Prospective studies of hormonal and life-style related factors and risk of cancer

Sara Wirén
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Västerbotten Intervention project (VIP), androstenediol glucuronide (α-diol-g), sex hormone binding globulin (SHBG), Prostate Cancer database Sweden (PCBaSe), Metabolic Syndrome and Cancer Project (McCan)
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Abstract

**Background:** Androgens are important in prostate cancer development but how circulating levels of androgens affect risk of prostate cancer of different aggressiveness is not clear. Being childless has been associated with a lower risk of prostate cancer, but it is not clear if this association is causal or a result of residual confounding. Fathering of dizygotic twins, a marker of high fertility, has not been studied in relation to risk of prostate cancer.

Another marker of life-long hormonal exposure is height, which has been associated with increased risk of cancer and cancer death. However, the association to separate cancer sites has not been consistent.

The aims of this thesis were to study hormonal factors (paper I), and proxies of hormonal factors (paper II and III), and risk of prostate cancer; as well as height and risk of cancer and cancer death by separate sites (paper IV).

**Methods:** Study designs were i) case-control studies, nested within the Västerbotten Intervention Project (paper I), and in Prostate Cancer database Sweden 2.0 (PCBaSe 2.0) (paper II and III), and ii) cohort study, in the Metabolic Syndrome and Cancer project (Me-Can) (paper IV).

**Results, prostate cancer:** In paper I, increasing levels of serum androgens were not associated with risk of prostate cancer overall or in tumor risk categories. In paper II, childless men had a lower risk of prostate cancer, overall and in all risk categories, compared to fathers, an association which was in part explained by differences in marital status and educational level. In paper III, fathers of dizygotic twins did not have an increased risk of prostate cancer, either overall or in risk categories, when compared to fathers of singletons.

**Results, cancer overall:** In paper IV, height was associated with an increased risk of cancer and cancer death overall in both women and men. The strongest association for cancer was to malignant melanoma in both women and men, and for cancer death to post-menopausal breast cancer in women and renal cell carcinoma in men.

**Conclusions:** These studies indicate that hormonal factors, when studied as serum levels or when studied using proxies of fertility, do not have a major impact on the risk of prostate cancer. The association between height and an increased risk of cancer appears robust for total cancer and cancer death, as well as for several separate cancer sites.
Abbreviations

40-y  Age 40 programme
A-diol-g  Androstanediol-glucuronide
BMI  Body mass index
CI  Confidence interval
CONOR  Cohort of Norway
IGF-1  Insulin like growth factor 1
Me-Can  Metabolic Syndrome and Cancer project
MPP  Malmö Preventive Project
NCS  Norway Counties study
OR  Odds ratio
Oslo  Oslo study 1
PCBaSe  Prostate Cancer database Sweden
PSA  Prostate specific antigen
SD  Standard deviation
SHBG  Sex hormone binding globuline
TNM  Tumor node metastasis classification
VHM&PP  Vorarlberg Health Monitoring and Prevention Programme
VIP  Västerbotten Intervention Project
WHO  World Health Organization
BPH  Benign prostate hyperplasia
PCPT  Prostate Cancer Prevention Trial
REDUCE  Reduction by Dutasteride of Prostate Cancer events
STR  Swedish Twin register
HR  Hazard ratio
ADT  Androgen deprivation therapy
PLCO  The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial
ERSPC  The European Randomized Study of Screening for Prostate Cancer
TUR-P  Transurethral resection of the prostate
LUTS  Lower urinary tract symptoms
IVF  In vitro fertilization
List of papers


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**Populärvetenskaplig sammanfattning på svenska**

**Introduktion, prostatacancer**
Prostatacancer är den vanligaste cancerformen bland svenska män och varje år diagnostiseras nästan 10 000 män med sjukdomen. Prostatacancer är en heterogen sjukdom med både så kallade låg- och högrisktumörer och dessa skiljer sig åt i fråga om behandling och prognos. I korthet betecknar låg-respektive högrisk tumörens sannolikhet för aggressiv tillväxt och spridning.

Låg- och högrisk prostatacancer kan delvis ses som olika sjukdomar och faktorer som påverkar risk för dessa sjukdomar kan skilja sig åt. När man studerar riskfaktorer för prostatacancer är det därför viktigt att veta vilken form av prostatacancer individen diagnostiserats med.


I delarbete II fann vi att män som inte fått barn hade en minskad risk för prostatacancer. Fäder hade generellt en något högre utbildningsnivå och var gifta i högre utsträckning än barnlösa män. Gifta män och män med en hög utbildningsnivå hade i vår studie en ökad risk för prostatacancer, något som kan förklaras av ett högre upptag av PSA test i dessa grupper.

För att vidare studera om det finns ett samband mellan fertilitet och risk för prostatacancer studerade vi risken för prostatacancer hos män som fått dizygota (fleräggs-) tvillingar, vilket är ett mått på ökad fertilitet, i delarbete III. Vi kunde här inte påvisa någon ökad risk för prostatacancer hos män med dizygota tvillingar.

**Längd och risk för cancer**

Hur lång man blir som vuxen påverkas till stor del av ens gener men även av olika hormoners påverkan under uppväxten. Längre individer har i tidigare studier haft en ökad risk för cancer och cancerdöd men det har inte varit klart för vilka cancerformer detta gäller.

Vi studerade sambandet mellan längd och risk för olika cancerformer och död i cancer bland en stor grupp människor från Sverige, Norge och Österrike (delarbete IV). Vi fann här ett samband mellan längd och ökad risk för cancer och cancerdöd bland både kvinnor och män. Sambandet kunde ses för flera olika cancerformer men var starkast för malignt melanom (den allvarligaste formen av hudcancer), bland både män och kvinnor. För cancerdöd var sambandet starkast för bröstcancer som uppkommer efter klimakteriet bland kvinnor, och njurcancer bland män.

Orsaken till sambandet mellan längd och cancer kan bero på genetiska eller hormonella faktorer och detta bör studeras vidare i andra typer av studier.
Slutsatser:

- Naturligt höga nivåer av manliga könshormoner påverkade inte risken att utveckla prostatacancer.

- Barnlösa män hade en lägre risk för alla typer av prostatacancer, både vad gäller låg- och högrisktumörer.

- Män som fått dizygota tvillingar hade liknande risk för prostatcancer som män som fått barn men inte dizygota tvillingar.

- På gruppnivå hade längre kvinnor och män en något ökad risk att diagnostiseras med eller dö av cancer.
Background

Human carcinogenesis

Cancer is a genetic disease that develops through multiple cellular mutations. These gradual mutations render the tumor cells new properties in the form of growth advantage over other cells, increased survival and the possibility to invade surrounding tissues and eventually metastasise [1]. Since solid tumors take decades to develop from the first mutation, there is ample time for influence from surrounding factors on this progression. It is likely that this progression is affected by known risk factors for cancer, even though the exact mechanisms remain to be elucidated.

Cancer epidemiology

In cancer epidemiology, the impact of various exposures’ on the risk of cancer is assessed. A goal of such studies is to identify risk factors, preferably those that are potentially avoidable. Epidemiological studies can also be used to generate hypothesis regarding tumorigenesis, which can subsequently be explored in experimental studies.

Global cancer incidence

Cancer is a global burden, with an estimated 12.7 million new cancer cases in 2008 [2]. The overall cancer incidence shows a steady increase, partly due to decreased mortality in other diseases, which has resulted in more people living to an age where the risk of cancer increases. It is also a reflection of increased obesity and other non-beneficial life-style factors [3].

Worldwide, the most common cancer diagnoses are breast cancer in women and lung cancer in men. There are large differences in incidence of cancer at different sites between countries, partly due to varying frequency of risk factors, as well as major differences in screening procedures and modes of detection of cancer between low and high income countries.

Global cancer mortality

Number of deaths from cancer has also increased and in 2010, 8 million people died of cancer worldwide, an increase of 38% in 20 years [4]. However, the age-standardized cancer death rate, which account for differences in age-distribution of the population over time, has actually decreased by 14% during the same time period. This reduction is largely due
to improved treatments for many cancers; with Hodgkin’s disease, as well as stomach and oesophagus cancer, showing the largest decreases in mortality rates. Survival in different forms of cancer differ largely, indicating the need for improvement of cancer care in many countries [5].

Risk factors for cancer

There are factors that increase risk of many types of cancer such as smoking and overweight [6, 7], whereas other risk factors only affect risk of one or a few cancer forms, such as Human Papillomavirus for mainly cervical cancer but also cancers of the anogenital area and oropharynx [8, 9], and Hepatitis B and C for liver cancer, and non-Hodgkins lymphoma [9, 10], as well as Helicobacter Pylori for gastric cancer [11].

Cancer incidence and mortality in Sweden

The age-standardized cancer incidence has risen in Sweden for both men and women, Figure 1 [12]. This is to a large part due to increased incidence of breast cancer in women and prostate cancer in men.

Figure 1. Age-standardized incidence of all-sites cancer in Sweden between 1960 and 2010, 0-85+ years. Standardized according to World Standard Population. NORDCAN © 2009 [12].
However, the age-standardized mortality in cancer has decreased in both men and women, Figure 2 [12]. This can be explained by increased survival for several common cancers, such as breast and colorectal cancer in women, and lung and colorectal cancer in men.

**Figure 2.** Age-standardized mortality of all-sites cancer in Sweden between 1952 and 2007, 0-85+ years. Standardized according to World Standard Population. NORDCAN © 2009 [12].
Prostate cancer

Adenocarcinoma of the prostate is a common disease in the older male population. The main risk factors for prostate cancer are old age, ethnicity and a family history of prostate cancer [13].

Prostate cancer incidence

Globally, prostate cancer is the second most common cancer in men, accounting for 15% of male cancer cases [2]. The incidence varies greatly, with the highest rates in the high-income countries of Oceania, Europe and North America, Figure 3. These differences are reflections of different uptake of prostate specific antigen (PSA) testing but also to some extent differences in genetic and environmental factors such as diet and general life-style.

PSA

PSA is a protease produced by prostate epithelial cells, which is secreted into the lumen where its function is to cleave semenogelin I and II in order to liquefy the semen [14]. A small fraction is leaked through the basement membrane and can be detected in serum. PSA is prostate-, but not prostate cancer specific, and it is elevated also by benign conditions such as benign prostate hyperplasia (BPH), and infections.

PSA can be measured both in asymptomatic men, so-called opportunistic screening, and in men where prostate cancer is suspected, often due to lower urinary tract symptoms (LUTS). An elevated PSA level will often give rise to further work-up in the form of trans-rectal biopsies in order to histologically evaluate the presence or absence of cancer. PSA can also be used to monitor treatment effects [15]. The use of PSA in screening is discussed on page 20-22.
Figure 3. Age-standardized incidence and mortality rates of prostate cancer by World area in 2008. Standardized according to World Standard Population. Jemal et al [2].
Prostate cancer is the most common male cancer in Sweden and in 2011, more than 9,600 cases were diagnosed [16]. The age-standardized incidence of prostate cancer has increased gradually in Sweden, with a major increase in the later part of the 1990s, Figure 4. This sharp increase is a result of a gradual increase of PSA testing for early diagnosis of prostate cancer during these years. This increased incidence, however, seem to have reached a peak and there has been a trend of decreased incidence the last years [16].

**Figure 4.** Age-standardized incidence and mortality of prostate cancer in Sweden between 1960 and 2010, 0-85+ years. Standardized according to World Standard Population. NORDCAN © 2009 [12].
Prostate cancer is classified according to the tumor, node and metastasis system (TNM), which is based on the pre-operative evaluation of the tumor extent, Table 1.

**Table 1.** Definition of pre-operative local clinical stage, lymph node status at imaging, and bone metastases at evaluation of skeleton

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<td>T1a</td>
<td>Not palpable, diagnosed at TUR-P, ≤5% of resected chips contained tumor tissue.</td>
</tr>
<tr>
<td>T1b</td>
<td>Not palpable, diagnosed at TUR-P, &gt;5% of resected chips contained tumor tissue.</td>
</tr>
<tr>
<td>T1c</td>
<td>Not palpable, diagnosed at biopsy due to e.g. elevated serum PSA.</td>
</tr>
<tr>
<td>T2</td>
<td>Palpable, intracapsular i.e. restricted to the prostate.</td>
</tr>
<tr>
<td>T3</td>
<td>Palpable, extracapsular i.e. growth outside of the prostate that may include invasion of seminal vesicles or bladder neck.</td>
</tr>
<tr>
<td>T4</td>
<td>Palpable, invades other structures than seminal vesicles or bladder neck.</td>
</tr>
<tr>
<td>N1</td>
<td>Regional lymph node metastasis.</td>
</tr>
<tr>
<td>M1</td>
<td>Bone metastases.</td>
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Transurethral resection of the prostate (TUR-P). NPCR annual report 2012 [17].

The TNM-classification can be used in combination with histological appearance (Gleason score), extent of tumor in biopsies and PSA level at diagnosis to create risk categories, table 2.
Table 2. Risk categories in the National Prostate Cancer register (NPCR)

1. Low risk

1a. Very low risk
T1c, PSA<10 μg/L, Gleason score 6 or lower, no more than 2 biopsy cores with cancer, total length of biopsies with cancer<4mm.

1b. Low risk (others)
Low risk that is not categorized to 1a.

1c. Low risk (missing)
Missing information for categorization of low risk according to 1a/b.

2. Intermediate risk
T1-2, Gleason score 7 and/or 10≤PSA<20 μg/L.

3a. Localized high risk
T1-2 and Gleason score 8-10.

3b. Locally advanced
T3 and/or 20≤PSA<50 μg/L.

4. Regionally metastatic
T4 and/or N1 and/or 50≤PSA<100 μg/L, no distant metastases (Mo or MX).

5. Distant metastases
M1, bone scan shows signs of metastases, and/or PSA≥100 μg/L.

6. Missing
Missing information for categorization.

NPCR annual report 2012 [17].

In NPCR, the main reason for the clinical investigation that resulted in the diagnosis of prostate cancer has been recorded since 2004. Between 2004 and 2012, there has been a sharp increase in the category health examination, i.e. PSA test in an asymptomatic man, Figure 5. This has led to a shift towards less aggressive tumors diagnosed in Sweden, Figure 6.
Figure 5. Main reason for the initiation of the medical investigation that led to the prostate cancer diagnosis by year of diagnosis, 2004-2012, graph based on data from the NPCR annual report 2012 [17]. Lower urinary tract symptoms (LUTS). The category Health examination usually means opportunistic PSA testing and the category Other symptoms can be for example pain from skeletal metastasis.

Figure 6. Proportion of men by risk category and year of diagnosis, 1998-2012, NPCR annual report 2012 [17]. Categories described in table 2, page 16.
Prostate cancer mortality

Globally, prostate cancer is the sixth most common cause of cancer death in men and the number of deaths has increased by 64% in 20 years [4]. However, the age-standardized mortality from prostate cancer has decreased by 3.1% in the same time period. In high-income countries, the overall prognosis in prostate cancer is comparatively good, with a 5 year survival rate in Sweden of over 90% [16].

Still, approximately 2,300 men died of prostate cancer in Sweden 2011 and the age-standardized overall mortality rate has been rather stable over the years, Figure 4, page 14. The last years there has been a trend of decreased mortality that has been most pronounced in men below 75 years of age [18]. However, the majority of prostate cancer deaths occur in men above 75 years of age.

The risk of dying of prostate cancer varies greatly across tumor risk categories, from individuals with low-risk tumors, where mortality rates do not differ from the general population, to individuals in higher risk categories, where mortality is clearly increased, Figure 7 [19].
Figure 7. Observed versus expected all-cause mortality according to risk category of prostate cancer, Rider et al [19].
Treatment of prostate cancer

Depending on the stage of the disease at diagnosis, as well as life expectancy related to age and comorbidity, different treatments are available. For the least aggressive tumors, active monitoring, i.e. where patients are offered delayed curative treatment in case of tumor progression, can be sufficient. How this monitoring should be performed is at the moment being evaluated in a randomized trial in Sweden [20].

Treatments that are given with a curative intent are surgery, in the form of radical prostatectomy, or radiation, mainly external. Androgen deprivation therapy (ADT), in combination with external radiation therapy, has been shown to increase survival in men with high-risk localized prostate cancer [21].

In later stages of the disease, when the intent is palliative, ADT is the first treatment of choice. Eventually, such tumors become castration resistant and then there are different forms of chemotherapy or hormone modulating drugs available [22, 23].

Screening using PSA

PSA can be used in screening for prostate cancer, as has been done in some countries, for example in the US. The aim of screening is to detect prostate cancer at a stage where it is accessible for curative treatment, with the hope that this will lead to fewer deaths from prostate cancer. However, the overdiagnosis, i.e. the detection of tumors which would never have progressed and surfaced clinically during a man's life, can be substantial [24]. One problem of this overdiagnosis is that these men might suffer treatment associated side effects such as incontinence, impotence and bowel problems, but no survival benefit.

Does PSA screening prevent death from prostate cancer?

There is an on-going debate on the benefits of screening with PSA and the two largest randomized trials on the matter show conflicting results.

The first, the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), a multi-center trial in the US of 76 000 men, showed no survival benefit for the screened arm [25]. The second, the European Randomized Study of Screening for Prostate Cancer (ERSPC), a pooled trial from Europe of over 180 000 men, showed a 20% decreased prostate-cancer mortality in the screened arm [26].
Another trial which was started separately but which was later included in ERSPC was performed in Gothenburg, Sweden, where 20,000 men were included [27]. Here, the risk reduction for prostate-cancer mortality in the screened arm was even larger than in ERSPC, 44%.

There are factors which might explain the large decrease in prostate-cancer mortality seen in the Gothenburg trial; this study included younger men that had a longer time to benefit effects from an early diagnosis, had longer follow-up, and a large part of the men included had never had a PSA test previous to the trial so there were opportunities to find even advanced tumors at the first round of screening.

The trials had different intervals between screening, annually in PLCO, every two years in the Gothenburg trial and every four years in ERSPC. All trials showed increased incidence of prostate cancer in the screened arms.

The rate of contamination by opportunistic screening in the non-screened arms of the trials varied largely; in ERSPC it was estimated to be less than 15%, while in PLCO, it was around 50%. A high rate of contamination of the control arm will dilute any true positive effect of the intervention.

Furthermore, in a Cochrane collaboration meta-analysis of five randomized controlled trials, including ERSPC and PLCO, there was no decrease in prostate cancer-specific mortality in men that had been screened [28]. However, this meta-analysis has received critique for comparing trials that differ too much in design, and to include trials that have severe methodological limitations [29].

Except for men included in the Gothenburg trial, there has been no organized screening with PSA in Sweden. Opportunistic screening however is common, and in 2011, over 40% of prostate cancer cases in Sweden were diagnosed as a work up of a health examination, i.e. PSA test in an asymptomatic man [17, 30].

How common opportunistic screening has been in Sweden has varied greatly between counties, and a recent study has shown that counties with high-intensity opportunistic screening, estimated from the estimated vs. observed incidence of prostate cancer, had approximately 20% lower prostate cancer-specific mortality [31]. Strengths of this study include the very large size, population-based setting, and high quality follow-up through nation-wide registers. Limitations include the use of an indirect measure of uptake of PSA test, and the lack of information on treatment received for the cancer.
In 2012, the US Preventive Services Task Force recommended against screening with PSA, with the motivation that the benefit did not outweigh the potential harm, a report which has received critique for containing errors [29]. Also in Sweden, recommendations from the National Board of Health and Welfare state that PSA screening is not recommended at the moment, with the motivation that the pros of early diagnosis do not outweigh the cons of overdiagnosis.
Androgens

In men, testosterone is the main sex steroid hormone androgen. Testosterone is mainly synthesized by Leydig cells in the testis but also to a small part in the adrenals [32]. Testosterone synthesis is stimulated by leutinizing hormone (LH), which in turn is regulated by gonadotropin releasing hormone (GnRH). In the circulation, testosterone is mainly bound to sex hormone binding globuline (SHBG) or to albumin. A smaller part is so-called free testosterone.

Testosterone can bind either directly to the androgen receptor, or be converted to dihydrotestosterone (DHT) by the enzyme 5α-reductase. DHT binds more strongly to the androgen receptor which gives tissues that express 5α-reductase a method of local regulation. The binding of testosterone or DHT to the androgen receptor activates transcription in androgen-regulated genes [33]. Androstanediol glucuronide (a-diol-g) is an end-product of DHT which is used to reflect intraprostatic androgen levels.

Androgens and prostate cancer

Androgens are necessary for the development of the normal prostate gland but are also involved in the development of prostate cancer. Androgens stimulate prostate cancer cell growth in vitro [34, 35], and in rodent models [36-38]. Indirect evidence also comes from the fact that prostate cancer is very rare in men with Klinefelter syndrome [39], a genetic syndrome associated with low circulating levels of androgens [40].

Prospective studies on androgens and risk of prostate cancer

Several prospective studies have studied if circulating levels of androgens affect risk of prostate cancer [41-47]. In short, these studies include measurements of circulating levels of androgens taken in middle-aged men, years before some of them develop prostate cancer. Individually, many of these studies have had limited power to assess this association.

In 2008, 18 of these studies were pooled, resulting in 3886 cases of prostate cancer and 6438 controls [48]. In this study, none of the androgens were associated with risk of prostate cancer, except for a tendency of a reduced risk with increasing levels of SHBG, Figure 8.
Figure 8. Associations between increasing fifths of hormone concentrations and risk of prostate cancer, Roddam et al [48].

There are studies indicating that androgen levels might affect risk of tumors of different aggressiveness differently, with high levels of testosterone being associated with an increased risk of non-aggressive tumors [46], but a decreased risk of aggressive tumors [43, 44, 46].

The effect of circulating testosterone and DHT on risk of prostate cancer has also been studied in the placebo-arm of the Reduction by Dutasteride of Prostate Cancer events (REDUCE) trial, a clinical trial on the use of 5α-reductase inhibitors which has been described below. This study included 3255 men that had at least one biopsy, either for symptoms and/or increased PSA, or at end of study [49].
This study did not show an overall association between levels of testosterone or DHT and risk of prostate cancer, but an indication of an increased risk of prostate cancer for higher testosterone levels in the sub group of men with clinically low testosterone levels (<10 nmol/L).

One limitation of this study is the short observation period (4 years), since the existence of a prostate tumor might affect testosterone levels. This has been shown in studies where androgen levels have been measured before radical prostatectomy, and again 3-12 months post-operatively. Here, testosterone levels increased post-operatively [50], and DHT decreased [50, 51], or remained unchanged [52]. However, it is not clear if these effects on androgen levels were the result of removal of the tumor or the prostate itself.

**5α-reductase inhibitors and risk of prostate cancer**

Strong evidence for the role of androgens in prostate tumorigenesis comes from two large randomized placebo-controlled trials, Prostate Cancer Prevention Trial (PCPT), and REDUCE, where 4-7 years use of 5α-reductase inhibitors, a drug which decreases levels of DHT and is used to treat benign prostate hyperplasia [53], resulted in approximately 25% lower incidence of prostate cancer [54, 55].

However, this decreased incidence was mainly evident for low-grade tumors and there was a small but significant increase in high-grade tumors. A similar decrease of low-grade tumors, but no increase in high-grade tumors was seen in a large population-based observational study on 5α-reductase inhibitors in PCBaSe 2.0 [56].

There is some evidence of a detection bias effect, where the use of 5α-reductase inhibitors would increase the likelihood of detecting a high-grade tumor at biopsy, without actually causing the high-grade tumor, to explain the increased risk of high-grade tumors in PCPT [57, 58]. If this was the case, these high-grade tumors would be less harmful than “true” high-grade tumors. This was not supported by a follow-up after 10 years, where there was no difference in survival of 619 men with high-grade tumors between treatment groups [59]. However, this analysis was based on 177 deaths of any cause and overall survival was estimated, not prostate cancer-specific mortality.

**Circulating vs prostatic androgens**

One limitation to studies using circulating levels of androgens is that it is not clear to what extent these levels reflect intra-prostatic levels of androgens.
Studies on this topic are scarce but in a study on 25 men that underwent radical prostatectomy, levels of androgens were measured in peripheral serum, serum from the prostatic veins and prostatic tissue. Here, the main finding was that the concentration of DHT was almost twice as high in serum from the prostatic veins as in the peripheral serum, while the concentrations of testosterone and SHBG did not differ [60].

Fatherhood status and risk of prostate cancer

In two large register-based studies in Sweden and Denmark, childless men had lower risk of prostate cancer than men who have fathered children [61, 62], whereas smaller studies have shown inconsistent results [63-67]. The hypothesis for this association is that some childless men are subfertile and have lower androgen levels [68], a factor which might affect risk of prostate cancer [69].

Many factors in addition to fertility affects if a man fathers children, such as marital status, lack of female partner etc. Factors like marital status and educational level might also affect the risk of prostate cancer through the influence on health seeking behavior, e.g. uptake of PSA test. A high uptake of PSA test will lead to a high incidence of low-risk tumors. Previous studies have not studied whether the association between fatherhood status and prostate cancer is restricted to low-risk tumors and has not been able to adjust for marital status or educational level.

Dizygotic twin fatherhood status and risk of prostate cancer

We wanted to further explore the potential link between fertility and risk of prostate cancer by using another proxy of fertility, fathering of dizygotic twins, a factor which is unaffected by issues of ability and/or wish to have children.

While monozygotic twinning occurs randomly, having dizygotic twins is considered a marker of high fertility and the rate of dizygotic vs monozygotic twinning has been used to monitor fertility trends in countries [70]. In a Danish study, infertile couple (defined as time to pregnancy>12 months), that later conceived naturally, had a clearly decreased rate of dizygotic twins, but the same rate of monozygotic twins as couples with normal fertility [71]. Also, one study of 37 fathers of dizygotic and 15 fathers of monozygotic twins showed that men with twins had better sperm quality then fathers of singletons [72].
A similar approach has been used in a study on testicular cancer risk, where the rate of dizygotic twinning was reduced in men that later developed testicular cancer [73]. To the best of our knowledge, this association has not been studied for prostate cancer.

**Height and risk of cancer**

**Determinants of adult height**

Genetic disposition determine more than 80% of the variation in adult height in men, and slightly less in women [74]. Apart from genes, growth hormone (GH), and the GH-regulated insulin like growth factors are essential for growth [75]. Other factors in childhood and adolescence, such as nutrition and general health, also play important roles [76], possibly through their influence on the GH/IGF-axis.

**Height and risk of cancer**

Adult height has in large studies been positively associated with an increased risk of all-sites cancer in both women and men [77-80]. For individual cancer sites, there is evidence of a positive association with risk of cancer of the prostate [81], breast [77], colorectum [82], ovary [83, 84], pancreas [85], kidney [86], testis [87], endometrium [88], malignant melanoma [89, 90], and to lymphohematopoietic malignancies [91], and negative associations to cancer of the head and neck [92], and esophagus [93].

There are a few large studies on height and risk of all-sites cancer in women [77-80]. Results for different cancer sites concur between these studies for some sites; such as cancer of the colon, breast and ovary, while the association is less clear for other sites. The by far largest of these studies, a study from UK of over 1 million women and 97 376 cancers, showed positive associations between height and 10 out of 17 sites studied while there were no significant negative associations, Figure 9.

There is only one study on all-sites cancer which also included men, a Korean study with 23 725 male cancer cases [80]. Here, height was positively associated with risk of 8 out of 15 cancer sites and there were no negative associations.
Figure 9. Relative risks (RRs) and 99% CIs per 10 cm increase in height for female incident cancer, adjusted for age, region, socioeconomic status, smoking, alcohol intake, BMI, strenuous exercise, age at menarche, parity, and age at first birth, by Green et al [77].
**Height and risk of cancer death**

In a large meta-analysis on height and risk of cause-specific death, which included over 1 million individuals and 47,502 cancer deaths, height was positively associated with increased risk of cancer death but decreased risk of total mortality and mortality from cardiovascular diseases [94].

In this study there were positive associations for height and risk of death from malignant melanoma, cancer of the pancreas, endocrine and nervous system, breast (female), ovary, prostate, colorectum, lung and hematological malignancies, Figure 10. There were also negative associations to risk of death from stomach and oral cancer. Notable is that results were not presented for women and men separately but analyses were instead adjusted for sex.

Another study with participants from Asia and Australasia which included 7,497 cancer deaths showed positive associations for height and risk of overall cancer death in both women and men [95]. For separate cancer sites, height was positively associated to risk of liver cancer in women and bladder cancer and malignant melanoma in men. However, for many of the other cancer sites, analyses were likely underpowered due to small number of deaths.

![Figure 10. Hazard ratios (HRs) for cause-specific non-vascular mortality per 1 SD (6.5 cm) higher baseline height, adjusted for age, sex, smoking and year of birth, by The Emerging Risk Factors Collaboration [94].](image-url)
Possible links between height and risk of cancer

There are mainly two pathways which are favored as potential links between height and risk of cancer. The first is factors in the growth hormone/insulin-like growth factor axis [96, 97], where IGF-1 has been most extensively studied. The second is common genes for increased height and tumorigenesis.

IGF-1 is a peptide secreted in the liver under regulation from growth hormone. IGF-1 has mitogenic and antiapoptotic properties [98], and prospective serum levels of IGF-1 has been associated with increased risk of cancer, most consistently for cancer of the breast, prostate and colorectum [99-102].

Genes that might be involved in this association are related both to increased height and to oncogenic pathways such as c-Myc, p53 and SMAD3 [103].
Register-based research; pros, cons and ethical considerations

Register-based research can use data collected for clinical and/or research purposes, such as biobanks and quality registers, often combined with nation-wide healthcare and demographic registers. In Sweden, linkages between different registers are possible through use of the national identification number [104].

A major advantage of using data from quality registers, such as NPCR, is that it includes more detailed tumor characteristics than the Cancer register does. Furthermore, as long as the quality register has a high coverage, the data reflect routine health care, not a more artificial setting such as that in a clinical trial. However, due to the observational design of many register-based studies, a con is the possibility for bias such as confounding by indication for treatment. For example, men with prostate cancer that undergo radical prostatectomy are generally healthier than those that undergo radiation therapy or surveillance. The selection of healthy men to surgery was evident in a study from NPCR, where the mortality in men that underwent radical prostatectomy was lower than the mortality in the general population, despite of their cancer diagnosis [105].

Furthermore, there is an on-going discussion on the use of personal data in such research, with the main focus being on the balance between the overall benefits of research for individuals and the society, and the risk of breach of personal integrity [106]. To limit this risk, it is crucial that data is handled professionally and with a high grade of computer safety.
Aims of this thesis

The overall aims of this thesis were to study the impact of androgens levels and indirect measures of androgen exposure on risk of prostate cancer, as well as the association between height and risk of cancer and cancer death.

Specific aims were to study:

- if prospective levels of androgens are associated with risk of prostate cancer, either overall or in subgroups of tumor aggressiveness (Paper I)

- if fatherhood status is associated with risk of prostate cancer, either overall or in tumor risk categories, also taking socioeconomic factors and comorbidity into consideration (Paper II)

- if dizygotic twin fatherhood status is associated with risk of prostate cancer, either overall or in tumor risk categories, also taking socioeconomic factors and comorbidity into consideration (Paper III)

- if height is associated with risk of cancer diagnosis and cancer death in women and men, overall and for specific cancer sites (Paper IV)
Materials and methods

Study settings

*The Västerbotten Intervention Project (VIP)*

VIP is an on-going project for health promotion in the county of Västerbotten. All individuals in Västerbotten are invited to a health examination at age 40, 50, and 60. Between 1985 and 1996, individuals were also invited at age 30. Participants in VIP complete a questionnaire regarding life-style related questions and diet, and are asked to donate a blood sample for future research. The participants blood sample is stored frozen at 70 degrees below zero and these samples, together with samples from the MONICA study and the mammary screening cohort make up The Northern Sweden Health and Disease Cohort: The Medical Biobank [107].

The intervention part of VIP is based on both community based health interventions, as well as an individual counselling where the participant and a trained nurse discuss the results of the health examination and the questionnaire [108].

The overall participation rate of VIP is for the years 1990-2006 61% in men and 67% in women [109]. Non-participation has been evaluated and is weakly associated with low education and a higher number of hospitalizations prior to the health examination, and slightly stronger associated with being single, having low income or being non-native Swede.

*The National Prostate Cancer Register (NPCR)*

NPCR started as a regional prostate cancer register in south-east Sweden in 1987 [110]. NPCR is nationwide since 1998 and the coverage is 98% in comparison to the Swedish Cancer Register to which registration is mandatory and regulated by law [17].

NPCR contains information on tumor characteristics according to the tumor, node, metastasis (TNM) classification, (Table 2, page 16), tumor differentiation (Gleason score), serum level of PSA at diagnosis; as well as diagnostic unit, date of diagnosis, and primary treatment delivered or decided within six months after diagnosis. Cause for diagnostic work-up leading to the prostate cancer diagnosis is recorded from 2004 and onwards.
The Prostate Cancer database Sweden (PCBaSe)

In 2008, NPCR was linked to several nationwide registers to form PcBaSe [111]. Linkage was performed using the personal identification number, unique to all Swedish citizens [104]. In 2011, a new linkage was performed to create PCBaSe 2.0, which include 119,777 cases with information from several registers, Figure 11 [112].

Figure 11. Registers linked to NPCR to form PcBaSe 2.0, by Van Hemelrijck et al [112].

Quality and coverage of selected registers included in PCBaSe 2.0

The Longitudinal Integration Database for Health Insurance and Labor Market Studies (LISA) includes all individuals above 16 years of age living in Sweden. In PCBaSe, LISA was used to obtain information on civil status and the highest attained educational level. The coverage of LISA is virtually complete.
The Swedish Multi-Generation register includes all subjects in Sweden born 1932 or later that were alive in 1961 [113]. The coverage of the register is generally very high, although for individuals that died before 1991, the completeness is approximately 50%. Information on number of children from The Swedish Multi-Generation Register was used in paper II and III.

The National Patient register includes all diagnosis from in-hospital care in Sweden since 1987 [114]. The coverage is almost 100%. In study II and III, all diagnosis in the National Patient register during the ten years preceding the diagnosis of prostate cancer in the cases were used to create a co-morbidity index according to Charlson [115].

**The Swedish Twin Register (STR)**

STR was established in the 1950s but includes twins born as early as 1886 [116]. Participation in STR is voluntary and inclusion is through invitation to the parents of twins. The parents’ response to questions on physical similarities in childhood is also the basis for the zygosity determination in a majority of same-sex twins.

Although the coverage of STR is generally high, the rate of same-sex twins with zygosity determination dropped to 30% in the late 1980s, mainly due to low response rate to questionnaires.

In 2013, STR was linked to PCBaSe 2.0 to obtain zygosity for same-sex twins born to men in the database, and this information was used in paper III.

**The Metabolic Syndrome and Cancer Project (Me-Can)**

Me-Can is a pooled cohort of seven cohorts; two from Sweden, four from Norway and one from Austria [117], Figure 12. In all cohorts, participants have been invited to a health examination where height, weight and metabolic factors have been measured. Participants also completed a questionnaire on lifestyle issues and from these, information on smoking was used.

The cohorts have been linked to cancer registers and cause of death registers in respective country [118-120], to obtain both incident and fatal cancers. In Norway and Sweden, data were also linked to the Registry of Total Population and Population Changes for assessment of vital status (not available in Austria).
**Figure 12.** Map with location of cohorts included in Me-Can. The 40-y cohort includes all counties in Norway (all grey- and black-marked areas in Norway), NCS includes areas □ and ■, the CONOR includes areas □ and ■, and further cohorts (marked in black) are; Oslo I, VHM&PP, VIP and the MPP, Stocks *et al* [121].
Study design and selection of cases

**Paper I**

This prospective case-control study is based on a linkage between the regional cancer register and VIP which identified cases of prostate cancer that had samples available for analysis. Further inclusion criteria for cases were; diagnosis of prostate cancer after the health examination, and no history of cancer (except non-melanoma skin cancer). One control per case was selected from men that were alive and cancer-free, and matched the case on age (±6 months) and date of health examination (±2 months). Tumor characteristics for cases were obtained from the northern part of NPCR, [122] and the tumors were categorised as either non-aggressive or aggressive. Cases were categorised as aggressive if they had any of these clinical characteristics: Gleason score 8-10, T3-4, lymph node metastasis, bone metastasis, serum PSA>50 ng/ml, or fatal disease up to October 2005.

**Paper II and III**

This case-control study included all cases of prostate cancer diagnosed between 1991 and 2009 in PCBaSe 2.0 and the tumors were classified into five risk categories according to a modification of the National Comprehensive Cancer Network, Table 3 [123].

<table>
<thead>
<tr>
<th>Risk categorya</th>
<th>Clinical stage</th>
<th>Gleason score</th>
<th>PSA value (ng/mL)</th>
<th>Metastasis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-risk</td>
<td>T1-2</td>
<td>≤6</td>
<td>&lt;10</td>
<td>None detected</td>
</tr>
<tr>
<td>Intermediate-risk</td>
<td>T1-2</td>
<td>7</td>
<td>10 to&lt;20</td>
<td>None detected</td>
</tr>
<tr>
<td>High-risk</td>
<td>T3</td>
<td>8-10</td>
<td>20 to&lt;50</td>
<td>None detected</td>
</tr>
<tr>
<td>Regionally metastatic disease</td>
<td>T4</td>
<td>Any</td>
<td>50 to&lt;100</td>
<td>N1</td>
</tr>
<tr>
<td>Distant metastases</td>
<td>Any</td>
<td>Any</td>
<td>≥100</td>
<td>M1</td>
</tr>
</tbody>
</table>

Throughout the table, presence of any non-beneficial variable renders a higher risk category. N1-lymph node metastasis, M1-distant metastasis.
Controls were randomly sampled among prostate cancer free men in the background population which matched the case for birth year (+/- 1 year) and county of residence. For the majority of cases, 5 controls were used but due to smaller population sizes available to sample, 2 controls were used for cases diagnosed 1991-95.

In paper III, a case-control study, analyses were restricted to men that had fathered children. Furthermore, to account for the increase in use of in vitro fertilization treatment in Sweden [124], only children born before 1991 were included in the analysis.

**Paper IV**

In this prospective pooled cohort study, there were two separate end-points, cancer diagnosis and cancer death. For cancer diagnosis, the first cancer after the health examination was used. For cancer death, only cancers recorded as the primary cause of death were used.

**Statistical methods**

**Paper I**

Conditional logistic regression was used to calculate odds ratios (ORs) and corresponding confidence intervals (95% CIs) for quartiles of androgens in the full study group, and for tertiles of androgens in subgroup analysis. These estimates were also adjusted for BMI. Cut-offs for tertiles and quartiles were based on the levels in the controls. Likelihood ratio tests were used to assess linear trends in ORs using mean values for each category.

**Paper II**

Conditional logistic regression was used to calculate ORs and 95% CIs for fatherhood status, marital status, education and comorbidity in the full study group as well as in subgroups of tumor aggressiveness. The analysis of fatherhood status were also adjusted for marital status and educational level, but not comorbidity index (CCI), since CCI was unrelated to risk of prostate cancer in univariable analysis.

**Paper III**

Conditional logistic regression was used to calculate ORs and 95% CIs for dizygotic twin fatherhood status in the full study group as well as in subgroups of tumor aggressiveness. The analysis of dizygotic twin
fatherhood status was not adjusted for marital status, education or comorbidity since these variables were evenly distributed in the groups fathers of dizygetic twins and fathers of singletons.

**Paper IV**

Hazard ratios (HRs) and 95% CI for increased height were analyzed with Cox proportional hazards regression with attained age as the time scale. The Cox models were adjusted for ten categories of date of birth and ten categories of age at health examination, and stratified for sub cohort within the model.

We calculated HRs in categories of height, and height as a continuous variable (5 cm per unit) for separate sites. We tested for multiplicative interactions between categories of BMI or smoking, and continuous height, using likelihood ratio test, and we adjusted the significance level for multiple testing using the Holm-Bonferroni correction.

We calculated HRs of breast cancer in groups according to age at diagnosis; assuming that women below age 50 had not undergone menopause (premenopausal), that women of age 50-60 were undergoing menopause (perimenopausal), and that women above age 60 years had undergone menopause (postmenopausal).

Throughout the studies, p-values below 0.05, and ORs as well as HRs with 95% CIs which did not include unity were considered statistically significant.

Statistical software used were the Statistical Analysis System (SAS)[125] in paper I, R statistical program package (2.12.0) [126] in paper II, and IV, and STATA 11 in paper II and STATA 12.1 in paper III (StataCorp LP, College Station, Texas).

**Ethical considerations**

Participants in VIP provided written informed consent at time of health examination and the project was approved by the regional ethics review board in Umeå.

For PcBaSe, information that data is collected to the NPCR is posted in all clinics that report to the register. Here, the possibility to decline participation is stated, the so-called opt-out principle. The project is approved by the Central Ethics review board in Sweden.
For Me-Can, participants provided informed consent at time of health examination, although for some years, the consent was verbal and not written. The Me-Can project was approved by regional ethics review boards in respective country.
Results

Paper I

This study included 392 age-matched cases and controls, Table 4. There was no significant difference in BMI between cases and controls. 27% of cases had aggressive tumors at diagnosis.

Table 4. Background characteristics for cases and controls in VIP

<table>
<thead>
<tr>
<th></th>
<th>Cases N=392</th>
<th>Controls N=392</th>
<th>P&lt;sub&gt;a&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ±SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years</td>
<td>57.1±4.5</td>
<td>57.1±4.6</td>
<td>Matched</td>
</tr>
<tr>
<td>BMI, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>26.0±3.0</td>
<td>26.5±3.4</td>
<td>0.053</td>
</tr>
</tbody>
</table>

Tumor characteristics of cases

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lagtime, years</td>
<td>Mean</td>
<td>6.2</td>
</tr>
<tr>
<td>Tumor aggressiveness&lt;sub&gt;b&lt;/sub&gt;</td>
<td>N (%)</td>
<td>230 (59)</td>
</tr>
<tr>
<td>Non-aggressive</td>
<td>230 (59)</td>
<td></td>
</tr>
<tr>
<td>Aggressive</td>
<td>107 (27)</td>
<td></td>
</tr>
<tr>
<td>No classification</td>
<td>55 (14)</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Wilcoxon´s signed-ranks test.
<sup>b</sup>Aggressive cancers defined as; T 3-4 or lymph node metastasis, or bone metastasis, or Gleason score 8-10, or serum PSA>50 mg/ml, or fatal disease up to October 2005.

Testosterone was strongly correlated with free testosterone, (correlation coefficients denoted r) (r=0.85), and SHBG (r=0.62), while there were significant but weak correlations to a-diol-g (r=0.13), and BMI (r=-0.26). BMI showed a statistically significant negative correlation also to free testosterone (r=-0.14), SHBG (r=-0.31), and a positive correlation to a-diol-g (r=0.08).
None of the androgens were significantly associated with risk of total prostate cancer, Figure 13.

**Figure 13.** Risk of total prostate cancer by quartiles of androgens, adjusted for BMI.

In subgroup analysis of tumor aggressiveness, there was an indication of increased risk of non-aggressive tumors, Figure 14a, and a decreased risk of aggressive tumors, Figure 14b, with the highest levels of testosterone and free testosterone. This pattern was not seen for a-diol-g or SHBG.
Figure 14. Risk of non-aggressive (A) and aggressive tumors (B) by tertiles of testosterone and free testosterone.
Aggressive disease defined as either Gleason score 8-10, T3-4, lymph node metastasis, bone metastasis, serum PSA>50 ng/ml, or fatal disease up to October 2005.

Paper II

This study included 117,328 cases of prostate cancer and 562,644 matched controls. Cases were more often fathers (84.1%, vs 81.0% among controls), were more often married (68%, vs 64.3% among controls), and slightly more often had a high educational level (18.2%, vs 16.7% among controls).

Prostate cancer was more often diagnosed as a consequence of a health examination, i.e. PSA test in the absence of symptoms, in fathers (22.1%), than in childless men (17.0%). Overall, 22.5% of cases had low-risk tumors, often detected by opportunistic PSA testing, Table 5.
Table 5. Tumor risk categories in PCBaSe 2.0

<table>
<thead>
<tr>
<th>Risk category</th>
<th>Number</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-risk</td>
<td>26,402</td>
<td>(22.5)</td>
</tr>
<tr>
<td>Intermediate-risk</td>
<td>26,611</td>
<td>(22.7)</td>
</tr>
<tr>
<td>High-risk</td>
<td>30,159</td>
<td>(25.7)</td>
</tr>
<tr>
<td>Regionally metastatic</td>
<td>10,315</td>
<td>(8.8 )</td>
</tr>
<tr>
<td>Distant metastases</td>
<td>20,391</td>
<td>(17.4)</td>
</tr>
<tr>
<td>Missing data</td>
<td>3,450</td>
<td>(2.9 )</td>
</tr>
</tbody>
</table>

Low-risk: T1–2, Gleason score 5-6 and PSA<10 ng/mL. Intermediate risk: T1–2, Gleason score 5-7 and/or PSA 10 to<20 ng/mL. High-risk: T3, Gleason score 8–10 and/or PSA 20 to <50 ng/mL. Regionally metastatic disease: T4 and/or N1 and/or PSA 50 to <100 ng/mL in the absence of distant metastases (M0 or Mx). Distant metastases: M1 and/or PSA>100 ng/mL.

Childless men had a decreased risk of prostate cancer across all risk categories, but which was strongest for low-risk tumors, Figure 15a. This association was attenuated, most pronounced for low-risk tumors, when adjusted for educational level and marital status, Figure 15b.

Married men and men with a high educational level had an increased risk of prostate cancer, with the strongest association for low-risk prostate cancer, Figure 16. The association between marital status and risk of prostate cancer was attenuated by increasing tumor aggressiveness category, and was not evident for metastatic disease. For educational level, a similar pattern was seen, but here the association was reversed to a weak negative association for metastatic disease.
Figure 15. Risk of prostate cancer for fatherhood status, overall and by risk category, unadjusted (a) and adjusted for marital status and educational level (b).
Figure 16. Risk of prostate cancer, for marital status (a) and educational level (b), overall and by risk category.
Paper III

This study included 96,301 prostate cancer cases and 378,583 controls, out of which 1,112 cases and 4,538 controls had fathered dizygotic twins. There were no major differences in tumor risk categories between fathers of dizygotic twins and fathers of singletons, Table 6.

Table 6. Characteristics of dizygotic twin fathers and fathers of singleton(s) in PCBaSe 2.0

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Fathers of dizygotic twins n=5,650</th>
<th>Fathers of singleton(s) n=469,234</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of cases</td>
<td>1,112 (19.7)</td>
<td>95,189 (20.3)</td>
</tr>
<tr>
<td>Number of controls</td>
<td>4,538 (80.3)</td>
<td>374,045 (79.7)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>72.9 (8.7)</td>
<td>71.3 (8.9)</td>
</tr>
<tr>
<td>Risk categories</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-risk</td>
<td>1,212 (21.5)</td>
<td>111,402 (23.7)</td>
</tr>
<tr>
<td>Intermediate-risk</td>
<td>1,231 (21.8)</td>
<td>109,896 (23.4)</td>
</tr>
<tr>
<td>High-risk</td>
<td>1,528 (27.0)</td>
<td>119,248 (25.4)</td>
</tr>
<tr>
<td>Regionally metastatic</td>
<td>525 (9.3)</td>
<td>40,397 (8.6)</td>
</tr>
<tr>
<td>Distant metastases</td>
<td>1,008 (17.8)</td>
<td>75,486 (16.1)</td>
</tr>
<tr>
<td>Missing data</td>
<td>146 (2.6)</td>
<td>12,805 (2.7)</td>
</tr>
</tbody>
</table>

*aLow-risk: T1-2, Gleason score 6 and PSA <10 ng/mL. Intermediate-risk: T1-2, Gleason score 7 and/or PSA 10 to <20 ng/mL. High-risk: T3 and/or Gleason score 8-10 and/or PSA 20 to <50 ng/mL. Regionally metastatic disease: T4 and/or N1 and/or PSA 50 to <100 ng/mL in the absence of distant metastases (M0 or Mx). Distant metastases: M1 and/or PSA ≥100 ng/mL.
There were no major differences between fathers of dizygotic twins and fathers of singletons with regard to educational level, marital status and comorbidity index.

There was no association between fathering of dizygotic twins and risk of prostate cancer compared to fathers of singletons, either overall, or in groups of tumor aggressiveness, Figure 17a.

Men with a high educational level had an increased risk of total prostate cancer compared to men with a low educational level, Figure 17b. Divorced, never married men as well as widowers were at decreased risk of total prostate cancer compared to married men, Figure 17c. For both marital status and educational level, the associations were reversed for metastatic disease, to an increased risk for non-married men and a decreased risk for men with a high education.

Increased comorbidity was associated with a decreased risk of total prostate cancer, while for metastatic disease, there was an indication of a reversed effect, Figure 17d.
Figure 17. Risk of prostate cancer, by risk category, for dizygotic twin fatherhood status (a), educational level (b), marital status (c), and comorbidity index (d).
**Paper IV**

This study included 585,928 individuals out of which 49% were men, Table 7.

**Table 7.** Characteristics of the Me-Can cohort

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>585,928</td>
</tr>
<tr>
<td>Age at measurement, years mean (SD)</td>
<td>43.1 (11.0)</td>
</tr>
<tr>
<td>% males</td>
<td>49</td>
</tr>
<tr>
<td>Follow-up, years mean (SD)</td>
<td>12.7 (7.2)</td>
</tr>
<tr>
<td>Cancer cases, women/men</td>
<td>17,549/21,313</td>
</tr>
<tr>
<td>Cancer deaths, women/men</td>
<td>5,431/8,116</td>
</tr>
</tbody>
</table>

There was an association between height and risk of cancer in both women, HR per 5-cm increment in height 1.07 (95% CI 1.06-1.09), and men, HR 1.04 (95% CI 1.03-1.06), Figure 18.

Height was also statistically significantly associated with an increased risk of 8 out of 21 cancer sites in women, and 9 out of 19 cancer sites in men, while only associated with a decreased risk of one cancer site in men. Among the sites with positive associations, the highest HR was for malignant melanoma in both women and men, Figure 18.
Figure 18. Height and risk of cancer by 5-cm increment in height for women (a), and men (b).
There was an association between height and risk of cancer death in both women, HR per 5-cm increment in height 1.03 (95% CI 1.01-1.06), and men HR 1.03 (95% CI 1.01-1.05), Figure 19.

Height was also statistically significantly associated with an increased risk of cancer death from 2 out of 17 cancer sites in women, and 2 out of 14 cancer sites in men, but only decreased risk of death of one cancer site in men. Among the sites with positive associations, the highest HR was for breast cancer in women and renal cell carcinoma in men, Figure 19. For breast cancer, this association was only observed for post-menopausal women (age>60), HR 1.10 (95% CI 1.00-1.21) for breast cancer death vs HR 1.02 (95% CI 0.94-1.11) in premenopausal women (<50 years).
Figure 19. Height and risk of cancer death by 5-cm increment in height for women (a), and men (b).
Discussion

Prostate cancer

Androgen levels

In paper I, prospective levels of androgens were not associated with risk of overall prostate cancer. In subgroup analysis, there were non-significant indications of associations between high levels of testosterone and free testosterone and an increased risk of non-aggressive tumors, but a decreased risk of aggressive tumors.

Strengths in this study include the prospective design, detailed information of tumor characteristics, as well as virtually complete follow-up through registers. Limitations include the use of a single blood sample to represent androgen exposure, and the lack of information on which cancers were detected through opportunistic PSA screening. However, this rate is considered to be relatively low for the years where these tumors were detected and the tumor characteristic can also give an indication of PSA uptake.

Furthermore, due to the slow growth of prostate cancer, it cannot be ruled out that some of the controls had an indolent prostate cancer, which might have affected their androgen levels. Also, although the number of cases in the full study group was rather large for a prospective serum study, the number of cases in subgroup analysis was limited.

Previous prospective studies on androgens and risk of prostate cancer, including a pooled analysis, have reported similar results, both for total prostate cancer [48, 49, 127-129], and for subgroup analysis of tumor aggressiveness [43, 44, 46]. All these studies share most of the above-mentioned limitations, as well as the use of serum instead of intra-prostatic levels of androgens. How well androgen levels at these localizations concur is not clear but one small study showed that the concentration of DHT was almost twice as high in serum from the prostatic veins then in the peripheral serum [60].

That androgen levels might affect risk of prostate cancer differently for tumors of different aggressiveness would concur with the finding that obesity is associated with a decreased risk of localized prostate cancer, but an increased risk of advanced tumors [130], since obesity is negatively associated with levels of testosterone, free testosterone and SHBG [131].
paper I, BMI was correlated to androgen levels, but risk estimates were not materially altered when adjusted for BMI. However, obesity is also associated with other metabolic aberrations, such as increased levels of insulin, which could influence risk of prostate cancer [132].

**Fatherhood status and risk of prostate cancer**

In paper II, childless men had a decreased risk of prostate cancer, overall and across risk categories. The association was strongest for low risk tumors and it was attenuated, but still statistically significant, after adjustment for marital status and education.

Our study on fatherhood status is the largest to date, and the first large study to include possible confounding factors. Other strengths include information on tumor characteristics, and the virtually complete coverage of the registers included. A limitation is the use of fatherhood status as a proxy for fertility, since childlessness can have many reasons; social, practical and medical.

The association between fatherhood status and risk of prostate cancer can have several possible explanations. The most plausible biological link is through lower androgen levels in those men that are childless due to male infertility [68, 133]. This is supported by a case-control study of 661 cases of prostate cancer, where men with self-reported life-long failure to conceive, and where known female infertility was excluded, had a decreased risk of prostate cancer [134]. However, these estimates were based on only 15 cases of prostate cancer with failure to conceive.

In contrast, one study which included 168 cases of prostate cancer out of more than 22 000 men that had been evaluated for failure to conceive, reported no association to overall prostate cancer risk, but an increased risk of high-grade prostate cancer, as compared to the background population [135].

Taken together, it is unlikely that differences in androgen levels could explain the full association observed for fatherhood status and risk of prostate cancer. Another possibility is the presence of residual confounding in adjusted risk estimates. In paper II, there were associations between educational level and marital status and risk of prostate cancer. For both these factors, the association was strongest for the least aggressive tumors and it was not evident, or reversed to an inverse association, for the more aggressive tumors.
This indicates a strong impact of opportunistic PSA testing; where men with a high educational level or men that are married have a higher uptake. This underscores the need for considering these factors when studying risk factors for prostate cancer.

Interestingly, being childless has recently been associated with an increased risk of male breast cancer, another hormone dependant cancer, in a pooled study of 11 case-control and 10 cohort studies [136]. In contrast, there was no association between infertility and risk of male breast cancer, but this estimate was based on small numbers.

**Dizygotic twin fatherhood status and risk of prostate cancer**

In paper III, dizygotic twin fatherhood status was not associated with risk of prostate cancer, either overall or in different risk categories.

Strengths of this study include its large size and high-quality information on zygosity for the twins. Also, using this proxy of fertility has fewer limitations than fatherhood status since the frequency of twinning is not affected by wish or ability to father children.

The main limitation is the use of a fathering of dizygotic twins as a proxy for fertility since it is not clear to what extent this proxy reflects male fertility and ensuing androgen levels. However, this proxy has been used successfully in a study on testicular cancer, where men that later developed testicular cancer were less likely to have fathered dizygotic twins [73].

Another concern in studies on twins is the increased frequency of assisted fertilization such as in vitro fertilization (IVF) in Sweden, since IVF treatment increase the occurrence of dizygotic twins [124]. If dizygotic twinning was a result of IVF treatment, this would be an indication of low fertility, not high fertility as we tried to assess. We did not have individual information on IVF treatments in PCBaSe 2.0. However, we restricted our analysis to the period before 1991, when IVF treatment was still uncommon in Sweden.

Our null finding can have several explanations; that there is no association between fertility and risk of prostate cancer and that the association observed between fatherhood status and risk of PC was caused by other factors. Alternatively, this approach was not sufficiently precise to study this association. Our study was large and there was no tendency of an association, and thus it is unlikely that expanding the study would add extra information.
Height and risk of cancer

In paper IV, height was associated with an increased risk of cancer and cancer death in both women and men. The highest risk of cancer was observed for malignant melanoma in both women and men, while for cancer death the highest risk was seen for breast cancer in postmenopausal women and renal cell carcinoma in men.

For some cancer forms, height was associated with an increased risk in both women and men, such as lymphohematopoietic malignancies, colo- and rectal cancer, non-melanoma and melanoma skin cancer, while for thyroid cancer and for renal cell carcinoma, the associations were only evident for women, and men, respectively.

Strengths in this study include the large size, the inclusion of both women and men, and the possibility to study both incident and fatal cancer. Weaknesses include the lack of tumor characteristics and socioeconomic and reproductive factors.

A high socioeconomic status is positively associated with height [77, 94], but negatively associated with several forms of cancer, such as lung and cervical cancer [137, 138]. However, socioeconomic status is positively associated with some other cancer forms such as female breast cancer, malignant melanoma [137], and low and intermediate risk prostate cancer (paper II). In previous large studies, adjustment for socioeconomic status and reproductive factors has not affected risk estimates for height and risk of cancer [77, 78].

It is likely that the link between height and risk of cancer is multi-factorial, and one of the proposed links is through the common effect of factors of the IGF/GH-axis on adult height, and risk of cancer [97, 139].

Another potential part of the explanation is through height-associated genes and risk of cancer [103], as seen in one study on testicular cancer [140].

Conclusions

Paper I does not provide strong support to the hypothesis that circulating androgens within the normal range affects risk of prostate cancer. In paper II, the association between fatherhood status and risk of prostate cancer can to some extent be explained by differences in socioeconomic factors, and uptake of PSA testing, between fathers and childless men. Whether the remaining association is causal remains to be elucidated. Furthermore, in
paper III, we found no support for the hypothesis that increased fertility is associated with an increased risk of prostate cancer.

The association between height and risk of cancer seen in paper IV is evident for many cancer forms and this finding merits further investigation.
**Future perspectives**

A unique property of prostate cancer is that through the increased use of PSA tests for early detection of prostate cancer, the panorama of tumors that are being diagnosed today differ from those diagnosed 20 years ago. This puts an extra challenge on research on prostate cancer, and especially on attempts to find risk factors. Thus, it is crucial that prostate cancer is not treated as one diagnosis, but instead analysed as potentially different diseases for low-risk and high-risk tumors. To do this, detailed tumor characteristics are required.

Also, although risk category can give an indication of whether the tumor was a result of opportunistic PSA screening, and main reason for the diagnostic work-up that lead to the diagnosis of prostate cancer is recorded in the NPCR, there can still be an uncertainty about which tumors were a result of opportunistic screening.

Since socioeconomic factors influence both the uptake of opportunistic PSA screening, and to some extent diagnostics and treatment after the diagnosis [141], these factors are also important to take into consideration.

In this thesis, paper II and III are based on the database PCBaSe 2.0, which in turn is based on NPCR. NPCR is continuously updated with new cases, a work which is performed by health care workers in their everyday work. There is an increasing number of national quality registers for different diagnosis and this is positive from many aspects such as quality assurance and research. Also, comparing health care across the nation is crucial in order to minimize inequalities in care between hospitals.

However, the time spent on registration is a problem. Hopefully this issue can be solved in the future by selected data being extracted directly from patient charts into quality registers. This would also likely improve coverage and data quality in the registers.

That androgens are involved in prostate tumorigenesis is undoubtable. Still, the best way of further exploring this topic is not as clear. Since androgen levels within the normal range does not seem to have a large impact on the risk of prostate cancer, very large studies would be needed to find any associations.

Perhaps using another proxy of androgen levels, which is more precise than fatherhood status and twin fatherhood status, would be of value. However,
the proxy must also be easy to assess in order to gain sufficiently large datasets.

The association between height and risk of cancer has now been seen in several large studies and this merits further investigation. Possibly studies with added information on prospective IGF-1, as well as height associated genes, might come closer to the mechanism behind this association. Preferably, the study would also include information on diagnostic procedures and tumor characteristics in studies on incident cancer, and treatments received for studies on cancer mortality.

However, making such a study with sufficiently large datasets would be hard due to the need for large biobanks with prospective samples available, as well as financial issues. Perhaps a first step would be to look closer at the cancer forms which have shown the strongest associations to risk.
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References


12. NORDCAN. (2010) Faktablad Cancerstatistik Sverige


