant inheritance. Odds-ratios with 95% CI to estimate genotype specific risks were also done by unconditional logistic regression.
Testing for specific haplotypes and the associated risk of prostate cancer were done by using the HAPLO.STAT program (http://www.mayo.edu/hsr/Sfunc.html).

Immunohistochemistry and Microscopic Analysis

Original sliced and mounted TURP specimens from 201 cases and 201 controls were evaluated for inflammation by using a two-grade scale. Extent and grade of inflammation were classified as either severe or mild-moderate. Definition of severe inflammation was infiltration of inflammatory cells covering one third or more of the specimen with either diffuse or multifocal pattern of inflammatory cells. Inflammatory cells should be present in confluent sheets in at least three large different tissue chips or covering one-third of the slide. Destructive inflammatory patterns with disruption of the epithelium due to acute inflammation fell into the severe category. The definition of mild to moderate inflammation was no areas of confluent sheets of cells or very small ones, no tissue destruction and less than a third of the area covered. Our definition of severe corresponds to the consensus classification by Nickel et al [177] of moderate and severe grade and to multifocal and diffuse extent. Our classification of mild to moderate relates to all other patterns defined by Nickel. Specimens were also evaluated for presence of cancer that possibly was missed during routine evaluation. To see what kind of inflammatory cells that were present, immunohistochemistry for CD3, CD20 and CD68 was performed. Types of cells were evaluated in both cases and controls.
To investigate in the smaller case – control study (50 cases and 50 controls), if there was an increased risk of subsequent prostate cancer associated with changes in expression of regulating genes and extent or grade of inflammation, immunohistochemistry for p53, AR, was performed. AR was analyzed as normal expression in the nucleus or loss/weakened expression. The definition of weakened expression was half or less the intensity of normal staining adjacent glands. Expression in each specimen was counted in 30-40 cells in 10-15 visual fields throughout the specimen in x 400 magnification. The total number of cells with loss or weakened expression was then divided with total number of cells counted, to create a percentage. AR expression was then turned into a continuous variable.

For evaluation of p53, 100 glands in every specimen were evaluated and all glands with a p53 positive cell were considered positive, creating a percentage. To grade the extent and strength of p53 staining, we created an index by multiplying number of p53 positive glands with percentage of positive cells in each gland and staining strength of p53. Staining strength was for weak (1) or strong (2) in each gland. Analyzing differences in inflammation statistically was done by using Fishers exact T-test for the two groups. AR and p53 as being continuous variables were analyzed by the non-parametric Mann-Whitney test.

Antibody	Company	Code	City, Country	Dilution
p53	Oncogene	Ab-6	San Diego, US	1:200
AR	BioCare		Concord, US	1:50
CD3	NovoCastra	NCL-CD3-565	UK	1:100
CD20	Ventana		Tucson, US	Ready-to-use
CD68	Dako	MO814	Stockholm, SWE	1:2000

Table 1. Antibodies used in paper IV.

Results

Paper I

In the cohort of 135,713 men, we observed 10,526 second primary cancers. The expected number was 8,984, resulting in a modest increased risk, SIR 1.17 95% CI (1.15-1.19), of second primary cancers after a prostate cancer diagnose. In the early period, up to six months after prostate cancer diagnose, almost a third of all second primary cancers were found n = 3,006. Exclusion of this period of follow-up instead revealed a decreased risk of being diagnosed with a second primary cancer, SIR 0.93 95% CI (0.91-0.95). For the two youngest cohorts in this study, below 60 and 60-64 years of age, the risk was not significantly decreased after exclusion of the first six months. Several diagnoses showed increased risk after prostate cancer in the whole period but only a few continued to demonstrate elevated risk after the first six months were removed. Increased risk was found for small intestine cancer SIR 1.39 95% CI (1.09-1.17), malignant melanoma SIR

1.33 95% CI (1.16-1.51), endocrine tumors SIR 1.41 95% CI (1.13-1.74) and for second primary male breast cancer SIR 1.95 95% CI (1.36-2.71). See table 2.

Cancer type	0-5 months	6- months	All cases
	SIR 95% CI	SIR 95% CI	SIR 95% CI
Male breast cancer	2.63 (0.85-6.14)	1.95 (1.36-2.71)	2.01 (1.44-2.74)
Small intestine tumors	9.76 (7.35-12.71)	1.39 (1.09-1.75)	2.20 (1.83-2.61)
Malignant melanoma	1.32 (0.85-1.97)	1.33 (1.16-1.51)	1.33 (1.17-1.50)
Endocrine tumors	5.37 (3.80-7.37)	1.41 (1.13-1.74)	1.81 (1.51-2.16)

Table 2. Tumor types with continuously elevated risks after exclusion of the first six months.

Endocrine tumors consist of parathyroid adenomas, endocrine pancreas, pituitary, adrenal gland and thymus with approximately half being parathyroid adenomas.
Malignant melanoma risk was elevated at the same level both in the early period up to six months and in the longer follow-up until ten years where the risk diminished.
Small intestine tumors had a very high risk in the early period after prostate cancer diagnose with SIR 9.76 95% CI (7.35-12.71) and then the risk dropped but continued to be significantly elevated. Male breast cancer had the highest increased risk among the cancers remaining after removal of the early time-period with an almost doubled risk. Depending on age at the time of the first tumor risk for male breast cancer were highest among young patients, below the ageof 60, SIR 5.29 95% CI (1.06-15.47), but numbers were small in each age group. Tumor types with high increased risk after prostate cancer diagnose that did not stay elevated was bladder cancer and kidney cancer. For young patients in the period after six months, there was an increased risk for bladder cancer, SIR 1.77 95% CI (1.30-2.36) that was not correspondingly present for kidney cancer. Smoke related cancer such as lung cancer and head & neck cancers displayed lower

numbers compared to expected numbers in the general population and thus decreased risk estimates.

Paper II

In paper II, we thoroughly evaluated the increased risk of second primary male breast cancer found in paper I. Our hypothesis that the excess risk could be due to the treatment administered proved to be right. In the nested case – control study significantly more cases than controls had been treated with estrogen (p = 0.03), corresponding to an Odds ratio of 3.91 95% CI (1.14-13.50). Among patients treated with estrogen, different estrogens were used, most prevalent was polyestradiol phosphate given as intramuscular injection. 85% of cases and 78% of controls had this treatment; the remaining had oral preparations, ethinyl estradiol or estramustine phosphate. Only one patient (control) in the actively treated group had received a LHRH-analog. Equally rare, (one case) was treatment with antiandrogen. Cases and controls did not differ regarding tumor grade or stage. For the case treated with antiandrogen, a note in the medical record indicates that the breast cancer possibly was present at the start of treatment. The vast majority had local, non-metastatic disease, 85.4% of cases and 79.0% of controls. Radiotherapy of mammary glands as a way to relieve swelling and tenderness of the breasts was uncommon, in spite of the frequent use of estrogen in these men. This treatment was used in four cases (11.1%) and three controls (4.5%), the difference was not significant. Median duration of estrogen treatment was 22.4 months in cases and 17.2 months in controls (p = 0.10). A majority of the breast cancers (29/41 = 71%) were diagnosed before 1985.

Of the 83 probands with both prostate and breast cancer, we could locate family data from the parish offices for 77 of them. Of the 878 relatives identified, 196 died before 1958 when the Swedish cancer registry was established and therefore not possible to include in the study. The unique personal registration number assigned to every citizen was missing in 90 relatives, which excluded them from analysis because it made the Cancer registry linkage impossible.

Linking and retrieval of cancer diagnoses was done for the remaining 592 relatives. Among them there were 18 prostate cancer diagnoses, a significant increased risk, SIR 2.14 95% CI (1.09-3.18). In total, 91 cancers were observed, and 73.7 were expected, SIR 1.23 95% CI (0.92-1.55). The second most common cancer was female breast cancer, 17

women had breast cancer, thereof six cases under the age 50. There was also a brother to a proband with breast cancer. No other individual site than prostate cancer had significant elevated risk.

Two individual families fulfilled familial/hereditary prostate cancer criteria with prostate cancer in two or more first-degree relatives (FDR). Two families had inheritance patterns suggestive of BRCA2 mutations. Analyzing these families with the BRCAPRO program http://astor.som.jhmi.edu/BayesMendel/brcapro.html revealed 100% and 49% probability of BRCA2 mutations. Patients or relatives have not been mutation screened.

Paper III

One of the ERβ SNPs analyzed, rs2987983, was associated with a small but significant increased risk for prostate cancer OR 1.23 95% CI. The genotype frequency of the C-allele was 27% in cases and 24% in controls, ($p = 0.03$). Corresponding frequency for TC or CC was 47.6% and 42.2%. The difference was largest between heterozygous, TC for cases 40.6%, controls 36.0% and CC for cases 7.0%, whereas controls had a frequency of 6.2% for CC. The other tested htSNPs did not differ significantly among cases and controls. All SNPs tested were in Hardy-Weinberg equilibrium among both cases and controls. Pair-wise LD between the htSNPs with the longest distance between them, rs2987983 and rs1266062 was 0.05. LD for the other combinations ranged 0.61-0.99. We also performed subgroup analyzes, localized vs. advanced and found an increased odds ratio, 1.35 95% CI (1.09-1.68) for being diagnosed with localized cancer. Other subgroup analyzes, age and family history, did not show any variation between cases and controls. Basing haplotype analysis on the four htSNPs, we found seven haplotypes with a frequency of ≥ 1%. None of the haplotypes associated with prostate cancer risk, the global test gave a p-value of 0.1.

Paper IV

We found an increased risk of prostate following TURP, SIR 1.26 95% CI (1.17-1.35), an increase that was pronounced in first six month of follow-up. After the first period, the risk was reduced but in the long follow-up, there was a 39% increased risk again. Although there was increased risk for all patients independent of age, the youngest cohort, age < 60, displayed the highest risk, SIR 1.78 955 CI (1.45 - 2.19). In contrast to the increased risk, mortality rates were decreased, the cohort experienced a 41% lower

than expected risk of dying in prostate cancer, SMR 0.59 95% CI (0.47 - 0.73). The median follow-up time in the cohort is 9.4 years, with 700 prostate cancer diagnoses observed compared to 557.5 expected. The TURP was performed in men aged 39.9 – 85 years where the median age was 70.1 years. 379 specimens out of 402 in the nested case – control study were possible to locate in the archive. Re-evaluating the specimens revealed 18 cancers missed in the first diagnostic examination, twelve of them were cases, six controls. Type of specimen, biopsies instead of TURP was the case in 25 specimens, thereof 20 cases and 5 controls, a significant difference (p = 0,001). These 43 specimens were excluded from further analysis. Evaluation of grade and extent of the inflammation revealed no difference between cases and controls, 48.3% of cases and 49.7% of controls had the severe inflammation classification (p = 0.83). Inflammation was present in all samples to some extent. Evaluation of loss or weakened AR expression showed that the staining was reduced in 20.2% ± 11.0% SD of cases and 18.5% ± 14.0% SD of controls. AR expression showed greater reduction in specimens with severe inflammation, although no difference was visualized regarding case - control status. P53 staining was limited to the basal layer in prostate glands and present in 14.4% ± 10.0% SD of cases and 12.9% ± 11.0% SD of controls (p = 0.52). Expression of p53 was not affected by inflammation nor did the age of the specimen affect the staining. All evaluations were done on the first TURP specimen a patent had, however, of the 402 primarily included, 62 (15.4%) had undergone two TURP and 15 (3.7%) had three or more TURP. No difference in number of TURPs between cases or control could be detected.

Discussion

Paper I

In this study, detection of a small overall increased risk of second primary cancers following prostate cancer was not expected, since at least four earlier, albeit smaller, studies did not show increased risk. In contrast, they showed decreased risk [56-59]. However, excluding cancers diagnosed in the first six months, allowing for diagnostic work-up and ruling out autopsy cases altered the results and made ours more in line with previous studies. Some site specific increased risks remained, although lower, in spite of

this modification. For example, there were increased risks for small intestine and endocrine tumors.

The younger a patient was at the time of prostate cancer diagnose the higher the risk of a second primary cancer was. This is probably a reflection of surveillance bias, young age of a patient leads to intensified examination. Young age at prostate cancer diagnose is associated with increased risk for second primary tumors also in the two other studies were age as a factor is studied [59, 178].

Another example where surveillance bias can be the explanation of increased risk is malignant melanoma, extra visits gives extra opportunities to discover skin tumors. This is somewhat contradicted by studies where prostate cancer is increased after malignant melanoma [179, 180]. The study by Tuohimaa et al [180] shows slightly higher risk of prostate cancer after melanoma in less sunny countries (Nordic countries and Canada) in comparison to sunny countries (Spain, Australia, and Singapore). The proposed explanation is a protective effect of D-vitamin that is produced in the skin; production is higher in countries with more sun.

The increased risk for male breast cancer following prostate cancer is reported only once before, in a study by Pawlish et al [60]. They included 9,794 men with prostate cancer and followed them for up to twenty years. Like us, they used a time limit for inclusion of second primary tumors and their calculations were done solely on tumors detected after one year from the index cancer. Their reported increased risk was not significantly elevated for male breast cancer and that can most likely be attributed to a much smaller sample size. One hallmark of second primary cancers with shared etiology is that they are present in both ways; this is the case for male breast cancer and prostate cancer. Studies have been done where male breast cancer acts as the index cancer [181, 182]. Pooling data from 13 cancer registries, allowed Hemminki et al to gather as many as 3,409 male breast cancers. Among these men, they discovered 426 second primaries. Increased risk was found for prostate cancer SIR 1.61 95% CI (1.34-1.93), small intestine SIR 4.95, 95% CI (1.35-12.7) and pancreas SIR1.93, 95% CI (1.14-3.05). The overall increased risk for prostate cancer was present in the first 9-year follow-up after male breast cancer and later ceased to be significantly elevated. They also studied male breast cancer as a second primary following both prostate cancer and other cancers and found increased risk for male breast cancer the first nine years but no overall increased risk, SIR 1.19 95% CI (0.90-1.54) after prostate cancer. This was based on 56 male breast cancer cases where cases from the Swedish cancer registry were included. The period of both these studies is

approximately the same and we found 40 cases of second primary male breast cancer, making the contribution of second male breast cancer from the Swedish registry substantial. Even if cases (n =5) diagnosed in the first six month in our study is excluded most of the risk can be attributed to the Swedish cases. This raises questions; can it depend on differences in the treatment of prostate cancer? The population in Sweden is homogenous, mostly Caucasian, and mutations in the BRCA2 gene can perhaps explain some of the excess risk in our study. Supportive of this explanation is reports on elevated prostate cancer risks in BRCA2 mutation carriers [91, 183]. The registries participating in the Hemminki [181]study apply different rules to characterize a tumor as a second primary but in the study a common set of rules were applied to overcome this concern so registration issues cannot be the reason for differences. The other large study of male breast cancer as the index cancer did not find elevated risks for prostate cancer nor did they see any excess male breast cancers after prostate cancer [182]. They used the SEER registries in California where the population is very mixed and that can perhaps explain part of the difference of the results. Breast tissue does in fact express PSA [184-186] and that can lead to difficulties in distinguishing between metastatic and primary cancers. In our study, the risk was higher in the years 1958-1980 where PSA was not available, so diagnostic mistakes cannot be ascribed to PSA immunohistochemistry. Furthermore, the Swedish cancer registry has been found to be very reliable regarding the reporting of second primary cancers [187] and therefore we think this excess risk is not a chance finding. Treatment provided for the first cancer can induce secondary malignancies. Common treatments for prostate cancer include hormonal manipulations, either with LHRH analogues or with estrogen. A well-recognized risk factor for male breast cancer is estrogen excess due to various factors [83, 84, 188]. Moreover, radiation to the mammary gland to prevent gynecomastia as a side effect of hormonal treatment may induce second primary tumors as radiation is associated with higher risk for breast cancer [86]. Problems associated with this study are the lack of treatment data and stage of the diseases. Information on treatment given would be most interesting since these known risk factors for male breast cancer then could be evaluated. Another finding in our study was the increased risk of a small intestine cancer as a second primary. This is supported by results from other studies where small intestine cancers acted as the index cancer [61-63] followed by prostate cancer. In the international cooperative study by Scélo et al [63] as well as the SEER study by Neugut et al[62], the excess risk was present both ways, further supporting an association between the cancer types. It also seems that

the carcinoid subtype stands for the major part of the association. This tumor is poorly understood regarding etiology and possibly can the common occurrence with prostate cancer shed some light. A possible connection could be the Insulin-like growth factor -1, where elevated circulating levels have been associated with prostate cancer risk [189] and IGF-1 also is important in promotion of carcinoid tumors [190]. Studies based on registries have both advantages and drawbacks and so is the case in our study as well. Strength in our study is the considerable size with 135,713 cases generating person-years at risk. This makes it possible to evaluate the risk of rare diseases such as male breast cancer and yet there are only forty cases in total. If we would include cases from more recent years where incidence of prostate cancer have risen, we will add many cases but not so many person-years due to short follow-up and possibly not gain many more male breast cancer cases. Population based cohort studies gives estimations of risk that are applicable to the population studied. By using the cohort design, we circumvent the problem of recall bias that is common in case – control studies making the estimations hopefully more accurate. In a study this large, where many associations are tested there is always the concern of multiple testing that can produce false positive associations. We think this problem is somewhat overcome when our major findings have been reported form other groups as well. It should be emphasized that studies of this kind are to be regarded as generators of hypotheses. In conclusion, we demonstrated in this study an increased risk of male breast cancer following prostate cancer, an increased risk we hypothesize to depend on treatment given or the possibility of a BRCA2 mutation in the patients.

Paper II

Before the publication of the present study, there have been only case-reports in the literature about male breast cancer following prostate cancer [85, 103, 191, 192]. In female breast cancer, hormone-replacement therapy in the menopause is a well-accepted risk factor for breast cancer; therefore, the result of this study pointing towards an increased risk of male breast cancer following estrogen therapy is not so surprising. Further support is gained from epidemiological studies on male breast cancer where increased risk is associated to relative estrogenic predominance. Factors associated with increased estrogen/androgen ratio are obesity, liver cirrhosis, testicular disorders such as undescended testes, mumps orchitis and Klinefelter syndrome [81-84, 188]. In our study,

these conditions are unlikely to be responsible for the excess male breast cancer risk as all men also have prostate cancer. At least Klinefelters syndrome is shown to be associated with a lower risk of prostate cancer [81]. Following antiandrogen treatment for prostate cancer there is at least two case reports of male breast cancer. In the first from Karamanakos et al [193], the patient had been on flutamide treatment for more than seven years. In addition, this patient was found to have a mutation in the BRCA1 gene. The second report, from Chianakwalam et al [194] described a case where treatment with bicalutamide had been ongoing for one year. The case in our study that was treated with antiandrogen had been on cyproteronacetat for two years before the breast cancer. The majority of patients in our study were diagnosed with breast cancer before 1985 (71%) where available treatment options were orchiectomy or estrogen. LHRH-analogs came in use in the mid-eighties and modern antiandrogens, flutamide and bicalutamide, were introduced ten years later. Therefore, evaluating antiandrogen treatment in regard of breast cancer risk might be too early, even though many thousands of patients have been treated. This is especially important to have in mind, since radiotherapy as a way to prevent or ease gynecomastia and pain induced by antiandrogens is widely used nowadays.

In a review by Dicker [195], the conclusion is that data are sparse regarding long-term safety. However, female breast cancer risk increases due to low-dose irradiation of various benign conditions where breast tissue is located in the radiotherapy field. This makes it important to be cautious about these treatments, especially when using a combination treatment of both radiotherapy and estrogen treatment. Radiation induced cancers have a long latency period and many prostate cancer patients will not live long enough to experience this risk but since antiandrogen are used earlier in the disease course it might become an issue. Data from our study could not confirm radiotherapy as a risk factor for male breast cancer. Numbers were too small to get enough power either to confirm or to reject the hypothesis.

In the family cohort study, increased risk of prostate cancer among the relatives can be regarded as a quality control since family history is one of the few verified risk factors for prostate cancer. The risk estimate, SIR 2.14 is in line with results from a meta-analysis where pooled risk ratio for a FDR was 2.5 (2.2-2.8) [13]. Previous studies of familial prostate cancer have shown partly divergent results regarding cancer risk among relatives. A French study by Valeri et al [89] examined the risk of breast cancer in FDR to prostate cancer cases. By comparing families including two or more prostate cancers with

families with only one case, they showed significant increased risk for breast cancer among female relatives in the families with many cases. Contradictory is a Swedish study where prostate cancer seems to be site-specific with no association of other cancer sites [196]. In our family study, there was a little more breast cancer than expected, 17 female breast cancers vs. 11 expected but the difference was not significant. Likewise, other sites like bladder and rectum had more than expected cases although not significant. There could be two explanations to this, one being that the number of relatives is quite small, allowing only detection of two-fold increased risk estimates. Therefore, there could still be a difference too small for our study to detect. Alternatively, both bladder and rectal cancer are common diseases and the result is due to chance and multiple testing.

In two of our families, the inheritance patterns together with the types of cancers suggest BRCA2 mutations. BRCA2 mutation carriers experience increased risk of prostate cancer [91, 183, 197] but screening of early-onset prostate cancer does not identify many mutation carriers [198]. Only two families in our study had high probability of mutations in BRCA2 as estimated by the BRCAPRO program. Conclusions to be drawn from this part of the study are that a small fraction of prostate cancer can be attributed to mutations in that gene. Nevertheless, it is important to identify prostate cancer cases with a mutation since two recent studies have shown worse outcome for carriers vs. non-carriers [97, 98].

Strength in this study is that retrieving medical records and systematically go through them to collect data gives good information on tumor stage, grade and treatment administered. A problem is that in old records, classifications may be different and translations into new systems must be done, which creates an uncertainty. Case – control studies gives good control over exposure and by collecting data directly from medical records, we avoided recall bias. This was a necessity anyway since most of both cases and controls were deceased.

Limitations connected to this approach are loss of follow-up, records might be too old still to be kept or they could not be found. This of course can create a bias if availability of records is unbalanced between cases and control but we saw no evidence for that in our study.

The Swedish personal registration number allows for good follow-up and makes linking to registries possible. This was missing in ninety relatives corresponding to about ten percent of relatives, making linkage impossible and can be of importance since numbers were not big.

The paternity question is always an issue in family studies and some of the offspring will be wrongly included in this study. Overcoming this problem needs genetic testing, but the problem is unlikely to be large.

In conclusion, this study reports of estrogen treatment as a possible cause of the increased risk for male breast cancer following prostate cancer. A small part of the cases can be found in families attributable to BRCA2 mutations. This finding is important for the future as a possible renaissance of estrogen in the treatment of prostate cancer is coming. A search at www.clinicaltrials.gov shows several studies ongoing treating prostate cancer. In spite of this, the doubled risk found and possibly related to estrogen treatment is small in absolute numbers and shall be taken in account when other possible benefits from the treatment is evaluated.

Paper III

This study was the first to report an ERβ association to prostate cancer, based on 1415 cases and 801 controls in Sweden. The study shows an association of a promoter SNP with the risk of prostate cancer. The risk inferred was not high, an overall odds ratio of 1.22 95% CI (1.02-1.46). This SNP, named rs2987983 (T/C), where C is the ancestral allele, is located in the upstream region, -13950 from the ATG where it is positioned between the two untranslated exons 0N and 0K. Recently two other studies, one from the National Cancer Institute Breast and Prostate Cancer Cohort Consortium [199] and the other from NCI's Cancer Genetic Markers of Susceptibility (CGEMS) [43] have analyzed the ERβ, considering prostate cancer risk. The first study, comprising 8.323 prostate cancer cases and 9.414 controls pooled from seven cohorts could not replicate the findings from the Swedish study. Due to genotyping failure of the rs2987983 SNP, they instead used rs3020450, a closely located SNP in complete LD. This SNP did not associate with prostate cancer risk, a finding also reported from the CGEMS project [43] examining the same SNP. Other ERβ SNPs that are examined in the pooled cohort study have not been significant; either analyzed separately or combined forming haplotypes [199] except for a small increased risk that was present for carriers of one haplotype in the recessive setting; both alleles had the risk haplotype TACC. However, this increased risk did not remain significant after correction for multiple testing. In the CGEMS study, two different SNPs did reach significance, thereof one, rs10137185, located upstream of rs2987983 [43]. In the follow-up on these SNPs, none of them remained significant,

http://caIntegrator.nci.nih.gov/cgems/ . There is also a study from Cunningham et al [200] aiming at investigating the androgen and estrogen metabolic pathways where they included one SNP in the ERβ. This SNP did not seem to influence the risk of prostate cancer; however, the sample sizes of both sporadic and familial prostate cancer cases were small.

Our selection of gene to test was based on a prior probability hypothesis, the documented importance of ERβ in the regulation of prostate proliferation. The result of the study with relatively weak association to prostate cancer risk can depend on several things. One explanation is of course that alterations of ERβ do not contribute to prostate cancer risk. Another is that epigenetic changes may vary between cases and controls, a variation not measured in our study. Promoter region methylation of ERβ is a common event in prostate tumor tissue [130, 131], leading to silencing of the gene. Methylation is more common in prostate cancer compared with normal prostate tissue [120, 130, 131]. A third explanation is that genes in the ERβ pathway are altered and indirect lead to changes. A proposed ligand to ERβ shown to have higher affinity than estradiol is 5α-androstane 3β,17β Diol. This is the breakdown product of DHT, the conversion is catalyzed by 3β-hydroxysteroid dehydrogenase (HSD3B1 and 2). The gene coding for HSD3B 2 contains an intronic repeat polymorphism and there are some SNPs in both HSD3B 1 and 2. Previous studies on these genes have shown weak associations with prostate cancer risk [201-203] but consistent replication have not been achieved [200]. A common problem in association studies, lack of replications [204] can depend on several things: First and most common are false positive results in the initial study, which is a regular problem with multiple testing. Following this, the level where p-values are considered significant should be more stringent. Together with false positive studies comes also the dilemma of publication bias, positive results tend to be published more often and the public will never know negative studies. Second, study population selection and ascertainment of cases vs. controls might not be adequate, thereby creating a possibility for admixture and distorted allele frequencies. Thirdly, there are often too small sample sizes, lacking power to detect differences or to negate differences [39]. Taking these things in consideration when trying to replicate a previous finding or evaluate a replication study will be most helpful. More so, a concise description of the methods used in the study will aid others in the pursuit of SNPs [40].

In conclusion, of our study, we found an association of risk for prostate cancer with a promoter SNP. An association strengthened in the sub analysis of localized vs. advanced

disease. However, in the light of the advances in the field of genetic epidemiology where genome wide SNP scans are frequent, compared to the time when we designed our study when the candidate gene approach was common, together with the results from more recent studies on ERβ, genetic variation in this gene does not seem to contribute to prostate cancer risk.

Paper IV

There might be several explanations to the increased risk for prostate cancer after TURP that we found in this study. The risk was present both immediately after TURP and in the long-term follow-up. However, the period between six months up to five years showed decreased risk, indicating a possible surveillance bias in the early period up to six months. Patients with lower urinary tract symptoms (LUTS) are more likely to seek medical care and therefore more prostate cancer is found in this group. If for example, the first TURP did not relieve symptoms, another surgical procedure was done resulting in more tissue to examine and possibly more cancer found. Supportive of this theory is the fact that 1772 (19.9%) of the patients underwent more than one TURP. On the other hand, we excluded prevalent prostate cancer patients from the study and also those who was diagnosed at the first TURP. By doing this, we possibly introduce a selection bias in the opposite direction towards lower cancer risk in the cohort, since the same cannot be done for the population. Comparing the cohort to the population revealed an 39% excess prostate cancer risk after ten years. Explanations of this could be that part of the follow-up period takes place in the era where PSA was introduced in Sweden. More and more prostate cancers that used to go undetected throughout life are found before death and the introduction of PSA testing is proposed as the reason [205]. PSA detected cancers of possibly non-significant clinical value can explain the decreased mortality found in the cohort. Decreased mortality is also reported in another large cohort study where the authors used the Swedish inpatient register to create a cohort. All patient with a discharge diagnose of BPH 1964-1984 were selected [153]. They separated patients into three different groups, non-surgically treated, TURP and transvesical adenomectomy, TA. Unlike us their follow-up period did not include the PSA introduction. For surgically treated patients the decreased mortality was 17-23% after five years. They also found a small increased risk of prostate cancer in the TURP group. They hypothesize that removal of prostate tissue in the surgical procedure is one reason for the lower mortality

in this group and supports that with less increase in risk in the later study period where the TURP procedure was more developed and more tissue was removed. In our study most of the patients are included during this period of developed tecniques and we still find an increased risk. This could possibly be due to that most of the tissue resected at TURP comes from the transition zone and not from the peripheral zone, where the majority of prostate cancers arise. Risk reduction due to extensive tissue removal then seems less likely. Other investigations have not found significantly increased risks of prostate cancer associated with BPH [154, 155, 157] with one exception[152]. The divergent study were a small hospital based study where not all patients included had a TURP in the beginning. Lacking pathological verification of a benign status in the beginning makes it difficult to exclude prevalent cases. These cases might later turn up as clinical patient and thereby inflate the risk. One of these studies did create a nested case – control study within their cohort to evlauate different pathological features such as dysplasia or atypi [154]. They did not see any significant differences. In our nested case – control study, examining if grade and extent of inflammation at the time of TURP was associated with later development of prostate cancer, we did not see any such associations. Supporting our finding are study results from Rotterdam, within the European Randomized Study of Screening for Prostate Cancer (ERSPC) this group examined pathologic features in biopsies taken within the screening program [206]. Biopsies that were negative for cancer in the first round were scored for inflammation, HGPIN, biopsy core length and glandular core length. Four years later in the second screening round, 61 cases diagnosed with cancer and 60 random controls that did not have cancer made up the study group. Comparing these groups, there was no evidence that any of these pathological features could predict cancer development. MacLennan et al produce contradictory results as they found chronic inflammation in prostate biopsies to be associated with cancer [207]. Of 177 patients with initial biopsy, there were evidence of chronic inflammation in 144 and of them 29 were diagnosed with cancer. Ten cancers were found in the remaining 33 cases that were devoid of inflammation. In repeat biopsies, cancer had developed in 29/144 (20%) in the inflammation group and 2/33 (6%) in the non-inflammation group. A limitation in this study is that not all were re-biopsied, 77 in the inflammation group and only 7 in the non-inflammation group, making numbers very different, 29/77 = 37% and 2/7 = 21%. Since both these studies evaluated biopsies instead of TURP material, they may not be fully comparable to our study. All studies suffer from the possibility of sampling bias. In our study, there is more

tissue available but mostly from the transition zone whereas in the other two, the biopsies sample a small volume of the peripheral zone. A majority of prostate cancer arises in the peripheral zone and there is evidence that peripheral zone cancer have higher Gleason score and have higher proliferation as measured by Ki-67 compared to transition zone cancers [208].

We found p53 expression to be common in our material, 14.4% of cases and 12.9% of controls had nuclear staining, whereas other studies find sparse p53 staining in BPH [159, 209]. In contrast, reviewing the literature Downing et al [160] found mutations and expression of p53 not to be confined to late stages and high-grade cancers. Stackhouse et al examined diagnostic biopsies for p53 in cancer lesions, patients later underwent prostatectomy[161]. They concluded that p53 expression in biopsies could not predict recurrence, but found p53 staining in the whole prostatatectomy specimen prognostic for relaps. P53 immunohistochemistry have some limitations, where one is the short half-life of the wild-type protein that is normally not detected while mutated p53 leads to stabilization of the protein and a possibility of staining. Not all antibodies stain all kinds of mutated proteins and some mutations lead to absence of expression and consequently no staining. Our finding of frequent expression in the tissue can be explained in at least two ways; one explanation is that we detect high amounts of wild-type protein as a measure of well-functioning cells reacting to cell damage, the other is that our evaluation method is too sensitive, overestimating the expression. However, application of this method to both cases and controls ensure equal evaluation of differences between them.

The AR, regulating growth and proliferation in the prostate and known to be affected by inflammation [147] was an evident candidate in our study. We did not notice any differences in loss or weakened expression among cases and controls, though we did see that severe inflammation reduced the expression compared to areas where the inflammation was graded as mild. A difference in the tissue reaction between cases and controls, as early as in our material where the mean time to prostate cancer after TURP was 7.5 years, might still be present. The reason for our null association of reduced AR expression may need to be sought in a downstream pathway or upstream, before the AR becomes involved. AR is involved in many processes in the cell [169] and is affected by inflammation, this taken together with the strong epidemiological evidence for inflammation as a risk factor for prostate cancer needs further evaluation. Strengths connected to our study are the relatively big size, pathological verification of cancer status and homogeneity of the cohort. By restricting to one region in Sweden, we

minimize differences in treatment of BPH. During the later inclusion period, medical treatment with α-blockers and finasteride was introduced but before active treatment was confined to surgery and surgery consisted mostly of TURP. Therefore, we think, our cohort is representative of BPH-patients. BPH-patient with mild symptoms, not needing treatment will not have surgery and for this reason not be included in this study but big differences would be more likely detected in a group with severe symptoms. Lacking clinical data regarding symptoms, stage and grade of the cancer as well as Gleason score and PSA is a limitation. Those data had been helpful in deciding whether the increased risk of prostate cancer following TURP mainly consisted of low-grade PSA-detected cancers. This is suggestive, since prostate cancer mortality is lower than expected comparing with the population. In conclusion of this study, we have found increased risk of prostate cancer following TURP in a BPH-cohort. This increased risk cannot be explained by differences in inflammation, AR or p53. The same cohort experiences a lower mortality from prostate cancer, possibly due to increased surveillance in the PSA-era.

Conclusions and General Discussion

Contrasting to the fact that prostate cancer is the most common male cancer in the developed world, little is known about risk factors and etiology. For a long time the mantra - age, race and heredity, have been the only well known factors predisposing for the disease.

Studies of second primary cancers show reciprocal associations for various tumors and raises questions about the way they are connected. One possible answer could be that it is genetic predisposition, another that it is the primary treatment and yet another answer, a combination of the two. It is also possible that the host reaction provoked by a combination of intrinsic factors, genetics and environmental exposures can give a clue to the etiology. All this are questions that we have tried to address in this thesis.

Our first study showed an increased risk for male breast cancer and generated a new question of why did we find this increased risk. Our hypothesis was that either treatment or genetic predisposition could explain the risk. Our hypothesis held true, we could show that most of the excess risk probably was due to estrogen treatment of the prostate

cancer. Then again a question, does genetic variation in the estrogen receptor, β in particular, have something to do with risk of prostate cancer. It is certainly involved in regulation of proliferation in the prostate. With the methods available at the time, we could show a weak association of a SNP in the promoter region of the ERβ gene with prostate cancer risk. Later studies have added to the information and now we do not believe that single nucleotide polymorphisms in the ERβ gene are associated with prostate cancer. Another central hypothesis is that inflammation acts as an initiator or propagator of cancer. It is a well-accepted model in other malignancies, and we studied it in prostate cancer. Epidemiological data together with morphological changes called PIA gave clues. In a large cohort, we could see increased prostate cancer risk after a TURP but the most plausible explanation to that was increased surveillance and diagnoses of clinically non-significant cancers. With the methods applied, we could not demonstrate a risk association with inflammation.

For the future, I think a combination of methods to be the best way forward using extensive laboratory and clinical data. A multidisciplinary effort will find prognostic and predictive factors by using the technique that makes genome wide SNP scans possible, in combination with clinically well-characterized large study cohorts. Characterizing newly diagnosed prostate cancer cases genetically, morphologically and with clinical data and to follow up on them would be very interesting. What responses do they get to the treatments administered and do specific subgroups experience other malignancies. A study combining the findings of disease associated SNPs with clinical data such as family history is already done [210] and more will come. Permitting a clinician as myself to dream, I would like to have both predictive and prognostic factors, giving the clinician and the patient guidance what treatment to choose when it is needed and support not to treat when it is not needed.

With this thesis, we have made a small contribution to science by showing an increased risk of male breast cancer following prostate cancer. This increased risk is possibly due to estrogen treatment and some small part can be attributed to mutation in the BRCA2 gene. We have, with added information from later studies found that genetic variation in the ERβ gene does not confer increased risk for prostate cancer. There is a small increased risk of prostate cancer after TURP but prostate cancer related mortality is decreased, this can be explained by increased surveillance and the diagnose of clinically non-significant cancers. Inflammation in the prostate at the time of TURP does not

associate with later development of prostate cancer, nor does changes in the AR or p53 expression associate.

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