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Genetic Epidemiology of  
Hereditary Prostate Cancer  
in Finland



ACADEMIC DISSERTATION

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## ACADEMIC DISSERTATION

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**To Satu and Markus**

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## LIST OF ORIGINAL COMMUNICATIONS

**I. Matikainen MP**, Pukkala E, Schleutker J, Tammela TLJ, Koivisto P, Sankila R, Kallioniemi O-P: Relatives of prostate cancer patients have an increased risk of prostate and stomach cancers: a population-based, cancer registry study in Finland. *Cancer Causes Contr* 12: 223-230, 2001.

**II. Matikainen MP**, Sankila R, Schleutker J, Kallioniemi O-P, Pukkala E: Nationwide cancer family ascertainment using Finnish Cancer Registry data on family names and places of birth for 35,761 prostate cancer patients. *Int J Cancer* 88: 307-312, 2000.

**III.** Xu J\*, Meyers D, Freije D, Isaacs S, Wiley K, Nusskern D, Ewing C, Wilkens E, Bujnovszky P, Bova GS, Walsh P, Isaacs W (Group 1)  
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Smith J\*, Bailey-Wilson J, Carpten J, Stephan D, Gillanders E, Admundson I, Kainu T, Freas-Lutz D, Baffoe-Bonnie A, Van Aucken A, Sood R, Collins F, Brownstein M, Trent J (Group 5)

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Evidence for a prostate cancer susceptibility locus on the X chromosome. *Nat Genet* 20: 175-179, 1998.

**IV.** Schleutker J \*, **Matikainen MP \***, Smith J \*, Koivisto P, Baffoe-Bonnie A, Kainu T, Gillanders E, Sankila R, Pukkala E, Carpten J, Stephan D, Tammela T, Brownstein M, Bailey-Wilson J, Trent J, Kallioniemi O-P

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A Genetic epidemiological study of hereditary prostate cancer (HPC) in Finland: Frequent HPCX linkage in families with late onset disease. *Clin Cancer Res* 6: 4810-4815, 2000.

**V.** Ikonen T, **Matikainen MP**, Mononen N, Hyytinen E-R, Helin HJ, Tommola S, Tammela TLJ, Pukkala E, Schleutker J, Kallioniemi O-P, Koivisto PA: Association of E-Cadherin germline alterations with prostate and gastric cancer. (submitted)

**VI. Matikainen MP**, Schleutker J, Mörsky P, Kallioniemi O-P, Tammela TLJ: Detection of subclinical cancers by prostate-specific antigen screening in asymptomatic men from high-risk prostate cancer families. *Clin Cancer Res* 5: 1275-1279, 1999.

## ABBREVIATIONS

AR	Androgen receptor (gene)
AT	Ataxia-telangiectasia
BPH	Benign prostatic hyperplasia
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
CAPB	Prostate and brain cancer locus at 1p36
CDH1	E-Cadherin gene
CI	Confidence interval
CGH	Comparative genomic hybridization
cM	CentiMorgan
CYP17	17-hydroxylase cytochrome P450 gene
DHT	Dihydrotestosterone
DNA	Deoxyribonucleic acid
FAP	Familial adenomatous polyposis
FCR	Finnish Cancer Registry
FDA	The U.S. Food and Drug Administration
HNPCC	Hereditary non-polyposis colorectal cancer
HPC	Hereditary prostate cancer
HPC1	Hereditary prostate cancer gene locus 1 at 1q24-q25
HPC2 / ELAC2	Hereditary prostate cancer gene 2
HPCX	Hereditary prostate cancer gene locus at Xq27-q28
IGF-1	Insulin-like growth factor 1
IR	Ionizing radiation
LD	Linkage disequilibrium
LH	Luteinizing hormone
NMM	No male-to-male (transmission)
OR	Odds ratio
PCAP	Prostate cancer gene locus at 1q42.2-q43
PCR	Polymerase chain reaction
PIN	Prostatic intraepithelial neoplasia
PSA	Prostate specific antigen
PTEN	Phosphatase and tensin homolog gene
RSR	Relative survival rate
SIR	Standardized incidence ratio
SNP	Single nucleotide polymorphism
SPR	Standardized prevalence ratio
SSCP	Single-strand conformation polymorphism (analysis)
TGS	Tumor suppressor gene
VDR	Vitamin D receptor (gene)
1,25-D	1,25-dihydroxyvitamin D

## ABSTRACT

Family history is one of the strongest risk factors for prostate cancer. Five to ten percent of prostate cancers may be strongly influenced by inherited genetic defects. The aim of this study was to search for genetic risk factors and susceptibility genes for human prostate cancer in Finland using epidemiological and molecular genetic methods.

The analysis of the standardized incidence ratios (SIR) of prostate cancer and other cancers in 11,427 first-degree relatives of 1,546 prostate cancer patients indicated that male relatives of prostate cancer patients had a significantly increased, approximately 2-fold, relative risk of prostate cancer. We also observed an association between gastric cancer and early-onset prostate cancer.

We then developed and validated a new method for rapid, nation-wide cancer family ascertainment using Finnish Cancer Registry data on 35,761 prostate cancer cases over a 40-year period. This "population array" is based on the sorting of the cancer registry data by family name and place of birth, as well as the determination of a higher than expected number of cancer cases (elevated standardized prevalence ratio, SPR) associated with such combinations. 468 candidate prostate cancer families were identified using this approach.

As a part of an international genetic linkage collaboration, we obtained evidence for the location of a prostate cancer susceptibility gene on the X chromosome at Xq27-q28 (HPCX). Linkage with a maximum two-point LOD score of 4.60 was observed in 360 families from Finland, Sweden and the USA. The HPCX locus on Xq27-q28 seems to explain a particularly large fraction of the Finnish hereditary prostate cancer cases, especially among families with no male-to-male transmission and late age of diagnosis. In contrast, we found no evidence of the involvement of the HPC1 locus at 1q24-q25 in Finnish families.

We also investigated, whether mutations of the E-Cadherin gene (CDH1) would be involved in cancer predisposition in families and individual patients with both gastric and prostate cancers. Fifteen of the 180 Finnish hereditary prostate cancer families (8.3%) had one or more gastric cancer cases. No truncating or splice-site CDH1 mutations were identified. However, a novel S270A missense mutation in exon 6 of the CDH1 was found in a small fraction of prostate cancer cases in Finland. Individual rare mutations and polymorphisms in the CDH1 gene may therefore contribute to the onset of prostate cancer, but the CDH1 gene does not explain the link between prostate and gastric cancers at the population level.

The improved knowledge of the epidemiology and molecular basis of hereditary prostate cancer has led to a need to provide counseling and clinical follow-up for men with a strong positive family history of prostate cancer. Serum PSA was elevated in 10% of unaffected men with positive family history of prostate cancer. Seven prostate cancers (3.3%) and two high-grade PIN lesions were diagnosed based on PSA screening in these families, with three cancers occurring in men aged 59 years or less. The results suggested that serum PSA screening may have the utility in the management and follow-up of unaffected male individuals especially in prostate cancer families with an early age of cancer diagnosis.

In summary, this thesis determined the role of familial risk factors in the development of prostate cancer in Finland. We also developed significant research resources for genetic studies of prostate cancer. A novel HPCX locus for prostate cancer predisposition was identified. This locus may be particularly important in prostate cancer causation in Finland. Finally, serum PSA screening may have the utility in the management and follow-up of unaffected male individuals especially in prostate cancer families with an average early age of cancer diagnosis.



## INTRODUCTION

Prostate cancer has become a major health problem in the western world during the last decades of the 20th century. It is now the most common cancer among Finnish men with 2,839 new cases reported in 1997. Prostate cancer is also second after lung cancer as a cause of cancer mortality in men (Finnish Cancer Registry, 2000). The incidence of prostate cancer has increased rapidly during the last 15 years, partly due to the widespread use of serum prostate specific antigen (PSA) measurements (Potosky et al., 1995; Jacobsen et al., 1995, Hankey et al., 1999). In spite of the high incidence and mortality rate of this malignancy, the etiology of prostate cancer has remained poorly understood.

The data on immigrant studies suggest a strong impact of environmental factors in the etiology of the disease (Armstrong and Doll, 1975; Shimizu et al., 1991). During the last years also evidence for genetic risk factors of prostate cancer has accumulated. One of the strongest risk factors is a positive family history (Steinberg et al., 1990; Carter et al., 1993). Most studies suggest that genetic factors are clearly involved in about 5-10 % of the prostate cancer cases (Carter et al., 1992; Schaid et al., 1998; Ostrander and Stanford, 2000). In contrast, a recent twin study (Lichtenstein et al., 2000) indicated that up to 40% of prostate cancers may be influenced by inherited effects. Regardless of the specific percentage of genetically determined cases, the interplay between genetic factors, endogenous hormones and environmental factors, including e.g. dietary fat is likely to be important in the pathogenesis of prostate cancer (Ross and Henderson, 1994; Kolonel, 1996; Ekman et al., 1999; Pentylala et al., 2000; Bosland, 2000).

A substantial progress has recently been made in the studies concerning genetic risk factors and development of prostate cancer. The first gene predisposing to human prostate cancer, HPC1, was linked to chromosome region 1q24-q25 by Smith et al. in 1996. At least six additional loci that may harbor susceptibility genes for prostate cancer have been identified during the last few years (Berthon et al., 1998; Xu et al., 1998; Gibbs et al., 1999; Tavtigian et al., 2000; Berry et al., 2000; Suarez et al., 2000). However, only one candidate susceptibility gene ELAC2 has so far been identified (Tavtigian et al., 2000; Rebbeck et al., 2000). The identification of these genes would enable a genetic diagnosis of the prostate cancer susceptibility. Studies of such genes could also shed light on the basic processes of prostate cancer development and progression process and form a basis for developing new effective targeted therapeutic methods.

In this thesis I have focussed on the search for genetic risk factors and susceptibility genes of prostate cancer using epidemiological and molecular methods in the Finnish population. We made use of the Finnish Cancer registry data, population registry data, parish records surveys, and genealogical data to study prostate cancer epidemiology in Finland, and to identify families with prostate cancer. The specific features of the Finnish population facilitated the genotyping studies designed to identify predisposition loci to prostate cancer in the Finnish population.

# **REVIEW OF THE LITERATURE**

## **1. Natural history of prostate cancer**

### **1.1. Histology and histogenesis of prostate cancer**

Prostate cancer is believed to arise from the secretory epithelial cells that line the luminal surface of the prostatic ducts and acini (Ware, 1994). Most carcinomas arise in the peripheral zone of the prostate gland, where also the earliest detectable precursor lesion of prostate cancer, prostate intraepithelial neoplasia (PIN), is found (Bostwick et al., 1987; Sakr et al., 1993; Ware, 1994). The likelihood that an individual PIN lesion progresses into clinical cancer is assumed to be low (Epstein, 1994). Another common early lesion is the indolent microscopic prostate cancer. In autopsy studies of prostates of 70-80 year-old men who have died from other causes than cancer, microscopic foci of adenocarcinoma are present in more than 50% of the cases (Breslow et al., 1977; Sheldon et al., 1980; Sakr et al., 1993). In most cases, these lesions never progress to clinical cancer in the life-time of the individual (Gittes, 1991). Histological prostate cancer is found at an equally high frequency in many different populations (e.g. in Japanese and American men), even though the incidence rates of clinical prostate cancer are very different in these populations (Breslow et al., 1977; Carter et al., 1990). Progression of latent histological cancers to clinically evident tumors seems to be the major rate-limiting step in prostate tumorigenesis.

Clinically detected prostate carcinomas display a variety of phenotypic features and malignant potential. Majority of all prostate carcinomas are typical adenocarcinomas, which can be divided into different tumor grades (Gleason, 1992). The histological differentiation together with tumor stage, determined by tumor size, as well as the presence of lymph-node and distal metastases are used to assess the prognosis of the patients (Gittes, 1991). The average 5-year survival of patients with clinically detected prostate cancer is largely dependent on the stage of the tumor at the time of diagnosis and varies from 84% for localized, early-stage, low grade disease to 25% for patients with advanced disease (Dickman et al., 1999).

An accumulation of genetic changes affecting critical genes is thought to underlie the gradual malignant transformation and cancer progression (Fearon and Vogelstein, 1990; Carter H et al., 1990; Solomon et al., 1991). Stem cell hypothesis with accumulating DNA damage has been suggested also in prostate cancer development (De Marzo et al., 1998). In prostate cancer the details of these genetic mechanisms are still unclear. Histological grade and tumor stage are still the critical prognostic factors at the present. There are ongoing efforts to develop additional prognostic indicators of prostate cancer based on improved understanding of the biology of the disease.

### **1.2. Androgen dependency and treatment of prostate cancer**

Androgens play a major role in the tumorigenesis of prostate cancer. Prostate cancer is considered to be the most hormone-dependent of all tumor types (Bosland, 2000). The androgen-dependency of prostate cancer growth was first reported by Huggins and Hodges 60 years ago (Huggins and Hodges, 1941). Clinical prostate cancer is usually highly dependent on the supply of bioactive androgens.

Prostate cancer is a curable disease only in its localized stage. The prognosis of localized prostate cancer is often good even without curative therapy (Gittes, 1991; Berner et al., 1999).

Radical prostatectomy and radical radiation therapy, the standard therapies for localized prostate cancer, are thus recommended only for patients with a life expectancy exceeding 10-15 years. Up to 20-40% of all prostate cancers are diagnosed at a clinically advanced stage (Scardino et al., 1994; Dickman et al., 1999; Määttänen et al., 1999), when curative treatment is no longer possible. For these patients, hormonal therapy is usually an effective treatment. About 70-90% of the prostate cancers initially respond to hormonal therapy (Greyhack et al., 1987; Mahler and Dennis, 1995; Palmberg et al., 1999). However, often hormone-refractory prostate cancer arises during hormonal therapy. There is a great need to develop new therapies to hormone-refractory prostate cancer.

## **2. Epidemiology of prostate cancer**

### **2.1. Trends in incidence and mortality of prostate cancer**

Prostate cancer has become a major health problem in industrialized world during the last decades of the 20<sup>th</sup> century. It is now the most common male cancer in the USA, with 317,000 new cases in 1996 (Ries et al., 2000), and it is estimated that one in eight men will develop clinical prostate cancer in their lifetime in the USA (Ries et al., 1999). In the European Union it is the second most common malignancy in men, with 134,865 new cases and 55,704 deaths in 1996 (Ferlay et al., 1999). In Finland prostate cancer has been the most common male malignancy since 1993 with 2,839 new cases in 1997 and it is second after lung cancer as a cause of cancer mortality (Finnish Cancer Registry, 2000).

During the last 20 years prostate cancer incidence has undergone some of the most dramatic swings observed in cancer statistics. In the USA the incidence of prostate cancer increased by 30% from 80 to 105 per 100,000 men between 1980 and 1988, with a 2.5% rise in the mortality from the disease (Ries et al., 1999). From 1989 to 1992 the incidence of prostate cancer increased, on average, 20% per year, reaching the peak incidence of 179 per 100,000 men in whites in 1992 and 250 per 100,000 in blacks in 1993 (Hankey et al., 1999). Since 1993 a decreasing incidence trend, at a rate of 10.8% per year, has been observed, and in 1997 the average incidence of prostate cancer in the USA was 149.7 per 100,000 men (Hankey et al., 1999; Ries et al., 2000). In Finland the incidence of prostate cancer increased slowly from the 1960s to the beginning of 1990s with age-adjusted incidence per 100,000 men increasing from 22.8 to 39.1. A rapid increase in prostate cancer incidence has been observed since 1991 with age-adjusted incidence per 100,000 men increasing from 43.2 in 1991 to 72.1 in 1997 (Finnish Cancer Registry, 2000). The annual number of prostate cancer cases is still increasing in Finland.

Age-adjusted prostate cancer mortality has also increased in the USA over the last several decades, with an acceleration in increase observed in the mid 1980s. The mortality started to decline in the USA in 1991, but in relation to the changes in incidence, the magnitude of the mortality decline has been small, from 26.7 deaths per 100,000 men in 1991 to 24.9 deaths per 100,000 men in 1995, a decrease of 6.7% (Ries et al., 1999). It has been estimated that at the age of 55 years, a US male has approximately 3% risk of dying from prostate cancer (Ries et al., 2000). In Finland the age-adjusted mortality of prostate cancer has been quite steady during the last decades. The relative survival rate (RSR) of prostate cancer patients has improved in Finland during the last 40 years. The increase in the 5-year RSR has been the slowest among the youngest patients (45-59 years) and fastest in the oldest group ( $\geq 75$  years). (Dickman et al., 1999) The increase in the RSR over time is probably mostly due to improved

diagnostics leading to a more favorable stage distribution of the tumors, and also to the diagnosis of small, intracapsular cancers, which otherwise would have not been detected clinically. The worse prognosis among younger men could be due to their more aggressive tumors, and also to diagnostic delays in those ages in which prostate cancer is rare (Dickman et al., 1999).

Like in all cancers, prostate cancer risk is strongly associated with age. In Finland 68% of prostate cancers were diagnosed after the age of 70 years in 1995, whereas only 0.4% were diagnosed before the age of 50 years (Finnish Cancer Registry, 1997). The mean age at diagnosis in Finland was 71 years. The incidence of latent prostate cancer begins to increase for men in their early 40s, and continues to increase throughout the remainder of their life (Sakr et al., 1993). A strong international and ethnic variation in incidence is another well established demographic characteristic of prostate cancer (Parkin, 1992). In 1988-1992 the incidence was highest in African Americans (137.0 per 100,000 men, age-standardized to the world population), high in Caucasian Americans (100.8 per 100,000 men) and Scandinavians (31-55 per 100,000 men) (Ferlay et al., 1997). Areas of low incidence are in Eastern Asia (2 per 100,000 men in Shanghai) (Parkin, 1992). Historically, the difference in incidence between high and low risk populations has been reported to be 50- to 100-fold. A part of this historical difference is likely to be due to differences in detection strategies used for finding prostate cancer in different populations (Shimizu et al., 1991). With the increased utilization of PSA as a detection method of prostate cancer, it has become increasingly difficult to determine the true range of prostate cancer incidence around the world. However, in studies investigating the differences in prostate cancer mortality, substantial international and ethnic variation remains (Zaridze et al., 1984).

The variation of incidence of prostate cancer in different ethnic groups and emigrants has also given clues to risk factors of prostate cancer. The high rate of prostate cancer in emigrants from Asia (with low incidence of prostate cancer) to the USA (with the highest incidence in the world) provides strong evidence in favor of environmental and life-style factors as risk factors of prostate cancer (Akazaki and Stemmermann, 1973; Kolonel et al., 1988; Shimizu et al., 1991; Cook et al., 1999). On the other hand, high prostate cancer incidence in African Americans has been suggested to be attributable to genetic factors (Irvine et al., 1995). Carter and colleagues (1990) have presented that although the age-specific prevalence of histologic prostate cancer is similar in Japan and in the USA, there is a marked difference in the age-specific prevalence of clinical prostate cancer between Japanese and American men. These data suggest that the initiation rate of prostate cancer may be the same in both groups but that there appear to be differences in the rate of promotion or progression to clinically evident prostate cancer.

## **2.2. PSA screening**

In the late 1980s, prostate specific antigen (PSA) came into wide use as a prostate cancer detection method (Stamey et al., 1987; Catalona et al., 1991). The U.S. Food and Drug Administration (FDA) approved the PSA test for the purpose of monitoring disease status of prostate cancer patients in 1986 and for aiding in the detection of the prostate cancer in men 50 years and older in 1994 (Hankey et al., 1999). However, the use of the PSA test for the diagnosis of prostate cancer, either in response to symptoms or for screening, increased dramatically in the USA from 1988 onwards (Potosky et al., 1995; Legler et al., 1998). The use of the PSA test is associated with a substantial increase in the incidence of prostate cancer in men 65 years and older during the late 1980s and early 1990s in the USA (Potosky et al.,

1995). In Finland, and many other European countries, this increase took place a bit later, in the early and mid 1990s (Auvinen et al., 1996).

Randomized prostate cancer screening studies are ongoing in several countries, including Finland (Schröder and Bangma, 1997; Määttänen et al., 1999). It is obvious that regular PSA testing of asymptomatic, middle-aged men reduces the number of men diagnosed with advanced or metastatic disease. However, it has not yet been established by ongoing randomized controlled PSA screening trials whether mortality of prostate cancer can be reduced by screening (Kramer et al., 1993; Gohagan et al., 1994; Hankey et al., 1999). The decrease in the incidence of the advanced stage disease in the USA since 1991, and the decline in the incidence of the earlier stage disease beginning in 1992 are consistent with PSA screening effect and give some support to the hopes that testing for PSA may lead to a sustained decline in prostate cancer mortality (Hankey et al., 1999).

There is also some concern that PSA screening leads to the diagnosis of many clinically insignificant (incidental/latent) cancers, which would not cause mortality or even cause symptoms to the patients (Wolf et al., 1996; Saksela, 1998). Etzioni et al. (1998) have estimated that 50% of the new prostate cancer cases would not have been clinically diagnosed in the absence of PSA testing. Also there will be false positive screening tests, which will lead to subsequent invasive procedures (Smith D et al., 1996). Despite these risks, PSA screening for prostate cancer is recommended by the American Cancer Society (von Eschenbach et al., 1997). Definitive results regarding usefulness of PSA screening in reduction of prostate cancer mortality will be available only in the future.

### **2.3. Risk factors for prostate cancer**

The etiology of prostate cancer is poorly known. Epidemiological studies have identified a number of risk factors. Most investigators agree that prostate cancer results from an interplay between genetic factors, endogenous hormones and environmental influences (Ross and Henderson, 1994; Kolonel, 1996; Ekman et al., 1999; Pentylala et al., 2000; Bosland, 2000). Together with race and age, family history is the best characterized of the currently identified risk factors (Carter et al., 1993). A complicating factor in dissecting risk factors for prostate cancer is that an individual's metabolism and response to dietary factors, the level of endogenous hormones, the changes of hormonal factors as a result of a diet and many other interactions may all be influenced by genetic factors as well.

#### ***Hormonal influences***

Because of the important role of hormones in controlling growth and proliferation of normal prostate cells as well as prostate cancer cells, the same hormones might be involved in abnormal growth of the prostate including carcinogenesis. Altered hormone metabolism could also play a role in the progression of prostate cancer from histologic to clinically significant forms. The incidence of prostate cancer is very low in eunuchs and castrated men (Wynder et al., 1984; Hovenian and Deming, 1948). There is also some evidence that serum testosterone and luteinizing hormone (LH) levels are correlated with prostate cancer risk (Bosland, 2000). The difference in prostate cancer incidence between African Americans and Caucasians has also been suggested to be due to higher serum testosterone levels in African Americans (Ross et al., 1986). However, higher circulating levels of testosterone in patients with prostate cancer have not been consistently observed (Bosland, 2000). Another hormone that has been linked with prostate cancer development is IGF-1, whose increased levels have been associated with prostate cancer (Chan et al., 1998; Wolk et al., 1998), but also conflicting

results have been presented (Pollak, 2000). Also other hormones, especially prolactin and estrogen, may play a role in prostate growth and differentiation (Bosland, 2000). However, further studies on these hormonal risk factors are required. Currently, serum-based biomarker assays do not reliably explain population differences in prostate cancer incidences and these markers cannot be used to identify individuals who are at a high risk for prostate cancer development (Chan et al., 1998).

### ***Benign prostatic hyperplasia***

An association between prostate cancer risk and prior occurrence of benign prostatic hyperplasia is biologically unlikely. Although both diseases appear to be androgen dependent, benign prostatic hyperplasia arises most often in the central or transitional zone of the prostate, whereas more than 80% of all cancers develop in the peripheral zone of the gland. Nevertheless, evidence that patients with history of benign prostatic hyperplasia have a higher risk for prostate cancer has been suggested in some studies (Armenian et al., 1974; Greenwald et al., 1974; Bosland, 2000).

### ***Vasectomy***

It has been suggested that vasectomy may increase the risk of prostate cancer. This hypothesis is based on observations that vasectomized men have higher levels of circulating testosterone (Honda et al., 1988). Vasectomy has been identified as a possible risk factor for prostate cancer in several case-control (John et al., 1995) and cohort studies (Sidney, 1987, Giovannucci et al., 1993). Meta-analysis (Bernal-Delgado et al., 1998) of 14 studies has indicated that there is no causal relation between vasectomy and prostate cancer. However, further studies will be required to rule out this risk factor.

### ***Sexual behavior***

Several studies have addressed the possibility that sexual factors play a role in prostate cancer etiology (Honda et al., 1988; Pienta and Esper, 1993). An association between total testosterone levels and sexual activity has been suggested in some of these studies (Bosland, 2000). The results of these studies suggest that prostate cancer risk may be associated with the level of sexual activity, but no direct evidence exist for such relation (Pienta and Esper, 1993).

### ***Dietary fat***

There is a considerable consistency across studies indicating that a high intake of fat, particularly total fat and saturated fat, is a risk factor for prostate cancer (Giovannucci et al., 1993; Kolonel, 1996; Lee et al., 1998). However, the strength of the associations is modest at best and may be greater for African-Americans than for European-Americans (Whittemore et al., 1995). It has been estimated that dietary fat intake may account for 10-15% of the difference in prostate cancer occurrence between Caucasians, African-Americans and Asians (Whittemore et al., 1995). The mechanisms that mediate the effect of fat on prostate carcinogenesis are not understood. The effects of dietary factors, such as that of fat, may be mediated through endogenous hormones (Bosland, 2000). A low-fat, high-fiber diet has been shown to affect male sex hormone metabolism by decreasing circulating testosterone (Adlercreutz, 1990, Hämäläinen et al., 1983, Hämäläinen et al., 1984). Besides fat, high intake of protein and energy and low intake of dietary fiber and complex carbohydrates have been found to be associated with the increased risk for prostate cancer (Kolonel, 1996). Also studies showing positive correlation between obesity (high body-mass index) and prostate cancer suggest significant role of fat and high energy diet as a risk factor for prostate cancer (Giles and Ireland, 1997).

### ***Vitamins and trace elements***

A variety of vitamins, trace elements and nutrients have been suggested to reduce the risk of prostate cancer, but the results of epidemiological studies are inconsistent (Kolonel, 1996; Gann, 1998). Intake of alpha-tocopherol (an E-vitamin) was found to significantly decrease the risk of prostate cancer in a large Finnish cancer prevention study (Heinonen et al., 1998). Prostate cancer incidence was 32% lower in the alpha-tocopherol group as compared to the controls. However, in the same study the incidence of prostate cancer was 23% higher and prostate cancer mortality 15% higher in a group receiving beta-carotene as compared to a control group receiving placebo. Epidemiological studies on the association between prostate cancer risk and intake of dietary vitamin A and beta-carotene are conflicting (Kolonel, 1996; Pentylala et al., 2000). It is possible that retinoids and carotenes enhance rather than inhibit development of prostate cancer under certain circumstances or in certain populations, although animal and in vitro studies have suggested a protective effect of retinoids (Kolonel, 1996). Association of vitamin D with prostate cancer has also been suggested (Peehl, 1999). An active human D-vitamin metabolite, 1,25-dihydroxyvitamin D (1,25-D), inhibits cell proliferation in cultured normal and malignant prostatic epithelium and plays a role in the differentiation of prostate cells (Skowronski et al., 1993). In a prospective study (Corder et al., 1993) levels of 1,25-D were found to be significantly lower among men who developed prostate cancer. Also trace elements, like selenium (Clark et al., 1998) have been significantly associated with a decreased risk of prostate cancer.

### ***Phytoestrogens***

Phytoestrogens include isoflavonoids that are found in soy products, and have weak estrogenic activity but also some estrogen agonistic activity. Higher levels of circulating levels of phytoestrogen metabolites have been observed in Asian men compared to European men (Adlercreutz et al., 1993). These estrogenic compounds could theoretically modulate androgenic action in the prostate, but their role remains unclear.

In general, the results from dietary intake studies support the concept that a high-fiber, low-fat diet may protect men against the development of prostate cancer. Associations with prostate cancer risk, reported for individual nutrients or foods, are not very strong. It is, therefore, conceivable that the combined effects of dietary factors on prostate cancer carcinogenesis are more important than the separate effects of any individual dietary factor (Pienta and Esper, 1993; Pentylala et al., 2000).

### ***Physical activity and anthropometric correlates***

There are studies suggesting that the level of physical activity may be a possible risk factor for prostate cancer, but the evidence for such an association is inconclusive (Andersson et al., 1997). Exercise may decrease or increase circulating androgen concentrations or have no effect, depending on the type of exercise and time of sampling. The hormonal influences may mediate the effect of exercise (Bosland, 2000). Evidence of the role of obesity or an increased body-mass index as a risk factor for prostate cancer is also controversial (Kolonel, 1996). A positive association between prostate cancer risk and muscle mass, but not fat mass, has been observed (Severson et al., 1988). This may suggest exposure to endogenous or exogenous androgenic hormones or other anabolic factors (Bosland, 2000).

### ***Socioeconomic factors***

Positive social class gradient has been suggested in prostate cancer (Rimpelä and Pukkala, 1987). In a study by Baquet and colleagues (Baquet et al., 1991), incidence of prostate cancer was generally higher in African-American men than in white men but no statistically

significant association was observed between socioeconomic status and prostate cancer incidence. Similar results were observed earlier in other studies (Ernster et al., 1978; McWhorter et al., 1989). The currently available, largely conflicting body of research reports, tend to support the concept that socioeconomic status is not an important risk factor for the development of prostate cancer (Pienta and Esper, 1993).

### ***Occupation***

Studies examining the risk of prostate cancer and occupation have also led to variable results. Industries and occupations that have been associated with higher incidence of prostate cancer include mechanics, newspaper workers, plumbers, rubber manufacturing industry workers and farmers, but many of these reports have not been confirmed (Tola et al., 1988; Pienta and Esper, 1993; Andersen et al., 1999). Industries in which workers are exposed to cadmium have been studied very intensively. Cadmium is a trace mineral found in cigarette smoke and alkaline batteries. People working in the welding and electroplating occupations are exposed to high levels of cadmium. Most of the studies investigating association of cadmium and prostate cancer risk support the hypothesis that cadmium exposure slightly increases the risk of prostate cancer (Pienta and Esper, 1993). It has been suggested that cadmium increases the risk for prostate cancer by interacting with zinc, a trace element necessary in many metabolic pathways (Pienta and Esper, 1993).

### ***Smoking***

Smoking is a strong risk factor for lung and bladder cancers. Several studies have suggested that cigarette smoking may be also a risk factor for prostate cancer. Hsing and co-workers (Hsing et al., 1990) observed an increased relative risk of prostate cancer for cigarette smoking (Odds ratio (OR) 1.8) and for chewing tobacco (OR 2.1). Coughlin and colleagues (1996) observed in their study of 348,874 men that the risk of developing prostate cancer was 1.21 to 1.45 -fold increased among men with a history of smoking as compared to non-smokers. However, compared to its very strong impact on carcinogenesis of other organs, it appears that cigarette smoking adds little, if any, to the risk for developing prostate cancer (Lumey, 1996).

### ***Infectious agents***

Links between prostate cancer and sexually transmitted diseases, including viral carcinogenesis have been suggested, but not proven (Pienta and Esper, 1993). Higher titers of herpesvirus, cytomegalovirus and human-papillomavirus in men with prostate cancer as compared to population controls have been observed in some studies (Dilner et al., 1998). The relationship between the risk of developing prostate cancer and a history of sexually transmitted disease or viral exposure remains unclear but warrants further studies.



**Table 1** Suggested etiologic factors for prostate cancer

Genetic factors	Race
	Family history
Internal factors	Hormones
	History of BPH *
	Vasectomy *
	Sexual activity, marital status *
External factors	Diet
	Fat
	Vitamins and trace elements *
	Physical activity, anthropometric correlates *
	Smoking
	Infectious agents *
	Socioeconomic factors *
	Occupation, environment

\* Evidence conflicting

## 2.4 Hereditary prostate cancer

The first reports of familial aggregation of prostate cancer were published by Morganti et al. (1956) and by Woolf (1960). These studies showed that the risk of prostate cancer was significantly increased in first-degree relatives of prostate cancer patients. A large number of studies over the past 30 years have confirmed these early observations (Table 2, page 19). These studies indicate that the risk of prostate cancer in brothers and sons of men with prostate cancer is two to ten fold increased. Particularly high risk has been associated with men having multiple affected relatives, or relatives diagnosed at an early age (Cannon et al., 1982; Steinberg et al., 1990; Goldgar et al., 1994; Hayes et al., 1995, Keetch et al., 1995, Mettlin et al., 1995; Whittemore et al., 1995; Grönberg et al., 1996; Lesko et al., 1996; Cerhan et al., 1998). This familial risk has been observed both in the low-risk (Asian Americans) (Whittemore et al., 1995) and high-risk populations (African Americans and Caucasians) (Hayes et al., 1995; Whittemore et al., 1995). Reports of familial aggregation of prostate cancer in Japan (Ohtake et al., 1998) and Jamaica (Glover et al., 1998) have also been published.

### 2.4.1 Case-control studies

Most of the studies on familial risks of prostate cancer have been case-control studies (Table 2, page 19). In case-control studies, the frequencies of exposure to one or more specific risk factors are assessed for a group of individuals (cases) who have developed a disease and for another group consisting of unaffected individuals (controls). The odds of exposure among cases is compared to the odds of exposure among controls, and the odds ratio is calculated. In case-control studies of prostate cancer relative prostate cancer risks for the relatives have ranged from 2.0 (Monroe et al., 1995) to 18 (McCahy et al., 1996). The higher risks found in

case-control studies as compared to cohort and registry-based studies reflect the possibility of biases in case-control studies. These may include selection and validation of the control group and detection bias in the case group. The cancer diagnoses of relatives in the control group are difficult to validate. Also patients with cancer do not always tell about their disease to relatives. Detection bias can also be assumed in case-control studies of familial diseases. Relatives of cancer patients are concerned about possible inheritance of disease and are, therefore, more likely to have examination earlier, already in the absence of symptoms.

#### **2.4.2 Cohort and registry based studies**

In cohort studies, defined separated groups, or cohorts, of exposed and non-exposed individuals are followed over time and the incidence of the investigated disease is observed. Only three registry-based cohort studies have been published, one in American population based on the Utah Population Database (Goldgar et al., 1994) and two in Sweden based on the Swedish Cancer Registry (Grönberg et al., 1996; Bratt et al., 1997). The relative risks presented in these cohort studies have been slightly lower compared to estimates presented in many case-control studies (Table 2, page 19). In the Utah study, which studied familial clustering of 28 distinct cancer sites among first-degree relatives of cancer probands, the relative risk of prostate cancer in male relatives of prostate cancer was 2.21 (95% confidence interval 2.05-2.38) (Goldgar et al., 1994). The estimated risk observed in the Swedish population was 1.7 (95% confidence interval 1.5-1.9) in the study by Grönberg et al. (1996) and 1.4 (95% confidence interval 1.5-1.9) in the study by Bratt et al. (1997). Grönberg et al. (1996) used a cohort of 5,496 sons of Swedish men found to have prostate cancer between 1959 and 1963, brothers of the index patients were not included in the study. The study of Bratt and co-workers was limited to very early-onset prostate cancer cases (diagnosed under 51 years). In another cohort study by Cerhan et al. (1998), a very high relative risk of prostate cancer was observed (3.2, 95% confidence interval 1.8 – 5.7). The study was based on a follow-up of a cohort of 1,557 Iowan men, ages 40-86 years, who were randomly selected as cancer free controls for a population-based case-control study conducted in Iowa during years 1987-1989. Family history of cancer in parents and siblings was obtained using a mailed questionnaire and incidental cancers and deaths were ascertained through linkages to state and national databases. A study using Swedish Family-Cancer Database suggested familial standardized incidence ratio of 2.4 (95% confidence interval 2.1 – 2.8) for prostate cancer and a strong impact of age of onset of prostate cancer on familial risk (Hemminki and Dong, 2000).

Since no major genes for prostate cancer have yet been identified, hereditary prostate cancer can be defined only by family history questionnaires and pedigree analysis. The definition of hereditary prostate cancer proposed initially by Carter et al. (1993), includes nuclear families with three or more cases of prostate cancer, occurrence of prostate cancer in each of the three generations in the paternal or maternal lineage, or a cluster of two first-degree relatives diagnosed with prostate cancer at the age of 55 or earlier.

**Table 2** Epidemiological studies of family history as a risk factor for prostate cancer

Author(s) and year	Study design	No. of cases	RR (95% CI)	Relationship and / or age of affected relative(s)
Morganti, 1956	Case-control		11 (1.4-84)	first degree
Woolf, 1960	Case-control, death certificate data	n = 228	3.0	first degree
Krain, 1974	Case-control	n = 221	6.1 (1.4-27)	first degree
Schuman et al., 1977	Case-control		2.3 (0.6-8.4)	first degree
Cannon et al., 1982	Cohort	n = 2,824	2.4	brother
			6.0	brother <65 years, proband <65 years
Meikle et al., 1985	Case-control	n = 150	4.0	all <62 years
Steinberg et al., 1990	Case-control	n = 691	2.0 (1.2-3.3)	1 first degree relative affected
			1.7 (1.0-2.9)	1 second degree relative affected
			8.8 (2.8-28)	First and second degree relatives affected
			2.0 (1.3-3.0)	Father only
			1.9 (0.7-5.2)	Brother only
			2.7 (0.5-13)	Father and brother
			2.2 (1.4-3.5)	1 first degree relative affected
			4.9 (2.0-12)	2 first degree relatives affected
			11 (2.7-43)	3 first degree relatives affected
Fincham et al., 1990	Case-control	n = 382	3.2	first degree
			3.1 (1.5-6.4)	father
			3.3 (1.9-5.9)	brother or son
Ghadirian et al., 1991	Case-control	n = 140	8.7 (2.0-38)	first degree (1-4)
Spitz et al., 1991	Case-control	n = 378	2.4 (1.3 - 4.5)	first degree
			2.2 (1.0-4.8)	father
			2.7 (1.0-6.9)	brother
			2.1 (0.8 - 5.7)	second degree
Carter et al., 1992	Case-control	n = 691	7.1 (3.7-14)	first degree, age of proband ≤ 50
same material as in Steinberg et al., 1990			5.2 (3.1-8.7)	first degree, age of proband ≤ 60
			3.8 (2.4-6.0)	first degree, age of proband ≤ 70
Goldgar et al., 1994	Cohort, registry	n = 6,350	2.2 (2.1-2.4)	all
Mettlin et al., 1995	Case-control		2.5 (1.6-3.9)	brother
			2.3 (1.5-3.3)	father
Whittemore et al., 1995	Case-control	n = 1,496	2.5 (1.9-3.3)	first degree
			2.9 (2.0-4.2)	brother
			2.0 (1.3-2.9)	father or son
			6.4 (1.9-22)	father/son and brother
Narod et al., 1995	Cohort	n = 6,390	1.7 (1.2-2.4)	first and second degree
			2.6 (1.7-4.1)	brother
			1.2 (0.8-1.9)	father
			1.2 (0.3-5.6)	second degree
Monroe et al. 1995	Cohort	n = 1,486	2.9	brother
			2.0	father
Isaacs et al., 1995	Case-control	n = 690	1.8 (1.3-2.4)	brothers and fathers

Author(s) and year	Study design	No. of cases	RR (95% CI)	Relationship and / or age of affected relative(s)
Keetch et al., 1995	Case-control	n = 1,084	3.4 (2.6-4.4)	first and second degree
			4.7 (3.0-7.5)	brother
			3.5 (2.4-5.0)	father
			2.5 (1.3-4.8)	grandfather
			2.7 (1.7-4.5)	uncle
Hayes et al., 1995	Case-control	n = 981	3.2 (2.0-5.0)	first degree
			5.3 (2.3-13)	brother
			2.5 (1.5-4.2)	father
Grönberg et al., 1996	Cohort, registry	n = 5,496	1.7 (1.5-1.9)	son
Lesko et al., 1996	Case-control	n = 564	2.3 (1.7-3.3)	first degree
			1.9 (1.2-3.0)	father
			3.0 (1.8-4.9)	brother
			2.2 (1.5-3.2)	1 first degree
			3.9 (1.7-5.2)	≥2 first degree
			5.3 (2.5-12)	<60
			2.7 (1.3-5.5)	60-64
		1.6 (1.0-2.5)	>65	
McCahy et al., 1996	Case-control	n = 209	18 (2.3-139)	first degree
Rodriguez et al., 1997	Cohort	n = 3141 / 480,802	1.5 (1.3-1.8)	first degree, RR for fatal prostate cancer
Ghadirian et al., 1997	Case-control	n = 640	3.3 (2.2-5.0)	first degree
Neuhausen et al., 1997	Cohort	n = 6,350	2.2 (2.1-2.4)	all
			4.1 (2.0-7.1)	<60
Bratt et al., 1997	Cohort	n = 89	1.4 (0.8-2.3)	first degree all, probands <51 years
			3.4 (1.4-6.9)	first degree relatives < 70 years
Glover et al., 1998	Case-control	n = 263	2.1 (1.1-4.4)	first degree
			3.1 (0.8-18)	second degree
Cerhan et al., 1998	Cohort	n = 1,557	3.2 (1.8-5.7)	first degree
			4.5 (2.1-9.7)	brother
			2.3 (1.0-3.0)	father
Bratt et al., 1999	Case-control	n = 356	3.2 (2.1-5.1)	first degree
			5.1 (2.4-10)	father and/or brother, proband <60 years
Hemminki & Dong, 2000	Cohort, registry	n = 76,447	2.4 (2.1-2.8)	all
			3.5 (2.2-5.0)	son, age of proband (father)<65
Matikainen et al., 2001	Cohort, registry	n = 1,547	1.7 (1.4-2.1)	all
			2.5 (1.9-3.2)	first degree relatives ≤60
			2.7 (1.9-3.7)	first degree relatives 61-69
			1.2 (0.8-1.6)	first degree relatives 70-79
			1.8 (1.3-2.6)	first degree relatives ≥80

### 2.4.3. Twin studies

Studies of identical and non-identical twins enable the identification and quantification of the impact inherited factors to cancer development. Several twin-studies of human prostate cancer have been published (Grönberg et al., 1994; Page et al., 1997; Ahlbom et al., 1997; Verkasalo et al., 1999; Lichtenstein et al., 2000). In these studies, concordance rates for prostate cancer have been substantially higher among monozygotic twin pairs, than among dizygotic twin pairs, indicating the importance of genetic factors for development of prostate cancer. In the largest of these studies (Lichtenstein et al., 2000), combined data of 44,788 pairs of twins from Swedish, Danish and Finnish twin registries was used. Statistically significant effect of heritable factors was observed for prostate cancer. Based on estimations in this study, 42% of the risk of prostate cancer may be explained by heritable factors. According to this extensive study, hereditary factors contribute to the development of prostate cancer much more than to most other cancers. A study of Finnish twins indicated that 43% of the development of prostate cancer could be attributed to genetic defects (95% confidence interval 12-67%) and 57% (95% confidence interval 33-88%) to environmental effects (Verkasalo et al., 1999).

### 2.4.4. Segregation analyses

Segregation analyses try to build a model for the inheritance pattern of disease based on epidemiological data. These analyses are very difficult to carry out for complex diseases that are likely to be caused by multiple predisposing genes. However, such estimates are required for carrying out parametric linkage analyses of cancer. Three formal segregation analyses have been published (Carter et al. 1992, Grönberg et al. 1998, Schaid et al. 1998). All these have suggested an autosomal dominant mode of inheritance of rare (population frequency 0.3 - 1.7%) high risk allele, conferring 63 - 89 % risk of prostate cancer by age of 85 years (Carter et al., 1992; Grönberg et al., 1998; Schaid et al., 1998) (Table 3, page 22). In the study by Carter and co-workers (1992) the risk of prostate cancer for heterozygous carriers of prostate cancer risk allele was estimated to be 88% by the age of 85 years, as compared to 5% for non-carriers. The proportion of prostate cancer cases caused by this high risk allele was estimated to be 43% for cases diagnosed by the age of 55, 34% by the age of 70 and 9% by the age of 85 years. In the study of Grönberg et al. (1997), a higher frequency of the susceptibility allele (1.67%) and a lower lifetime penetrance (63%) was suggested. Schaid et al. (1998) reported that no single-gene model of inheritance clearly explained the observed familial clustering but the best fitting model was also a rare autosomal dominant gene, with the best fit observed in probands diagnosed under 60 years of age. Also autosomal recessive and X-chromosomal mode of inheritance have been suggested (Narod et al., 1995; Monroe et al., 1995). In the study of Narod et al. (1995) the prevalence of prostate cancer was increased in those men with any first-degree relative affected (prevalence = 6.7%; relative risk = 1.72 as compared with men with no first-degree relative affected; prevalence = 3.89; relative risk = 1.00). Most of the increase in relative risk was contributed by affected brothers (prevalence = 10.2%; relative risk = 2.62;  $P = 0.0002$ ), which was concluded to be suggestive to recessive or X-linked, genetic component to prostate cancer inheritance. Also in the study of Monroe et al. (1995) an excess risk of prostate cancer in men with affected brothers compared to those with affected fathers was observed, consistently with the hypothesis of an X-linked, or recessive, model of inheritance.

Based on the fact that autosomal dominant, recessive as well as X-linked modes of inheritance have been suggested for prostate cancer, prostate cancer is most likely caused by a number of genes, each with different models of inheritance, population frequencies and

penetrance. Reliable estimates of gene frequencies and penetrance cannot be made until the genes have been cloned and the frequencies of mutations have been screened at the population level.

**Table 3** Segregation analyses of prostate cancer

Study	Population	No. of families	Model suggested	Gene frequency	Cumulative risk at 85 years
Carter et al. 1992	American	691	Autosomal dominant	0.003	88%
Grönberg et al., 1997	Swedish	2857	Autosomal dominant	0.0167	63%
Schaid et al., 1998	American	4288	Autosomal dominant	0.006	89%

### 2.4.5. Association of other cancers with prostate cancer

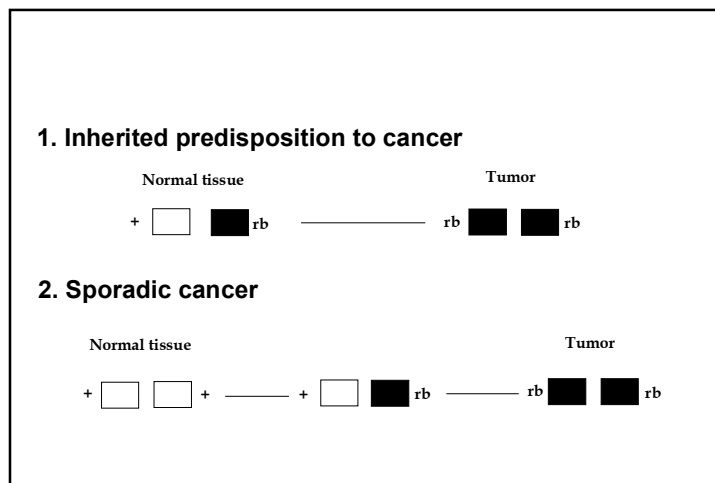
Whereas an increased risk of prostate cancer for the first-degree relatives of the men with prostate cancer has been a constant epidemiological finding, associations reported between prostate cancer and cancers at other sites have been quite conflicting. Some studies have indicated that hereditary prostate cancer is very site-specific and that no other malignancies exist at a higher than expected incidence (Isaacs et al., 1995). However, the risk of breast cancer has been shown to be slightly elevated in close relatives of men with prostate cancer (Cannon et al., 1982; Anderson and Badzioch, 1993; Goldgar et al. 1994; Tulinius et al., 1994; McCahy et al., 1996; Cerhan et al., 1998; Vaittinen and Hemminki, 1999; Valeri et al., 2000; Hemminki and Dong, 2000). The increased risk of tumors of the central nervous system in the relatives of the prostate cancer patients and families with hereditary prostate cancer has been suggested (Goldgar et al., 1994, Isaacs et al., 1995 Gibbs et al., 1999). Familial associations have been observed also between prostate cancer and stomach, liver and kidney cancers and myeloma in the study of familial cancer risks between discordant sites (Vaittinen and Hemminki, 1999). An association of prostate cancer in one generation and stomach, liver and skin cancer and myeloma in another generation was observed also in the cohort study based on Swedish family-cancer database (Hemminki and Dong, 2000). No association between prostate cancer and colon cancer was observed in a cohort study by Cerhan et al. (1998). However, in families potentially linked to HPC1, modest excess of breast and also colon cancer has been reported (Grönberg et al., 1997, Damber et al., 1998). Also in members of the families meeting the criteria of hereditary prostate cancer, the risk of breast and gastric carcinoma was found to be slightly increased (Grönberg et al., 2000).

## 3. Genes predisposing to cancer

### 3.1. Knudson's hypothesis and cancer-predisposition genes

Knudson published the "two-hit model" of cancer development in 1971 (Knudson, 1971). Normal human cells have two copies of all somatic genes, one inherited from the father and one from the mother. According to Knudson's hypothesis, inherited susceptibility to cancer can be caused by germ-line mutations leading to malfunction of one of the two alleles of a tumor suppressor gene (TSG) (Figure 1). Loss of one TSG allele is not sufficient to cause cancer, but if the other allele is somatically mutated, the cell will undergo malignant

transformation. In hereditary cancer cases the first mutation is present in all cells of the body. Since only one additional mutation is required for cancer formation, the likelihood of a second, somatic, hit is so high that the mode of inheritance of malignancy appears dominant at the family level.



**Figure 1** Knudson's two-hit hypothesis

In hereditary cancers the high likelihood of malignant cell transformation also leads to earlier onset and higher rate of tumor formation as compared to sporadic cancers. These features, bilaterality/multiplicity and early onset of a disease are also recognized as general characteristics of hereditary cancer syndromes (Bishop, 1996).

Cancer susceptibility caused by germ-line mutations in tumor suppressor genes is inherited dominantly in a Mendelian way. The offspring of the mutation carriers have a 50% risk of inheriting the trait. The penetrance of many of the genes predisposing to common cancer types is relatively high, ranging from 70% to 90% (Houlston and Peto, 1996). All currently known hereditary cancer forms are not caused by mutated tumor suppressor genes. For example, in hereditary non-polyposis colon cancer (HNPCC) the genes involved in tumorigenesis (e.g. hMLH1, hMSH2, hPMS1 and hPMS2) are DNA mismatch repair genes (Aaltonen et al., 1993; Parson et al., 1993; Farrington and Dunlop, 1996). Mutations of these genes lead to deficient repair. Accumulation of errors during DNA replication will lead to the damage of other genes, such as tumor suppressor genes. Another class of genes involved in carcinogenesis are oncogenes. These genes promote cell proliferation, and are inactivated in non-proliferating cells. Hereditary cancer syndrome Multiple endocrine neoplasia type 2 is caused by over-activity in the RET oncogene (Mulligan et al., 1993), and it is as yet the only known cancer syndrome caused by dysfunction of oncogene.

In some cancer syndromes the higher risk of malignancies is related to the higher sensitivity to environmental risk factors. For example, ataxia-telangiectasia (AT) is an autosomal recessive disorder characterized by cerebellar ataxia, telangiectases, immune defects, and a predisposition to malignancy (Savitsky et al., 1995). Chromosomal breakage is a typical feature of the disease. In AT patients cells are abnormally sensitive to killing by ionizing radiation (IR), and abnormally resistant to inhibition of DNA synthesis by ionizing radiation (Savitsky et al., 1995).

Of the common malignancies with known inherited predisposition breast and gastric cancers are discussed in more detail in the next chapter. Examples of currently known hereditary cancer syndromes are summarized in Table 4.

**Table 4.** Examples of currently known hereditary cancer syndromes with predisposing gene(s) identified

Disease or syndrome	Gene	Chromosomal location	References
Retinoblastoma	RB1	13q14	Sparkes et al., 1980; Friend et al., 1986
Wilm's tumor	WT1	11q13	Riccardi et al., 1978; Call et al., 1990
FAP	APC	5q21	Bodmer et al., 1987; Kinzler et al., 1991
HNPCC	MLH1	3p21	Lindblom et al., 1993; Bronner et al., 1994
	MSH2	2p16	Peltomäki et al., 1993; Nyström-Lahti et al., 1994
	PMS1	2q31-q33	Nicolaidis et al., 1994
	PMS2	7p22	Nicolaidis et al., 1994
Breast cancer	BRCA1	17q21	Hall et al., 1990; Miki et al., 1994
	BRCA2	13q12-q13	Wooster et al., 1995; Tavtigian et al., 1996
Melanoma	MLM/ MTS1	9p13-p22	Cannon-Albright et al., 1992
Gastric cancer	CDH1	16q22	Guilford et al., 1998
Li-Fraumeni syndrome	TP53	17p13	Malin et al., 1990
Neurofibromatosis1	NF1	17q11	Wallace et al., 1990
Neurofibromatosis2	NF2	22q12	Trofatter et al., 1993
von Hippel-Lindau syndrome	VHL	3p25	Hosoe et al., 1990
Multiple endocrine neoplasia type 2A	MEN2A	10q11	Mulligan et al., 1993
Multiple endocrine neoplasia type 1	MEN1	11q13	Larsson et al., 1988; Chandrasekharappa et al., 1997
Inherited renal cell carcinoma	RCC	3p14	Boldog et al., 1993
Ataxia telangiectasia	ATM	11q23	Savitsky et al., 1995
Peutz-Jeghers syndrome	PJS	19p	Hemminki et al., 1997; Hemminki et al., 1998

### 3.2. Multistep development and progression of cancer

The pathogenesis of most hereditary and sporadic cancers is more complicated than that of retinoblastoma and similar single gene disorders. A mutated tumor suppressor gene is only one of many “hits” in the multistep process of malignant transformation. Probably the best defined multistep pathway of cancer development is that of colorectal cancer (Vogelstein et al., 1988; Midgley and Kerr, 1999), which involves several ordered steps from normal epithelium, via adenoma formation, to invasive cancer. Different hereditary colorectal cancer



syndromes are caused by genetic changes affecting different steps of the pathway. Development of a large number of colon polyps is typical in familial adenomatous polyposis (FAP). Due to the large number of polyps, almost all patients with FAP develop colorectal cancer before the age of 40. In an other type of colorectal cancer syndrome, hereditary non-polyposis colorectal cancer (HNPCC), multiple polyps are not observed, but when a single polyp has developed in a patient with HNPCC, malignant transformation of the polyp occurs rapidly (Mecklin et al., 1986; Kinzler and Vogelstein, 1996).

#### **4. Genetic basis of hereditary predisposition to prostate cancer**

##### **4.1 HPC1 locus at 1q24-q25**

The first genome-wide genetic linkage analysis of prostate cancer susceptibility loci was performed by Smith et al. (1996) in 79 North American and 12 Swedish pedigrees, each having at least three first-degree relatives with prostate cancer. Affected individuals had an average age of 65 years at diagnosis. The highest 2-point LOD score observed was 2.75 with a marker that maps to the distal long arm of chromosome 1 at 1q24-q25. The investigators typed additional markers in this region and provided additional evidence for linkage with a maximum 2-point LOD score of 3.65 at  $\theta = 0.18$  with marker D1S2883 (called HPC1). Significant evidence for locus heterogeneity was obtained with an estimated 34% of the families linked to the 1q24-q25 region. The maximum multipoint LOD score under the assumption of heterogeneity was 5.43.

The HPC1 locus has been confirmed by 3 other groups (Cooney et al., 1997; Hsieh et al., 1997; Neuhausen et al., 1999) and through reanalysis and extension of the original study (Grönberg et al., 1997). Conversely, HPC1 was not confirmed by two other investigations (Eeles et al., 1997; McIndoe et al., 1997). Cooney et al. (1997) confirmed 1q24-q25 as a likely location of a prostate cancer susceptibility gene with a maximum LOD = 1.58 in 59 families. Six African-American families in this study contributed disproportionately to the observation of linkage.

Grönberg et al. (1999) analyzed 40 (12 original and 28 newly identified) Swedish families with hereditary prostate cancer. A maximum 2-point LOD score of 1.10 was observed at D1S413 (at a recombination fraction of 0.1), with a maximum NPL Z score of 1.64 at D1S202 ( $P = 0.5$ ). The evidence for linkage to this region originated almost exclusively in a subset of 12 early-onset (age less than 65 years) families, which yielded a maximum LOD score of 2.38 at D1S413 ( $\theta = 0.0$ ). Estimates from heterogeneity tests suggested that as many as 50% of the early-onset families have linkage to the HPC1 region.

Neuhausen et al. (1999) examined evidence for linkage to the 1q24-q25 region in a set of 41 extended multi-case prostate cancer pedigrees from Utah containing 440 members with prostate cancer. In comparison to other studies, the Utah pedigrees were generally much larger (average of 10.7 vs 5.1 cases) and had an older average age at diagnosis (69 vs 65 years). The authors found that the youngest quartile (by median age at diagnosis) yielded a maximum LOD of 2.82,  $P = 0.0003$  (at D1S215 to D1S222).

Combined analysis of 6 HPC1 markers in 772 families segregating hereditary prostate cancer (Xu and the International Consortium for Prostate Cancer Genetics, 2000), found evidence for linkage with a peak parametric multipoint LOD score assuming heterogeneity (HLOD) of 1.4

( $p = 0.01$ ) at D1S212. The estimated proportion of families linked to the locus ( $\alpha$ ) was 0.06. Parametric analysis revealed a significant effect of the presence of male-to-male transmission within the families. In the subset of 491 families with male-to-male disease transmission, the peak HLOD score was 2.56 ( $p = 0.0006$ ). Families that had an early mean age at diagnosis (<65 years) had a peak HLOD of 2.28 at D1S212, with  $\alpha=0.19$ , whereas the 330 families that had members with a late age at diagnosis (>65 years) had a maximum HLOD of 0.79, with  $\alpha=0.07$ . Families with five or more affected family members ( $n=141$ ) had a peak HLOD of 2.01 at D1S212, with  $\alpha=0.15$ , whereas 350 families with four or fewer affected family members had a maximum HLOD of 0.71 at D1S212, with  $\alpha=0.08$ . The highest value of  $\alpha$  was observed for the 48 families that met all three criteria (peak HLOD 2.25,  $\alpha=0.29$ ). The results supported the finding of a prostate cancer susceptibility gene linked to 1q24-q25 in a defined subset of families in which several members are affected at an early age and in which there is evidence of male-to-male transmission.

#### **4.2 PCAP locus at 1q42-q43**

Berthon et al. (1998) found a second putative predisposing gene for prostate cancer at 1q42.2-q43 (PCAP), significantly distant from the proposed HPC1 locus. They performed a linkage study using a set of European families (French and German) with 3 or more prostate cancer patients per family. They obtained a maximum 2-point LOD score of 2.7 with marker D1S2785. The estimated proportion of families with linkage to the 1q42.2-q43 locus was approximately 50%. Furthermore, 9 of the 47 families with early-onset prostate cancer (< 60 years of age) gave multipoint LOD and NPL-scores of 3.31 and 3.32, respectively, with  $P=0.001$ .

Gibbs et al. (1999) analyzed 152 families with prostate cancer for linkage to markers spanning a 20-cM region of 1q42.2-q43. No significant evidence for linkage was found. Suggestive evidence of linkage was seen in families with at least 5 affected men. If heterogeneity is assumed, an estimated 4 to 9 % of these 152 families may show linkage in this region.

#### **4.3. HPCX locus at Xq27-q28**

Population-based studies have suggested an X-linked mode of inheritance of prostate cancer (Narod et al., 1995; Monroe et al., 1995). Xu et al. (1998) presented evidence for the location of a prostate cancer susceptibility gene at Xq27-q28 and termed this locus HPCX. This finding was based on 360 US, Swedish and Finnish prostate cancer families and is discussed in more detail in the results part of this thesis. A maximum 2-point LOD score of 4.60 was observed at DXS1113 at  $\theta = 0.26$  in the combined data set. Heterogeneity estimates suggested that the gene accounts for approximately 16% of the hereditary prostate cancer cases in American and Swedish population and up to 41% in Finnish population. Linkage to Xq27-q28 was observed in a combined study population of 360 prostate cancer families collected at 4 independent sites in North America, Finland, and Sweden.

Lange et al. (1999) reported positive LOD scores over a 30 cM region containing HPCX locus at Xq27-q28, with a subset of cases with no evidence of male-to-male transmission and with early onset of disease ( $\leq 65$  years) contributing strongest for linkage.

#### **4.4. CAPB locus at 1p36**

A prostate cancer/brain cancer susceptibility locus (CAPB) at 1p36 was proposed by Gibbs et al. (1999), who evaluated 12 families with both a history of prostate cancer and a relative with primary brain cancer. The overall LOD score in these 12 families was 3.22 at a theta 0.06 with marker D1S507. In the younger age group (mean age at diagnosis less than 66 years), a maximum 2-point LOD score of 3.65 at theta = 0.0 was observed with marker D1S407. This linkage was rejected in both early- and late-onset families without a history of brain cancer. Gibbs et al. (1999) concluded that a significant proportion of families with a high risk for prostate cancer and a family member with brain cancer show linkage to the 1p36 region.

#### **4.5. HPC2/ELAC2 gene locus at 17p**

Tavtigian et al. (2000) cloned and characterized the hereditary prostate cancer susceptibility gene ELAC2 on chromosome 17p. Linkage analysis of a genome-wide scan of large, high risk Utah pedigrees provided evidence for a prostate cancer predisposition locus on chromosome 17. Positional cloning and mutation screening within the refined interval identified a gene, HPC2/ELAC2, harboring mutations that segregated with prostate cancer in two pedigrees. They identified two common missense variants in the ELAC2 gene that are associated with a diagnosis of prostate cancer: a Ser-to-Leu change at amino acid 217, and an Ala-to-Thr change at amino acid 541. In a sample of cases unselected for family history, Rebbeck et al. (2000) studied the relationship of the two variants identified by Tavtigian et al. (2000) with the probability of having prostate cancer. They studied 359 subjects with prostate cancer and 266 male control subjects matched for age and race. Among control subjects, the Thr541 frequency was 2.9%, and the Leu217 frequency was 31.6%, with no significant differences in frequency across racial groups. Thr541 was observed only in men who also carried Leu217. The probability of having prostate cancer was increased in men who carried the Leu217/Thr541 variant (odds ratio = 2.37; 95% confidence interval 1.06-5.29). The risk did not differ significantly by family history or race. Genotypes at HPC2/ELAC2 were estimated to cause 5% of prostate cancer in the general population studied.

#### **4.6. Recent genome-wide linkage scan results and putative HPC loci at 20q13, 16q, and other sites**

In a genome-wide linkage study of 162 families Berry et al. (2000) found evidence for a prostate cancer susceptibility locus on 20q13. The linkage was suggested particularly in families having less than 5 affected members, a later average age of diagnosis, and no male-to-male transmission. The group of patients having all three of these characteristics had a multipoint NLP score of 3.69 ( $p=0.0001$ ). Weak evidence for linkage to HPC1 was observed in the subset of families with male-to-male transmission. Also suggestive evidence for linkage to PCAP was observed in this study. No evidence for linkage to CAPB in the brain cancer-prostate cancer subset was observed.

Suarez et al. (2000) performed a genome-wide screen of 504 brothers with prostate cancer. The patients were from 230 multiplex sibships. Their study identified positive linkage on chromosomes 2q, 12p, 15q, 16p and 16q with strongest linkage on chromosome 16q between markers D16S515 and D16S3040 with peak multipoint Z score 3.15. Subgroup analysis of the late-age-at-onset group gave evidence of linkage on 4q and a subgroup of families with history of breast cancer revealed a strong linkage at 1p35.1 with Z score 3.78 at D1S1622.

Witte et al. (2000) conducted a genomewide linkage analysis of 513 brothers with prostate cancer, using the Gleason score, as a quantitative measure of prostate cancer aggressiveness. This was the first study in which a measure of prostate cancer aggressiveness had been investigated as a quantitative trait in a genomewide scan. Candidate regions were found on 5q, 7q, and 19q.

Gibbs et al. (2000) also found evidence of linkage at multiple sites in a genomic scan of 94 prostate cancer families, including 432 affected men. Stratification by a variety of factors appeared to improve the chances of identifying relevant genes. When the entire dataset is considered, regions of interest (LOD score >1.5) were identified on chromosomes 10, 12 and 14, with dominant mode of inheritance. Under recessive model LOD scores > 1.5 were found on chromosomes 1, 8, 10 and 16. Stratification by age at diagnosis suggested susceptibility locus on chromosome 11, among the later-onset families, with a LOD score of 3.02 at marker ATA34E08.

#### **4.7. Linkage studies at previously identified loci**

The role of HPC1 (1q24-q25), PCAP (1q42.2-q43) and CAPB (1p36) was studied in 144 prostate cancer families in the study by Berry et al. (2000a). No significant evidence of linkage to the HPC1, PCAP or CAPB region was found when the entire dataset was analyzed. However, weak evidence for linkage to HPC1 was observed in the subset of families with no male-to-male transmission with maximum multipoint nonparametric (NPL) linkage score 1.99. In addition, in families with male-to-male transmission, average age at diagnosis >66 years, and  $\geq 5$  affected individuals in family maximum multipoint NPL score 1.45 was observed on PCAP region.

In a study by Berghthorson et al. (2000) prostate cancer susceptibility loci HPC1, PCAP and HPCX were studied in 87 Icelandic prostate cancer families. They concluded that the putative cancer susceptibility genes at chromosomes 1q24-q25, 1q43-q44 and Xq27-q28 are unlikely to contribute significantly to hereditary prostate cancer in Iceland. Also the prevalence of allelic imbalance was relatively low in both the HPC1 (0%-9%) and PCAP (5%-20%) regions and was not elevated in tumors from positively linked families.

Allelic imbalance at 1q24-q25 and 1p36 region was studied in Swedish material by Ahman et al. (2000). Frequencies of allelic imbalance at the two investigated loci were quite low (3 of 27 informative tumors at the 1p36 locus and 3 of 27 informative tumors at the HPC1 locus), which makes it unlikely that these loci encode genes that are acting as classic tumor suppressor genes in the initiation or progression of hereditary prostate cancer.

Based on a current data from linkage analyses it seems that prostate cancer is a complex, heterogeneous disease with multiple genes and factors contributing to its development. Most of the loci reported in the previous studies remain unconfirmed for the moment. Furthermore, even those that are definitively (HPC1) or tentatively (PCAP, HPCX, CAPB) confirmed, the linkage has only been evident in very small subgroups, amounting to no more than 10% of all prostate cancer families.

**Table 5** Linkage analyses of prostate cancer

<b>Loci / Studies</b>	<b>No. of families</b>	<b>Chromosome region(s)</b>	<b>Gene(s)</b>	<b>Comment</b>
<b>HPC1</b>				
Smith et al., 1996	91	1q24-q25	HPC1	Genome wide scan
Cooney et al., 1997	59	1q24-q25	HPC1	Confirmatory study
Hsieh et al., 1997	92	1q24-q25	HPC1	Confirmatory study
McIndoe et al., 1997	49	1q24-q25		Negative results
Eeles et al., 1998	136	1q24-q25		Negative results
Neuhausen et al. 1999	41	1q24-q25	HPC1	Confirmatory study
Grönberg et al. 1999	40	1q24-q25	HPC1	Confirmatory study
Xu and the ICPCG, 2000	772	1q24-q25	HPC1	Confirmatory study
Goode et al., 2000	150	1q24-q25		Negative results
<b>PCAP</b>				
Berthon et al., 1998	47	1q42.2-q43	PCAP	
Gibbs et al., 1999	152	1q42.2-q43		Negative results
<b>HPCX</b>				
Xu et al., 1998	360	Xq27-q28	HPCX	
Lange et al., 1999	153	Xq27-q28	HPCX	Confirmatory study
<b>CAPB</b>				
Gibbs et al., 1999	12	1p36	CAPB	Prostate-Brain cancer locus
<b>HPC2/ELAC2</b>				
Tavtigian et al., 2000	33	17p	HPC2/ ELAC2	Based on genome wide scan
<b>Studies with multiple loci</b>				
Berry et al., 2000a	144	1q24-q25, 1q42.2-q43, 1p36		Weak evidence for linkage to HPC1
Bergthorson et al., 2000	87	1q24-q25, 1q42.2-q43, Xq27-q28		Negative results
Schleutker et al, 2000	57	1q24-q25, Xq27-q28	HPCX	Positive on HPCX
<b>Additional genomewide scans</b>				
Berry et al., 2000b	162	20q13	HPC20	Genomewide scan
Suarez et al., 2000	230 (sibships)	16q, (1p35.1, 2q, 4q, 12p, 15q, 16p)		Genomewide scan
Witte et al., 2000	233	5q, 7q, 19q		Genomewide scan
Gibbs et al., 2000	94	11p (10, 12, 14, 1, 8, 10, 16)		Genomewide scan

#### **4.8. BRCA1 and BRCA2 genes**

In a population based study, it has been shown that in male relatives of breast cancer patients, the risk of getting prostate cancer is higher compared to the risk in the normal population, with a risk ratio of 1.4 (95% CI 1.1-1.9) (Tulinius et al., 1992). Also the risk of breast cancer in close relatives of prostate cancer patients has been shown to be slightly elevated (Cannon et al., 1982; Anderson and Badzioch, 1993; Goldgar et al. 1994; Tulinius et al., 1994; McCahy et al., 1996; Cerhan et al., 1998). Several studies have provided evidence that germline mutations in the BRCA1 and BRCA2 genes confer an increased risk of prostate cancer (Ford et al., 1994; Langston et al., 1996; Gayther, Ponder 1997). A single BRCA2 mutation (999del15) was found in 8 of 12 analysed PCA patients in Icelandic BRCA2-families. (Sigurdsson et al., 1997). Functionally BRCA1 has been shown to act as a potential human prostate tumor suppressor by modulating proliferation, damage responses and expression of cell regulatory proteins (Wang et al., 2000). BRCA1 has been shown to play a significant role in repair of oxidative DNA damage (Gowen et al., 1998).

In the Ashkenazi Jewish population, a significantly elevated risk of prostate cancer has been observed among the carriers of BRCA1 or BRCA2 mutations (Struewing et al., 1997). In this population, three founder mutations, 185delAG and 5382insC in the BRCA1 gene and 6174delT in the BRCA2 gene, exist at a relatively high frequency as predisposing mutations for breast cancer and ovarian cancer. However, Hubert et al. (1999) found no evidence of an increased frequency of prostate cancer in association with these mutations in Ashkenazi males. Also Wilkens et al. (1999) observed that the frequency of founder BRCA1 and BRCA2 mutations was not elevated in a sample of Ashkenazi prostate cancer families. In their study of BRCA1 and BRCA2 mutations in 22 high-risk prostate cancer families with at least two cases of breast and/or ovarian cancer per family, Sinclair et al. (2000) concluded that BRCA1 and BRCA2 appear to have a limited role in familial prostate cancer.

#### **4.9. Other candidate prostate cancer susceptibility genes**

A candidate prostate cancer susceptibility gene on the X chromosome is the androgen receptor gene (AR). AR, however, is located at Xq12, over 50 cM from the region implicated in the study by Xu et al. (1998), but seems not to be the target gene pointed out by the linkage study. Besides linkage, the AR gene has been intensively studied as a candidate prostate cancer susceptibility gene.

One of the critical functions of the product of the androgen receptor gene (AR) is to activate the expression of target genes. This transactivation activity resides in the N-terminal domain of the protein, which is encoded in exon 1 and contains polymorphic CAG and GGC repeats (microsatellites). These repeats are coded as glutamine (Glu) and glycine (Gly) in the encoded protein. A smaller size of the CAG repeat is associated with a higher level of receptor transactivation function of the encoded protein, thereby possibly resulting in a higher risk of prostate cancer (Giovannucci et al., 1997). Schoenberg et al. (1994) demonstrated contraction in this microsatellite from 24 to 18 CAG units in an adenocarcinoma of the prostate. The shorter alleles were implicated in the development of the tumor. Edwards et al. (1992) and Irvine et al. (1995) showed that the prevalence of short CAG alleles was highest in African-American males with the highest risk for prostate cancer, intermediate in intermediate-risk non-Hispanic whites, and lowest in Asians at a very low risk for prostate cancer. Irvine et al. (1995) found that high-risk African Americans also had the lowest frequency of the GGC allele. Consistent with the interracial variation in CAG and GGC distributions, there was an

excess of white patients with short CAG repeats relative to the white controls. Irvine et al. (1995) found a statistically significant negative association between the number of CAG and GGC repeats among the prostate cancer patients. Giovannucci et al. (1997) showed that a shorter CAG repeat sequence in the AR gene predicts higher grade and advanced stage of prostate cancer at diagnosis, and metastasis and mortality from diseases. In the Finnish study, Wallen et al. (1999) found a microsatellite mutation (CAG(20) to CAG(18)) in prostate cancer containing the AR gene amplification. In this study, the mean lengths of the polymorphic CAG and GGC repeats were similar to those observed in the normal population. Short CAG repeats in the AR gene correlated with the younger age at diagnosis of sporadic and familial prostate cancer, but not with the increased risk of prostate cancer in the study by Bratt et al. (1999). However, no association between AR CAG allele length and prostate cancer was detected in the recent study by Lange et al. (2000). Overall, the current data has been interpreted to suggest a possible association between the microsatellites of the androgen receptor gene and the development of prostate cancer.

Mutations of the androgen receptor (*AR*) gene have also been reported in prostate cancer (Bosland et al., 2000). Occasionally, germ-line mutations have been found, but a link between *AR* mutations and predisposition to human prostate cancer has not been firmly established. Recently, two independent studies reported the same germline mutation at codon 726 in exon E (CGC to CTC) in two apparently unrelated Finnish prostate cancer patients (Elo et al.; 1995; Koivisto et al., 1999). A recent study (Mononen et al., 2000) indicates that the Arg726Leu substitution in the AR may confer an up to 6-fold increased risk of prostate cancer and may contribute to cancer development in up to 2% of the Finnish prostate cancer patients.

Polymorphisms in a number of other genes important in steroid metabolism and signaling have been suggested to be associated with prostate cancer. These include polymorphisms in the vitamin D receptor (Taylor et al., 1996; Ingles et al., 1997), in 17-hydroxylase cytochrome P450 gene (*CYP17*) (Lunn et al., 1999), in 3 $\beta$ -hydroxysteroid dehydrogenase type II (*HSD3B2*) (Devgan et al., 1997), as well as the 5 $\alpha$ -reductase type II (*SRD5A2*) (Reichardt et al., 1995; Makridakis et al., 1997). Some examples of these polymorphisms and mutations are discussed here briefly.

A germline missense mutation (A49T) in the human prostatic steroid 5-alpha-reductase gene (*SRD5A2*) (Makridakis et al., 1997) has shown to be associated with an 3.3-fold and 2.5-fold risk of prostate cancer in both African-American and Hispanic men, respectively (Makridakis et al., 1999), and perhaps even contributing to cancer development in up to 8% of the advanced prostate cancer cases. However, recent results in Finnish population argue against a prominent role of this variant as a genetic risk factor for prostate cancer (Mononen et al., 2001).

Elevated prostatic dihydrotestosterone (DHT) has been suggested to increase the risk of prostate cancer. The *HSD3B2* gene encodes the type II 3 beta-hydroxysteroid dehydrogenase, one of the enzymes that initiate the inactivation of DHT. Devgan et al. (1997) determined the distribution of a complex dinucleotide repeat in the *HSD3B2* gene in high-risk African-Americans, intermediate-risk Euro-Americans, and low-risk Asians. They suggested that allelic variants of the *HSD3B2* gene may play a role in predisposition to prostate cancer, and in explaining the substantial racial/ethnic variation in risk.

Polymorphisms have been identified also in the 17-hydroxylase cytochrome P450 gene (*CYP17*) which is involved in androgen biosynthesis and metabolism. Lunn et al. (1999)

found that the CYP17 A2 allele occurred at a higher frequency in Caucasian patients with prostate cancer (70%) than in Caucasian controls (57%), suggesting that the A2 allele may convey an increased risk for prostate cancer (odds ratio (OR) = 1.7, 95% confidence interval (CI) = 1.0-3.0). Blacks and Caucasians had a similar frequency of the A2 genotype (16 and 17%, respectively) while Taiwanese had the highest frequency (27%).

Several lines of evidence suggest that vitamin D may be an important determinant of prostate cancer risk. Vitamin D receptor (VDR) is therefore a strong candidate for prostate cancer susceptibility gene. VDR polymorphisms have been associated with prostate cancer. Taylor et al. (1996) showed that VDR genotype correlated to the risk of prostate cancer, with up to three-fold difference between cases and controls. Also Ingles et al. (1997) found that genetic variation in the VDR gene associated with prostate cancer, and appeared to preferentially confer risk for advanced disease.

**Table 6** Examples of low penetrance genes associated with a elevated risk of prostate cancer

Androgen receptor (AR) mutations	Elo et al., 1995; Koivisto et al., 1999; Mononen et al., 2000
AR polymorphisms (CAG, GGC)	Irvine et al., 1995; Giovannucci et al., 1997; Lange et al., 2000
5 $\alpha$ -reductase II	Reichardt et al., 1995; Makridakis et al., 1997, Makridakis et al., 1999
CYP17	Lunn et al., 1999
HSD3B2	Devgan et al., 1997
Vitamin D receptor	Taylor et al., 1996; Ingles et al., 1997

Another suggested candidate gene for prostate cancer has been PTEN located at chromosome 10q23 (Li et al., 1997). However, it has been reported that PTEN is unlikely to contribute in a significant way to the inherited predisposition to prostate cancer (Cooney et al., 1999; Forrest et al., 2000).

A recent genome-wide linkage search of 134 families with two or more testicular germ-cell tumors localized the susceptibility gene for testicular cancer on chromosome Xq27 (Rapley et al., 2000) close to HPCX region on Xq27-q28. However, a connection between these loci has not been reported.

#### 4.10. Pathology and clinical course of hereditary prostate cancer

The increased number of precursor lesions and tumor multifocality are commonly associated with hereditary cancers (Bastacky et al., 1995). However, in prostate cancer multifocality or multiple precursor lesions are not specifically related to familial types of this malignancy (Bastacky et al., 1995). Studies have not demonstrated substantial pathological differences among hereditary, familial and sporadic forms of prostate cancer based on specimens from radical prostatectomies (Steinberg et al., 1990; Bastacky et al., 1995; Keetch et al., 1996; Kupelian et al., 1997a; Bauer et al., 1998; Bova et al., 1998; Schaid et al., 1998). The higher number of low grade tumors among hereditary prostate cancer cases compared to sporadic cases have been reported in two studies (Bastacky et al., 1995; Keetch et al., 1996).

Most of the studies investigating the clinical course of hereditary prostate cancer have not provided evidence for any substantial difference between familial and sporadic prostate cancer in response to treatment, or in ultimate outcome (Bauer et al., 1998; Bova et al., 1998;



Hanlon and Hanks, 1998; Hanus et al., 1999). Also population-based cohort study by Grönberg et al. (1998) showed no differences in survival between familial and sporadic prostate cancer cases. In addition, family history of prostate cancer has not been associated with prognosis in early-onset (<51 years) prostate cancer patients (Aprikian et al., 1994; Bratt et al., 1998). However, Kupelian et al. (1997a; 1997b) have reported that patients with familial prostate cancer have a higher likelihood of biochemical failure after radical prostatectomy, than patients with sporadic cancer, and that familial prostate cancer may have a more aggressive course than nonfamilial prostate cancer.

In the study by Norrish et al. (1999) familial prostate cancer appeared to be diagnosed at an earlier stage of disease progression, possibly as the result of higher socio-economic status and greater use of screening and investigative procedures among patients reporting positive family history. This could partly explain the high proportion of low grade tumors observed also in the studies of Bastacky et al. (1995) and Keetch et al. (1996).

In families potentially linked to HPC1, younger age at diagnosis, higher-grade tumors, and more advanced-stage disease have been reported (Grönberg et al., 1997). Also in a subset of prostate cancer families, co-aggregation of breast and gastric carcinoma has been observed (Valeri et al., 2000; Grönberg et al., 2000).

## **5. Challenges for the genetic dissection of complex traits**

During the past two decades there has been a rapid progress towards the mapping and identification of genes involved in inherited predisposition to cancer. Genetic linkage analysis has also led to the identification of several susceptibility genes to common cancers, such as the mismatch repair genes hMSH2 and hMLH1 in colorectal cancer (Peltomäki et al., 1993; Nyström-Lahti et al., 1994; Lindblom et al., 1993; Bronner et al., 1994), the MTS1 gene on chromosome 9p responsible for familial melanoma (Cannon-Albright et al., 1992), as well as the BRCA1 and BRCA2 genes in breast cancer (Hall et al., 1990; Wooster et al., 1994). In these examples, linkage mapping led to the subsequent identification of the gene by positional cloning. After the strongest and most prevalent cancer predisposition genes have been found, identification of additional susceptibility loci for the common cancer forms is becoming increasingly challenging.

Statistical methods for family-based parametric linkage analysis are based on the assumption that a single major gene is causing most diseases in the family as well as on the assumption that the prevalence and mode of inheritance of this disease gene are known (Terwilliger and Ott, 1994). The gene is inherited in a certain fashion, and causes disease with a certain probability. Such mode of penetrance and the pattern disease transmission can be estimated by segregation analysis (Nicholas, 1982). In most cases such estimates remain very inaccurate or downright misleading (Clerget-Darpoux et al., 1986). Non-parametric linkage analyses do not require this level of detailed information on the gene of interest and are characterized by less assumptions (Kruglyak et al., 1996). However, these methods can also only find the predominant predisposition genes. Most investigators readily accept that complex diseases, such as cancer, arise from the effects of a number of disease genes, including environmental influences, as well as interactions between the two. In most cases, traditional family-based analyses are not able to take into account the simultaneous effect of multiple loci, and confounding environmental effects in particular (Lander and Kruglyak, 1995).

## 5.1 Factors complicating linkage analysis of prostate cancer

In prostate cancer, there is a number of complicating factors that influence linkage studies.

First, the disease is very common in the population. It has been estimated that more than 10% of males get the prostate cancer in their life-time (Ries et al., 1999). Therefore, the likelihood that there are clusters of sporadic cases that look like they are caused by an inherited gene defect is very high. Furthermore, there may be individual sporadic cases with a family where a disease predisposing mutation also segregates.

Second, prostate cancer is a very late-onset disease. The mean age at diagnosis of prostate cancer is 71 years in Finland and even a man over 80 years can get a prostate cancer. Many gene carriers also die of other causes before the disease has had a chance to manifest itself.

Third, prostate cancer has a wide spectrum of disease phenotype, starting from a small indolent, microscopic cancer, which is found at autopsy in up to 50% of elderly men, to prostatic intraepithelial cancer, localized cancer, and aggressive, highly metastatic prostate cancer (Gittes, 1991). Usually one does not make a major distinction between these phenotypes, when a family-based disease transmission is evaluated. However, assessment of the phenotype is essential to linkage studies.

Fourth, linkage studies of prostate cancer have suggested locus heterogeneity (Ostrander and Stanford, 2000). The same phenotype may be due to different genetic defects. Furthermore, many genetic effects may have a prominent role in the population, but have such a low penetrance that they do not cause clear familial clustering. Chance clustering of sporadic cases, incomplete penetrance of germline mutations, and substantial genetic heterogeneity make classical linkage analysis difficult, and require very large numbers of informative pedigrees (Xu and ICPCG, 2000). Incorrect assumptions on estimates of penetrance, the selection bias from preferential sampling of probands with heavily affected families, the misclassification of the disease status of relatives and the possible violation of Hardy-Weinberg equilibrium (Gail et al., 1999) are problematic in linkage analysis of complex diseases. For example, twin studies find that 42% of prostate cancers may have a genetic influence (Lichtenstein et al., 2000; Verkasalo et al., 2000). This is substantially more than obtained from any segregation analyses published so far (Carter et al. 1992, Grönberg et al. 1998, Schaid et al. 1998). This may reflect the influence of a number of factors: 1) Several individual, but highly penetrant genes, each causing cancer in a very small fraction, 2) genes with recessive, and other modes of inheritance that are usually difficult to detect by linkage analysis and 3) multiple low-penetrance genes, many of which can be very common in the population.

Fifth, there is a profound cohort effect on prostate cancer caused by the wide-spread application of PSA screening during the 1990s. Prostate cancer incidence doubled during a 20-year period from 1975 to 1995 (e.g. in Finland from 31.7 / 100,000 to 61.5 / 100,000) (Finnish Cancer Registry, 2000). This has substantial implications for genetic studies, where one looks at the disease transmission in a given family across multiple generations. Statistical approaches to taking these effects into account are difficult to develop. One can only speculate, what the effect of this PSA screening is on studies of inheritance. It is possible that the increased PSA screening substantially elevates the background sporadic rate, which makes it more difficult to identify true genetically determined familial clusters from clusters caused

by chance clustering. For example in studies of HPC1 the primary linkage result of the study of Smith et al. (1996) has been difficult to confirm in other materials (Eeles et al., 1997; McIndoe et al., 1997; Berry et al., 2000a; Bergthorson et al., 2000). One suggested explanation has been the differences in the time-period of a family collection. The family collection for the original study was done in Johns Hopkins during 1980s, before the large-scale use of PSA testing, and the family material for later studies has been done during 1990s, after the introduction of the PSA test (Xu et al., 2000).

## 5.2 Strategies for complex traits

There are multiple strategies with which one can try to address these multiple problems of linkage analysis in prostate cancer.

First, one can carry out stratified analyses in the families defined by clinical, phenotypic or biological features of the tumors. In the case of prostate cancer, stratification based on the mode of inheritance was shown to be effective (Xu et al., 1998). In breast cancer, the high risk and phenotype of male breast cancer in certain families has been shown to be due to the BRCA2 gene but not the BRCA1 gene (Easton et al., 1997).

Second, one can analyze families from ethnically, geographically or otherwise isolated populations, where the likelihood of multiple genes clustering in the population is lower than in highly heterogeneous populations, such as that of the USA. The population of Finland represents a case example of such a population that fulfills many of the criteria for an ideal population in this respect (de la Chapelle, 1993; Peltonen, 1997). It is historically isolated and genetically homogeneous, providing unique advantages for the search of susceptibility genes for cancer and other complex traits. The special characteristics of the Finnish population have been successfully used in the past to identify causative loci and genes for a large number of rare monogenic diseases that are enriched in the Finnish population (Norio et al., 1973; Peltonen, 1997). Other founder populations used in genetic studies include Ashkenazy Jews and Icelandics (Struwing et al., 1997; Sigurdsson et al., 1997).

Finally, one can develop methods and approaches for determining risk genes that are not dependent on the genetic linkage analysis. Substantial hopes in this respect have been based on the association analysis, where one in essence compares allele frequencies of two or more markers in cases and controls. In an isolated and genetically homogeneous population, such as that of Finland, this is expected to be easier than in mixed populations (Hästbacka et al., 1992; Jorde, 1995). Allelic associations caused by linkage disequilibrium (LD) are seen only when a significant proportion of the apparently independent chromosomes in a population are in fact copies of the same ancestral chromosome (Jorde, 1995). In Finland the geographical distribution of families with a genetic disease is often used to identify putative founder regions where a particular ancient disease mutation is highly enriched. There are numerous examples of such disease clusters representing distantly related carriers of the same ancient founder mutation (Nyström-Lahti et al., 1994; Hästbacka et al., 1994; Vesa et al., 1995; Moisio et al., 1996). Association analysis in the founder populations could be one key also to genetic dissection of complex diseases. The introduction of single nucleotide polymorphism markers (Kruglyak, 1997) and DNA microarray technologies (Wang et al., 1998) now provides a new possibility to carry out such analyses in large-scale population samples.

Also approaches to combining different methods of molecular genetics, for example the use of comparative genomic hybridization (CGH), followed by allelic loss studies and linkage

studies in localization of the gene for Peutz-Jeghers syndrome by Hemminki et al., (1997), and familial breast cancer (Kainu et al., 2000) have been successful and may help to deal with the limitations of classical linkage analysis.

## **AIMS OF THE STUDY**

The aim of this study is to search for epidemiological and genetic clues to the hereditary prostate cancer in Finnish population. The specific aims are:

1. To determine the risks of prostate cancer and other malignancies in relatives of prostate cancer patients (I)
2. To develop novel cancer registry-based methods and strategies for rapid, nation-wide, identification of prostate cancer families (II)
3. To ascertain prostate cancer families in Finland and identify the loci predisposing to prostate cancer in the Finnish population by linkage analysis (III, IV)
4. To investigate association of *E-Cadherin* germline alterations with prostate and gastric cancers. (V)
5. To evaluate the role of the serum prostate specific antigen measurements in the early detection of prostate cancer in genetically predisposed individuals (VI)

# **MATERIALS AND METHODS**

## **1. Data sources**

The data for this thesis were ascertained from the following sources: the Finnish Cancer Registry (FCR), the Population Register Centre and local population registries. Diagnoses of cancer patients were confirmed using the Finnish Cancer Registry or individual patient records from the regional hospitals where the patients had been treated.

The population-based and nation-wide Finnish Cancer Registry (FCR) was founded in 1952, and cancer registration started in 1953. Reporting of cancer to the FCR was made obligatory in 1961 but the coverage was excellent even before this legislation. Physicians, hospitals and pathology laboratories send their reports to the registry independently. In addition, the FCR receives information on each death certificate in which cancer is mentioned. On average five notifications are received per case. The FCR registers over 99% of all solid tumors diagnosed in Finland (Teppo et al, 1994). The FCR files are linked to the file of death and immigrations issued by the Population Register Centre, Finland. The coding of cancer case in the FCR includes the primary site, laterality, histologic type, stage and grade.

Population registration in Finland has traditions dating back to the 16<sup>th</sup> century and is considered to be of an excellent quality. Population registries are kept by the church parishes and for persons not belonging to any official religious community, by local authorities. From 1964, a centralized, nationwide, computer-based population registry has been run by the national Population Register Centre. This registry reached complete coverage of all Finns on January 1, 1967. The registration in this national Population Registry is based on unique personal identifiers, which are nowadays used as main keys in every major person registry, including FCR. The population registry contains detailed demographic information, including the place of birth and history of residency. Genealogical data for these studies are obtained from local parish records and National Archives of Finland.

## **2. Index patients and relatives**

### **2.1. Estimation of standardized incidence ratios (Study I)**

We identified 9,142 men with newly diagnosed prostate cancer (cancers diagnosed between January 1, 1988 and December 31, 1993) from the FCR. All 557 patients diagnosed at an age of 60 years or less during that time-period were chosen as the index patients (“Cohort 1”). In addition, all prostate cancer cases diagnosed at an older age (>60 years) (“Cohort 2”) were selected from three central hospital regions in Mid-Finland (Pirkanmaa) and along the Eastern border of Finland (North-Carelia and Kainuu), called here as East-Finland. There were 659 index patients from the Pirkanmaa region, and 320 in the East-Finland region, altogether 989 patients in cohort 2.

The information on the birthplaces of the index patients was received from the Central Population Registry. The local registries (church parishes and local authorities) were contacted to obtain the names and birth dates of their parents, siblings, spouses and children. The parents and siblings were followed up through the parish records until death or until they obtained a personal identification code in 1967. Tracing of relatives was based on the computerized records of the Central Population Register for all people who were alive in 1967. All data on relatives who had not emigrated or died before 1967 were linked with the

Central Population Register (in June 1998) to obtain dates of death and to check the validity of the identification codes obtained from local authorities.

Tracing of family members was successful for 94% of the index patients. Altogether 11,427 first-degree relatives were identified from registries. Of these, 10,650 (93%) were traced successfully, while 777 (7%) were lost to follow-up. In the families with persons lost to follow-up (715 families) data for a mean of 1.2 persons per family was missing (range 1-6 persons). Of completely followed relatives, 8628 (81%) were alive during the period (1953-1997) when FCR data were available.

## **2.2. Cancer registry -based method for cancer family ascertainment (Study II)**

A cohort of all 35,761 patients with prostate cancer diagnosed by the end of 1996, excluding those who had died before 1967, was identified from the Finnish Cancer Registry and linked to the Finnish Population Registry to obtain the municipality of birth for each patient. There were 10,721 different last names and 596 different places of birth among the prostate cancer patients. The entire Finnish male population (3.3 million), excluding those who had died before 1967 was used as a reference data. This data set was obtained from the Population Register Centre. There were 95,000 different last names and 596 different places of birth in the entire male population.

In a separate project, we have ascertained Finnish prostate cancer families using a number of methods described later in this chapter. All diagnoses of cancer in these families were confirmed either from the Finnish Cancer Registry or from the patient records of local hospitals. Sixty-seven known prostate cancer families with three or more affected cases per family were used in this study as a reference to evaluate the sensitivity of the population array strategy. Out of these families, 16 contained cancer cases diagnosed outside the time window of our present study (1967-1996), and in one family cancers were diagnosed in family members who had moved out of the country, and therefore were not included in the registries used here. The fifty remaining families were used as a reference group to ascertain the sensitivity of the "population array" approach in the identification of nuclear prostate cancer families.

## **2.3. Collection of Finnish hereditary prostate cancer families for linkage studies (Studies III and IV)**

Since January 1995 prostate cancer families with two or more affecteds have been collected in the Laboratory of Cancer Genetics at Tampere University Hospital, Finland. The families have been identified through referrals from physicians, family questionnaires sent to patients, a nationwide registry-based searches (Studies I and II) and advertisements in newspapers, radio and television. A total of 292 Finnish prostate cancer families meeting the criteria by Carter et al. (1993) and Walsh and Partin (1997) were identified in our nation-wide scan verifying families for linkage studies. In short, these criteria were: 1) prostate cancer present in three different generations, or 2) three first degree relatives with prostate cancer in a family, or 3) two affecteds diagnosed at the age of 55 years or earlier. First, a questionnaire-based pilot approach at the Tampere University Hospital area on 355 living prostate cancer patients diagnosed during 1988-1993 identified 26 prostate cancer patients with a positive family history. Second, the first round of a cancer registry -based search (Study I) identified 93 families with two or more affected cases. Third, articles and advertisements in major Finnish newspapers, TV and radio resulted in 500 contacts. Of these, 151 families met our HPC

criteria. Finally, letters were sent to all 120 urologists in Finland. Fifty-two families were identified in this manner. The degree of overlap (30 families) between the different ascertainment methods suggested that the 292 individual families, which were identified during the collection of HPC families for linkage studies, represent a significant fraction of all hereditary prostate cancer families in Finland.

Diagnoses were confirmed using the Finnish Cancer Registry or individual patient records from regional hospitals. If the family met the Carter et al. (1993) criteria, all living affected family members, as well as the spouse and adult-aged offspring of deceased patients were contacted to obtain informed consent and to request a blood sample for linkage analyses. All male individuals of 45 years or older who participated the study were tested for total serum PSA (Study VI) in accordance with the informed consents. If abnormal age-correlated values were obtained, the men were referred to a local urology department for subsequent urological examination to exclude the presence of subclinical prostate cancer. Seven histologically confirmed prostate cancer cases were identified by PSA screening (Study VI). All individuals participating in the study gave full informed consent.

Fifty-seven of the collected and sampled families in our nation-wide family collection of prostate cancer families were informative for linkage studies (Studies III and IV). To carry out Study III, an international collaboration group for was established. 360 prostate cancer families altogether collected in the USA (Johns Hopkins University (JHU) at Baltimore, Maryland and the Mayo Clinic in Rochester, Minnesota), Finland (University of Tampere, Tampere) and Sweden (Umeå University, Umeå).

**Table 7** Characteristics of Finnish prostate cancer families in studies III and IV

Number of families	57
Number of individuals typed	548
Number of affected individuals typed	137
Average number of affected/family (range)	3.2 (2-9)
Average number of affected individuals typed/family (range)	2.4 (2-9)
Average age at diagnosis (range)	68.2 (45-90)

In addition, the “population array” approach (Study II) identified over 400 candidate prostate cancer families, which are now studied and collected in more detail. Also additional families identified by Finnish urologists have been collected continuously. In December 2000 360 prostate cancer families with two or more affected members have been identified in Finland. We have been able to collect blood samples from the members of 168 families. Sixty-eight of the sampled families are families with three or more affected family members and 92 families with two affected members.

#### **2.4. Association of *E-Cadherin* germline alterations with prostate and gastric cancers (Study V)**

Personal identification codes of members of 180 previously identified and sampled Finnish hereditary prostate cancer (HPC) families with two or more affected first- or second-degree relatives per family were linked to the Finnish Cancer Registry (FCR) to identify those families which had also gastric cancer cases. Fifteen of the 180 families had both prostate and gastric cancer cases according to the FCR data. These prostate-gastric cancer families had two



or more cases of prostate cancer and at least one gastric cancer per family. One prostate cancer patient from each of the 15 families was analyzed for germline *CDHI* mutations. In three families, the sample of the affected individual was not available and a sample from a first-degree relative was used. We also reviewed the database of the Department of Pathology at the Tampere University Hospital to identify individual patients, who were diagnosed with both prostate and gastric cancers since 1993. From 1738 prostate cancer patients recorded between 1993-1999, 18 patients had also been diagnosed with gastric carcinoma. DNA sample was available from eight of these prostate-gastric double cancer patients. To carry out a population-based screening of the frequency of the missense mutation S270A, the screening was extended to the following groups: 1) one affected individual from 120 Finnish prostate cancer families with two or more affected family members (regardless of the gastric cancer status), 2) 472 unselected, consecutive prostate cancer patients from the Tampere University Hospital, 3) 140 gastric cancer patients from Tampere University Hospital, 4) 923 anonymous, unselected healthy male blood donors.

## **2.5. Prostate specific antigen measurements in high-risk prostate cancer families (Study VI)**

Of the identified Finnish prostate cancer families, 103 were selected for this study based on the criterion that at least one unaffected first-degree male relative aged over 44 years was available. The study material consisted of 226 unaffected first-degree male relatives from the 103 families, of which 209 (107 brothers and 102 sons of probands) agreed to participate (92.5%) and gave written informed consent. The mean age of the 209 unaffected men was 56.0 years (range 45-75). The mean number of unaffected cases studied per family was 2.0 (range 1-9). 120 men came from families with 3-7 prostate cancers and 89 from families with two affected cases. The mean age of diagnosis of prostate cancer in these families was 69.7 years, range 55 to 86 years.

## **3. Methods**

### **3.1. Estimation of standardized incidence ratios (Study I)**

The relatives were followed up for cancer through the files of the FCR. For relatives who died in 1953 – 1966, the record linkage was done by the patient name, with the date of birth, place of birth and place of residence as additional keys. For those alive after January 1, 1967, the linkage was done automatically using the personal identification code as the key. The follow-up for cancer among parents of the index patients started on the date of birth of the index patient or on January 1, 1953, whichever was later. For siblings and children, the follow-up started at the date of their birth or on January 1, 1953, whichever was later. Follow-up for every cohort member ended at emigration, death or on December 31, 1997, whichever was first. The numbers of observed cases and person-years at risk in each relative category were counted, by sex and by five-year age groups. The expected numbers of cases for total cancer and for specific cancer types were calculated by multiplying the number of person-years in each stratum by the corresponding cancer incidence rates in Finland. Adjustment for sex, age and calendar period was included in the calculation of the expected numbers of cases.

The specific cancer sites selected for the analysis included all common cancer types (see Table 2 in Study II). Standardized incidence ratios (SIRs) were calculated as the ratios of the observed to the expected numbers of cases. 95% confidence intervals (CIs) were calculated on

the presumption that the number of observed cases followed a Poisson distribution. Age-adjusted comparisons of incidence ratios between different subgroups were calculated using Poisson regression analysis.

To validate the potential selection bias in the families, the families with persons lost to follow-up were first analyzed separately. The standardized incidence ratios (SIRs) for overall cancer in males were 1.28 (95% CI 1.08-1.49) in complete families and 1.37 (95% CI 1.04-1.75) in incomplete families. In the females, the corresponding values were 0.85 (95% CI 0.69-1.04) and 0.94 (95% CI 0.69-1.25). Based on the small, statistically insignificant difference in overall cancer ratios, the incomplete families were included in the final analysis.

### **3.2. Cancer registry -based method for cancer family ascertainment (Study II)**

The cohort of prostate cancer patients was used to calculate the observed number of prostate cancer for each family name and place of birth -combination. There were 28,459 family name and place of birth -combinations in this cohort. Expected numbers were determined from the prevalence of each family name, place of birth and their combinations in the entire Finnish male population (3.3 million), excluding those died before 1967. There were 688,000 combinations altogether. Standardized prevalence ratios (SPR) were counted as a ratio of observed and expected numbers of prostate cancer cases per each family name and place of birth combination. The calculation was further stratified by the year of birth. Exact 95% confidence intervals were defined under Poisson assumption.

Using parish records we identified all first and second degree relatives for those patients who shared the family name and place of birth and were associated with a substantial excess of prostate cancer cases (high SPR). The attempt was to validate the presence of a close familial relationship between such patients. If no such associations can be ascertained, it is possible that individual prostate cancer patients shared the same family name and place of birth by chance. Alternatively, the patients may be very distant relatives to one another. To explore this latter possibility, genealogy research was extended back to the 17th century to identify more distant links between the patients.

### **3.3. Linkage analyses in Finnish prostate cancer families (Studies III and IV)**

#### **3.3.1. Genotyping**

In the studies III and IV, we genotyped DNA samples from 57 Finnish prostate cancer families with at least two living affected cases (Table 7, page 40). The same families first analyzed in Study III were used also for Study IV. No bilinear families were included. A large number of unaffected cases were also collected to infer phase and to construct the haplotypes of deceased patients. Altogether, 869 blood specimens were obtained for DNA isolation. In addition, four specimens from formalin-fixed tumor specimens from selected families were studied. For population controls, anonymous, whole blood specimens from 160 normal healthy blood donors were obtained from the Blood Center of the Finnish Red Cross (Helsinki, Finland).

Genomic DNA for studies III and IV was prepared from a 10 ml whole blood sample or from a paraffin embedded tissue sample using Puregene™- kit (Gentra Systems, Inc., Minneapolis, MN, USA). Linkage studies of the HPC1 and HPCX regions were performed on 57

informative families. The markers applied were derived from the Genome Database (GDB) (Johns Hopkins University School of Medicine, Baltimore, MD). For the Study III, the marker data were obtained for the 20 polymorphic loci available in the GDB spanning the ~40 centimorgan (cM) interval between *DXS1212* and *DXS1108*. Allele frequencies were estimated from independent individuals in the families and unrelated individuals separately for the North American and Finnish families in Study III. Twenty to 60 ng of genomic DNA was used per PCR reaction and fluorescently-labeled PCR primers for 20 different microsatellite markers at Xq in Study III, and 39 at 1q (Smith et al., 1996) and 22 at Xq for Study IV, were employed. High-throughput, semi-automated genotyping was accomplished by means of ABI 377 sequencers. Additional archived tissue specimens were analyzed using an ABI 310 DNA sequencer for Study IV. GENESCAN and GENOTYPER programs (Applied Biosystems, Foster City, CA, USA) were used in data analysis as described in Smith et al. (1996).

### 3.3.2. Linkage analyses

#### Study III

Both parametric and non-parametric linkage approaches were used in the Study III. For the parametric analysis, the same model was used as in the original HPC1 linkage study by Smith et al. (1996), which assumes a dominant disease allele with an allele frequency of 0.003 and age-specific penetrances, although affected men were assumed to be carriers of an X-linked, sex-limited, dominant gene. A fixed 15% phenocopy rate was assumed, while all unaffected men under 75 and all women were assumed to be of an unknown phenotype. In men over the age of 75, the lifetime penetrance of gene carriers was estimated to be 63%, and the lifetime risk of prostate cancer for a non-carrier was 16% in this age class. FASTLINK (Cottingham et al., 1993; Lathrop et al., 1984) and ANALYZE (<ftp://link-age.cpmc.columbia.edu/software/analyze>) were used for parametric two-point analysis. For the non-parametric analysis, affected sibpairs were used for the two-point analysis as implemented by ANALYZE, using the mean test and likelihood-based test. The simulation study was performed using FASTLINK (<ftp://watson.hgen.pitt.edu.pub>). The parametric multipoint analysis was performed using FASTLINK (LINKMAP; Cottingham et al., 1993; Lathrop et al., 1984). A sliding multipoint approach was used as described (Terwilliger and Ott, 1994). Heterogeneity analysis was then performed using HOMOG (Ott, 1991). The admixture model was used to test for several hypotheses for genetic locus heterogeneity (computer software HOMOG3R; Ott, 1991). (For further details of the hypotheses, see Study III.)

#### Study IV

Linkage analysis was carried out with 39 microsatellite markers for the HPC1 and 22 markers for the HPCX region. Statistical analyses were carried out in subgroups defined by family size, mode of transmission and the age of prostate cancer diagnosis. The standard two-point and multipoint parametric likelihood analysis was performed using the computer program FASTLINK (Cottingham et al., 1993). The parameters of the trait model used in the linkage analyses were identical to those used by Smith et al. (1996). In brief, only individuals with verified diagnoses of prostate cancer were considered to be affected. Males under the age of 75 with normal (age-adjusted) PSA values were treated as having an unknown status. Males over 75 years with normal PSA values were considered unaffected. Age-dependent penetrance values with three liability classes were used. The frequency of the HPC1 and HPCX genes were set to 0.003. In the sliding four-point FASTLINK analysis for HPCX, markers DXS1232, DXS1205 and DXS6571 were used with recombination fractions of 0.02 and 0.006, respectively. Additional parametric and non-parametric multipoint analyses were

performed with GENEHUNTER (Kruglayk et al., 1995). The X chromosome version of GENEHUNTER was used in X chromosomal analyses. For the subgroup analyses, 33 families were classified as having "no-male-to-male" (NMM) transmission of the disease (no affected fathers or affected uncles in the paternal side of the family) and 24 families as "male-to-male" (affected father or paternal uncle). Finnish allele frequencies for each marker were estimated from the founders of the 57 linkage families and from 160 anonymous control samples obtained from blood donors. In the subgroup linkage analyses, families with a mean age of onset of 65 years or less were considered as early onset families and all others were considered as late onset families. For subgroup analyses, one representative, positive marker for each HPC candidate area was selected; D1S158 for HPC1 (Smith et al., 1996) and DXS1205 for HPCX (Study III). Non-parametric sib-pair tests of linkage were performed with the program package ANALYZE, using POLYLOCUS program (<ftp://ftp.well.ox.ac.uk/pub/genetics/analyze> or <ftp://linkage.cmpc.columbia.edu/software/analyze>). HOMOG3R was used to calculate the log-likelihoods of heterogeneity (for further details, see Study IV). The predivided sample test (PS-test) was used to test for heterogeneity between the various subgroups described in Table 2 (Morton, 1956; Leal, 1997). A Bonferroni correction was performed to correct multiple testing.

### **3.4. Association of *E-Cadherin* germline alterations with prostate and gastric cancers (Study V)**

DNA was extracted from whole blood using the Puregene kit (Gentra Systems, Minneapolis, MN) following the manufacturer's protocol. DNA extractions from paraffin-embedded, formalin-fixed tissues were carried out using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). A written informed consent was obtained from all patients giving a blood sample for the study.

To exclude the HNPCC phenotype as a cause for the prostate-gastric cancer association, Finnish founder mutations for *MLH1* gene were screened from the members of 15 prostate-gastric cancer families and 8 patients diagnosed to have both prostate and gastric cancers. These two *MLH1* mutations together account for 51% of all Finnish kindreds with verified or putative HNPCC (Nyström-Lahti et al., 1996). Mutation 1 in the *MLH1* (a 3.5-kb genomic deletion affecting exon 16 and flanking introns) (Nyström-Lahti et al., 1995) gene was detected as described by Nyström-Lahti *et al.* (1995) except that the annealing temperature was 65°C in PCR. Mutation 2 in the *MLH1* gene (a single-base change at the splice-acceptor site of exon 6) (Nyström-Lahti et al., 1995) was screened by minisequencing, performed as described by Syvänen (1998). Both in mutation 1 and 2 screening the positive controls were included.

SSCP (single-strand conformation polymorphism) analysis of the entire coding sequence of the *CDHI* gene was performed from genomic DNA samples from 15 prostate-gastric cancer families and 8 prostate-gastric cancer patients. Primer sequences and PCR conditions were based on those described by Berx *et al.* (1995). All samples where a variant band were detected were sequenced using the original PCR primers with ABI PRISM 310 Genetic Analyzer (PE Biosystems, Foster City, CA ).

PCR-SSCP analysis of the *CDHI* gene revealed a novel S270A missense mutation in one prostate-gastric cancer family. Trying to examine the association of the S270A variant in the prostate cancer predisposition at the population level we studied the frequency of the variant among population controls and prostate cancer cases. Minisequencing was performed as

described by Syvänen (1998). Minisequencing positive results were confirmed by sequencing with ABI PRISM 310 Genetic Analyzer (PE Biosystems, Foster City, CA) using the same primers as in PCR.

### **3.5. Prostate specific antigen measurements in high-risk prostate cancer families (Study VI)**

One 10 ml blood specimen was obtained for DNA isolation and another one to prepare serum for PSA measurement from each participant. Total serum PSA concentrations were determined with AutoDELFIA™ PSAkit (Wallac, Turku, Finland). Age-specific reference values were used as a criterion for cut-off values of raised serum PSA-levels. These reference values are utilized in the clinical routine at the Tampere University Hospital and are based on the results from the Gothenburg (Hugonsson et al., 1997) and Rotterdam EORTC (Bangma et al., 1997; Standaert et al., 1997) screening studies with 5845 and 1726 unselected participants (95 percentile of the distribution). If serum PSA was elevated, the subject was referred to a local urology department for subsequent urological examination, which included a new PSA measurement, digital rectal examination, ultrasound, and one set of random biopsies.

## **4. Statistical methods (Studies I-VI)**

**In Study I** standardized incidence ratios (SIRs) were calculated as the ratios of the observed to the expected numbers of cases. 95% confidence intervals (CIs) were calculated on the presumption that the number of observed cases followed a Poisson distribution. Age-adjusted comparisons of incidence ratios between different subgroups were calculated using Poisson regression analysis.

**In Study II** standardized prevalence ratios (SPR) were counted as a ratio of observed and expected numbers of prostate cancer cases per each family name and place of birth combination. The calculation was further stratified by the year of birth. Exact 95% confidence intervals were defined under Poisson assumption.

Statistical methods used in **studies III and IV** have been described in the chapter **3.3.2**.

**In Study V** statistical analyses were performed using the GraphPad InStat™ version 2.04a (GraphPad Software, San Diego, CA, USA) and the StatView for Windows version 5.0.1. (SAS Institute Inc., USA). Correlations were made with two-tailed Fisher's exact test.

**In Study VI** statistical analyses were performed using Fisher's Exact Test calculated with GraphPad InStat™ software (GraphPad Software, San Diego, CA, USA)

## **5. Ethical considerations**

Permission to utilize Finnish Cancer Registry data in the studies of this thesis was granted by the Ministry of Health and Social Affairs (Dnro 59/08/95) and ethics committee of Tampere University Hospital (95062). HPC families in Finland were ascertained by a number of methods with the appropriate approval from the Ministry of Health and Social Affairs and local ethics committees of regional hospitals. A written informed consent was obtained from all patients and relatives giving a blood sample for the studies III, IV, V and VI. Linkage studies were carried out with the approvals from the Ministry of Health and Social Affairs, ethics committee of Tampere University Hospital and local ethics committees of regional hospitals. The genetic epidemiological study of hereditary prostate cancer in Finland (including all studies of this thesis) has been annually reviewed by Institutional Review Board of National Human Genome Research Institute at National Institutes of Health (NIH) and approved by US OPRR (Office for Protection from Research Risks) (no. S-6849-03).

## RESULTS

### 1. Estimation of standardized incidence ratios (Study I)

#### 1.1. Risk of prostate cancer in relatives of prostate cancer patients

Male relatives of prostate cancer patients had a significantly increased, approximately 2-fold, relative risk of prostate cancer. The age-adjusted SIR for the relatives in cohort 1 was 1.33 times higher (not significant) compared to the relatives in cohort 2. SIR in the brothers of the prostate cancer patients was higher than that in the fathers (SIR was 2.81 in brothers vs. 2.23 in fathers of cohort 1 and 1.80 in brothers vs. 1.53 in fathers of cohort 2). However, when the age-adjusted SIRs were compared, there was no difference in the prostate cancer risk between brothers and fathers (brother / father ratio 1.02; 95% CI 0.68-1.54). Relatives of the youngest index patients (age of diagnosis  $\leq$  69 years) had statistically significant elevated risks, ranging from 2.4 to 2.7. In relatives of the index patients aged 70-79 years, which covers the mean age of prostate cancer diagnosis in Finland (71 years), SIR was not significantly elevated (1.15). In contrast, in the relatives of the oldest index patients (over 80 years), SIR was again significantly elevated, 1.83, and almost at the same magnitude as in the young age of the onset group.

#### 1.2. Risk of other malignancies in relatives of prostate cancer patients

The risk of stomach cancer among male relatives of early age of onset prostate cancer patients was significantly elevated (SIR 1.88, 95% CI 1.29-2.65). All of this excess risk originated from the relatives of prostate cancer patients diagnosed at an age of 55 years or less. In this group, the relative risk of gastric cancer was 5-fold (4.96, 95% CI 2.77-8.17), which is substantial, compared to the relative risk of prostate cancer in this age group (SIR 2.61, 95% CI 1.25-4.80). None of the other cancer types investigated showed any significant excess of risk in male relatives, and no statistically significantly increased risks were observed for any type of cancer in the female relatives.

### 2. Cancer registry -based method for cancer family ascertainment (Study II)

#### 2.1. Development of "Population array" method

We first analyzed the distribution of the 35,761 prostate cancer cases by family name, and compared this with the family name distribution of all of the 3.3 million Finnish males. SPRs were calculated for each of the 10,721 family names included in the database. Eight of our 67 previously known families (10.4%) were flagged using this approach. The low sensitivity is due to the fact that the familial cancer cases do not lead to an increase in the prevalence ratio, if the family name is very common in the entire Finnish population. The combination of the family name and the place of birth ("population array") provided a powerful indicator of prostate cancer families. There were 28,459 different combinations of family name and place of birth with at least one prostate cancer. Of these combinations, 468 (1.6%) produced a significantly increased SPR ( $p < 0.05$ ) with three or more prostate cancer cases. Such prostate cancer cases are likely to represent prostate cancer families, not only because the patients share the same family name and place of birth, but because they are associated with a substantially higher number of prostate cancer cases than expected.

Out of the 50 previously known Finnish prostate cancer families, 33 (66%) were included among the 468 combinations with elevated SPR values. Of the 17 previously known prostate cancer families that were missed with the population array, 16 were due to the fact that all cancer cases within a family did not have the same place of birth. In one family, the cancer patients had a different family name. Nineteen of the highest 20 SPRs represented true prostate cancer families based on a parish records survey. This indicates 95% specificity of the method for the top 20 SPRs. Except for one sibpair, all of the identified 13 new families had three or more affected cases. In many cases, all the known affected individuals in a family could be directly ascertained from the population array results. In others, new affected cases that belonged to our previously ascertained families were identified. The data of 468 elevated SPRs therefore provide a rich resource for identification of new prostate cancer families, as well as for increasing the information content of the previously collected family materials.

## **2.2. Founder region suggested by the “Population array” method**

The geographical distribution of the families with a genetic disease is often used to identify putative founder regions where a particular ancient disease mutation is highly enriched. Figure 3A (in Study II) shows a plot of the geographical distribution of the places of birth for the top 300 SPR values. Suggestions of a clustering were seen in Western and South-Western Finland. In the Western cluster, three families with an elevated SPR were subsequently linked together with the ancestors born in the 1620s (see Fig. 3B in Study II). Several additional branches of this pedigree were subsequently demonstrated. This indicates that regional clusters of the families with prostate cancer, as identified by the population array method, may identify distantly related individuals who may share the same founder mutation.

## **3. Linkage analyses in Finnish prostate cancer families**

### **3.1. Discovery of HPCX susceptibility region (Study III)**

The results from the original 10cM genome-wide screen using 66 North American prostate cancer families by Smith et al. (1996) implicated a broad, 40 cM interval from *DXS1001* to *DXS1108*, reaching a maximum two-point LOD score of 1.08 at marker *DXS1193* at *Xq27-q28*. Twenty-six markers, spanning 19cM from *DXS984* to *DXS1108* (140-159 cM from Xpter) were genotyped for the 57 Finnish HPC families.

Two-point parametric LOD scores are presented in table 2 of Study III. Twelve of the markers tested had LOD scores greater than 1 in the combined data set, with maximum LOD score of 4.6 at marker *DXS1113*, at theta 0.26. The results were supported also by non-parametric LOD scores with maximum LOD score of 3.20 at marker *DXS1113*. In Finnish material the highest two-point parametric LOD score 2.05 was observed at marker *DXS1205*, proximal from *DXS1113*. At *DXS1113* LOD score of the Finnish families was only 0.49.

Simulation studies were performed to estimate the probability of obtaining a two-point parametric LOD score of 4.6 or greater or a p-value less than 0.00006 for the non-parametric affected sibpair analysis (mean test), at a single marker on the X chromosome in the absence of a linkage (false positive rate). Among 10,000 replicates in simulation, there were no two-point parametric LOD scores greater than 4.0, nor were there any p-values smaller than 0.00006 for the affected sibpair analysis.

Results from the parametric multipoint linkage analyses were consistent with the two-point analyses. The maximum LOD score assuming heterogeneity was 3.85 between loci DXS1200 and DXS297. Significant evidence of locus heterogeneity was obtained, with the proportion ( $\alpha$ ) of the families linked, estimated at 16% ( $\chi^2=17.73$ ,  $df=1$ ,  $p=0.00002$ ) (see Table 4 in Study III). Estimates of the proportions of the linked families ranged from 15% (Johns Hopkins) to 41% in the Finnish material.

Families with no male to male (NMM) transmission ( $n=129$ ) contributed disproportionately to the evidence of linkage to this region (maximum LOD score assuming heterogeneity = 2.46 at 151 cM from Xpter; estimated proportion linked = 19%). In contrast, for the families with male to male transmission ( $n=190$ ), the maximum LOD score assuming heterogeneity was 1.47 with the estimated proportion of the families linked 13%. Thus, the majority of evidence of linkage to *Xq27-q28* originates from the families with inheritance patterns consistent with the X chromosome linkage. The observation of positive LOD scores in the families with apparent male-to-male transmission may result from the presence of phenocopies as affected fathers or other relatives.

### **3.2. HPC1 and HPCX loci in the Finnish population (Study IV)**

HPC1 and HPCX linkage studies were carried out using the 57 informative families as described in the Study III. HPC1 linkage studies of the families demonstrated two-point parametric LOD scores ranging from  $-20.74$  (D1S518) to  $-3.55$  (D1S230) ( $\theta = 0$ ) for the 39 markers utilized. Multi-point parametric LOD scores and non-parametric NPL scores from the GENEHUNTER program were also negative. Two-point non-parametric affected sibpair tests using ANALYZE were not significant.

The maximum parametric LOD score for the HPC-X locus was 2.05 and occurred at  $\theta = 0.14$  with marker DXS1205. Five other markers on both sides of DXS1205 also showed positive LOD scores. Sliding four-point linkage using FASTLINK gave a maximum parametric multipoint LOD of 1.39 near DXS1205. The maximal NPL score obtained with GENEHUNTER for this region was 1.97 at position 2.62 corresponding to the location of marker DXS8043 ( $p = 0.024$ ). The two-point non-parametric affected sib-pair tests were most significant at DXS1205 ( $p=0.006$ ).

### **3.3. Stratified analyses of HPC1 and HPCX in the Finnish population (Study IV)**

Using HOMOG3R analyses, significant evidence of heterogeneity was observed ( $p<0.05$ ) with an estimated  $a_1$  of 0.5 for HPCX and an  $a_2$  of 0.3 for HPC1, assuming that there are two known loci (HPCX and HPC1) and  $a_3 = 1 - a_1 - a_2 = 0.20$  families unlinked to either loci. The test for two loci versus at one locus was not significant, yielding most parsimonious estimates of  $a_1 = 0.45$ ,  $a_2 = 0$  and  $a_3 = 0.55$ . We then performed subgroup analysis of these families by the age of onset and by the number of affected individuals (Table 2 in Study IV).

For HPC1 all subgroups defined in this manner had only strongly negative two-point LOD scores. In contrast, HPCX LOD scores were positive in several subgroups. The thirty-three families that were classified as having NMM transmission of the disease accounted for most of the positive LOD scores for the HPCX region with a maximum two-point LOD score of 2.16 ( $\theta = 0.079$ ) for DXS1205. In non-parametric two-point sib-pair analysis of the NMM group, the LOD score for this marker was even higher, 3.04 ( $p<0.00093$ ). In contrast, the



remaining families with “male-to-male” transmission had a peak two-point LOD score of 0.17 (theta 0.49) at DXS1205 and a non-significant affected sib-pair test.

Further stratification of the data indicated that most of the HPCX positivity came from the subgroup of families having NMM transmission and a late age of diagnosis (>65 years) (Table 2). These families were also relatively small, having two to three affected cases. Late-onset NMM families gave an overall two-point maximum LOD score of 3.12 (theta = 0.001) for DXS1205 and a non-parametric affected sib-pair LOD of 2.23 ( $p < 0.00068$ ).

To further evaluate whether the subgroups described above were the source of the observed heterogeneity, Morton’s pre-divided sample test was used. After adjusting for multiple testing, only the division based on the presence of male-to-male transmission and age of diagnosis was of statistical significance (adjusted  $p$ -value  $< 0.05$ ).

#### **4. Association of *E-Cadherin* germline alterations with prostate and gastric cancers (Study V)**

We identified 15 families (8.3%) with both prostate and gastric cancer among the 180 previously ascertained Finnish HPC (hereditary prostate cancer) families. These prostate-gastric cancer families had an average of 2.9 (range 2-5) cases of prostate cancer and 1.4 (range 1-4) of gastric cancer cases. Four patients in these families had both gastric and prostate cancers. The average age of the prostate cancer diagnosis in these families was 70 years (range 45-99) and that of gastric cancer 70 (range 34-88).

The database of the Department of Pathology of the Tampere University Hospital identified 18 patients out of the 1738 (1.0%) prostate cancer cases diagnosed during 1993-1999, who also had a diagnosis of gastric cancer. The average age of the prostate cancer diagnosis in these patients was 72 years (range 58-87) and that of gastric cancer 72 years (range 62-84). The histological type of gastric cancer was diffuse in two cases, intestinal in three cases, and in three cases the type could not be ascertained from the records.

To exclude HNPCC families and patients, we screened the Finnish founder mutations 1 and 2 of the *MLH1* gene from members of 15 prostate-gastric cancer families and eight patients diagnosed to have both prostate and gastric carcinomas. These two founder mutations cover 51% of all Finnish kindreds with verified or putative HNPCC (Nyström-Lahti et al., 1996). No *MLH1* mutations were detected, which is compatible with the observation that there is no increase of the colorectal and endometrial cancer cases in our HPC families.

Samples of the same cohort of 15 families and 8 double cancer patients were further screened for germline mutations in the 16 exons of the *CDH1* gene by PCR-SSCP from genomic DNA. PCR-SSCP analysis of all of the 16 exons of the *E-Cadherin* gene from genomic DNA revealed no splice site or truncating mutations. One missense mutation leading to a serine (TCT)→alanine (GCT) substitution at codon 270 in exon 6 was detected in family 215 (see Fig. 1 in Study V). The family 215 has four prostate cancer and two gastric cancer cases. Three brothers diagnosed to have prostate cancer were also S270A variant carriers. The fourth brother was also a S270A variant carrier but did not have prostate cancer.

The S270A missense variant is changing an evolutionary conserved amino acid, which is located in the extracellular domain of the E-Cadherin protein. In the mature protein, the

missense variant S270A is located at the beginning of the second extracellular cadherin-motif subdomain (C1-C5, where C1 is the most distant from the membrane). S270A is apparently not part of the highly conserved sequence motifs required for  $\text{Ca}^{2+}$  binding or dimerization. We performed a large-scale population screening of the S270A variant in over 1500 individuals to determine, whether the S270A variant would be associated with prostate cancer. The frequency of the S270A substitution was 0.5% (5/923) in the genomic DNA from population controls. The prevalence was significantly higher among familial prostate carcinoma patients (4/120, 3.3%;  $p = 0.01$ ) and also increased in an unselected series of prostate cancer patients (7/472, 1.5%;  $p = 0.12$ ). The increased frequencies of the S270A variant were detected among all gastric cancer patients (2/140, 1.4%;  $p = 0.24$ ) and among patients with diffuse type gastric cancer (2/68, 2.9%;  $p = 0.08$ ), but because of the small sample size, these differences remained statistically insignificant.

## **5. Prostate specific antigen measurements in high-risk prostate cancer families (Study VI)**

Twenty-one of the 209 apparently healthy men (10.0%) from prostate cancer families had elevated serum PSA values as compared to the age-specific reference values. The elevated values ranged from 2.6 to 28.3 mg/l. A subsequent urological examination with the first set of random biopsies revealed seven patients (3.3%) with prostate adenocarcinoma and two (1.0%) with prostatic intraepithelial neoplasia (PIN). One of the cancers was of advanced stage, whereas others were intra-capsular. The mean age of the PSA-detected cancer patients was 65.1 years (range 52-75), which is approximately seven years lower than the average age of diagnosis of prostate cancer patients in Finland. Three of the cases (43 % of all screen-detected cases) were detected under the age of 60 years.

The frequency of PSA-positivity (28.6%) and subclinical prostate cancer (14.3%) were significantly higher ( $p=0.010$  for PSA and  $p=0.024$  for cancer by Fisher's Exact test) in men from the families with an average age of the onset of 60 years or less, as compared to those from the families with a mean age of the onset  $>60$  (see Fig. 2 in Study VI). Furthermore, five of the seven new prostate cancers found in this screening study came from large families where three or more men were previously known to be affected with prostate cancer. Overall, six of the seven new cancer cases detected were detected in the families that either had an average age of the onset under 60 years of age or three or more previously known affected cases.

## DISCUSSION

This thesis studied the role of familial risk factors of prostate cancer in Finland. Special characteristics of the Finnish population and cancer registration system facilitated the studies in this thesis. The population-based and nation-wide Finnish Cancer Registry has collected data of malignancies since 1953 and the coverage of the register is currently over 99% of all solid tumors in Finland (Teppo et al., 1994). This high-quality nation-wide cancer registry data formed the basis of the studies I and II. The Finnish population is an attractive target population for genetic studies (de la Chapelle 1995, Peltonen 1997). The current inhabitation of Finland started about 2000 years ago with a small founder population, expanding in isolation to the present population of five million (Norio et al., 1973). A low population density, and a high degree of national and local isolation due to Finnish language, religion, difficult climate and geographical position are typical features of the history of the Finnish population. These have led to a subsequent genetic uniformity. Well-defined populations with well-characterized histories are considered the most useful for linkage analysis (Jorde et al., 1994). Also the population registration with electronic databases available since 1967 and parish records available for genealogical studies even from 16<sup>th</sup> century has been advantageous for these studies. In the Study II the development of the “population array” method demonstrates the advantages that Finland has to offer for genetic and epidemiological studies of cancers. The population array provides a rapid means to identify retrospectively a potential familial clustering of prostate cancer or any other cancer type. Geographical clustering, which is a typical phenomenon in hereditary diseases in Finland (Norio et al., 1973) and reflects founder populations formed by the local isolation, was also observed with “population array”. This combination of special characteristics of the Finnish population, research resources and the new strategies developed in this study will hopefully help in the dissection of the genetic basis of complex diseases in the near future.

### 1. Epidemiological evidence of genetic predisposition

A number of studies have shown an elevated risk of prostate cancer among the relatives of prostate cancer patients (Table 2, page 19). Most of these studies have been case-control studies, and only five cohort studies have been published before the Study I. Since the genes and environmental factors influencing cancer development may substantially vary from one population to another, it is important to understand the specific epidemiological features of hereditary prostate cancer specifically in the Finnish population. Studies of the genetic factors predisposing to cancer can optimally be carried out if the epidemiological features of cancer in that population are accurately defined. Finally, epidemiology often provides a basis to form hypotheses that can be tested using genetic studies.

In our population-based cancer registry study we found a significantly elevated risk of prostate cancer in the relatives of both early- (<70) and late-age (≥80) of onset prostate cancers patients. The risk ratios for prostate cancer observed in this study were of similar magnitude as previously reported in population-based registry studies (Table 2, page 21). No increased risk of prostate cancer, however, was observed for relatives of patients diagnosed at the age of 70-79 years, which covers the median age of onset (71 years) of prostate cancer in Finland. Several studies (Table 2, page 19) have emphasized the significance of the early age of onset of the disease as a predictor of familial prostate cancer risk, but little attention has been paid to the genetics of the late onset disease. The elevated prostate cancer risk among the relatives of late onset patients suggests that there could be a separate risk factor for familial prostate cancer, which is primarily associated with the late age of onset. This hypothesis is

supported by the Study IV, which suggests that the HPCX locus primarily contributes to the late-age of onset disease.

Study I reported that male relatives of the early onset prostate cancer patients have a significantly increased risk of stomach cancer. An association of prostate cancer and gastric cancer has also been observed by Vaittinen & Hemminki (1999) and Hemminki & Dong (2000) in the family-cancer database studies and by Grönberg et al. (2000) in Swedish families with hereditary prostate cancer. Contrary to the prostate cancer risk, the gastric cancer risk showed a strong association with the age of the index patient at the diagnosis. The 5-fold SIR was observed in the relatives of patients with the age of onset of 55 years or less, while there was no excess risk among relatives of older index patients. In previous studies, associations between prostate cancer and breast, ovarian, and colorectal cancers as well as tumors of the central nervous system have been reported (Cannon et al., 1982; Anderson and Badzioch, 1993; Goldgar et al. 1994; Tulinius et al., 1994; Isaacs et al., 1995 ; McCahy et al., 1996; Cerhan et al., 1998; Valeri et al., 2000). We did not find any significant excess of risk for these types of cancers. This may reflect differences in the study populations, as well as the heterogeneity of the prostate cancer predisposing genes in different populations. The Study I was based on all prostate cancers in a population, not just the prostate cancer families where the genetic component contributing to other cancer types may not only be stronger, but also biased by the specific family collection criteria. The association of gastric and prostate cancers could be due to shared genetic or environmental risk factors but could also be due to a chance. One would not expect that shared environmental risk factors would lead to cancer development exclusively at an early-age. The role of the shared genetic risk factors appears more likely, based on the observation that all the excess gastric cancer risk was confined to the relatives of the prostate cancer patients diagnosed at an extremely early age. Chromosome region 16q has been pointed out in two genome-wide linkage studies of prostate cancer families (Suarez et al., 2000; Gibbs et al., 2000). This is also the chromosomal site of the *CDH1* gene, whose mutations have been found in hereditary gastric cancer (Guilford et al., 1998). This led us to the hypothesis that mutations of the *CDH1* gene may be contributing to the observed association of prostate and gastric cancers. This was further investigated in Study V.

## **2. Genetic loci predisposing to hereditary prostate cancer in the Finnish population**

During the past five years, numerous chromosomal regions with a genetic predisposition to hereditary prostate cancer has been suggested (Table 5, page 29). Multiple candidate loci, incomplete penetrance and differences in clinical characteristics of prostate cancer make studies of the genetic basis of prostate cancer susceptibility challenging. Heterogeneity could be reduced by analyzing genetically homogeneous populations or by identifying subgroups of patients that are more likely to be linked to a particular locus.

Our conclusion based on studies III and IV is that there is significant evidence of heterogeneity in the loci causing prostate cancer, even in the genetically homogeneous Finnish population. The HPCX locus on the chromosome region Xq27-q28 seems to explain a large proportion of the Finnish hereditary prostate cancer cases, especially among families with no male-to-male transmission and the late age of onset. In contrast to some other studies, our family collection process in Finland was not heavily biased towards the early age of onset cases or cases with male to male inheritance. Our observations could mean that the HPCX gene has a higher prevalence also in other populations than Study III suggested. In the Study III, the HPCX locus was estimated to account for 15-16% of the families collected in Johns

Hopkins University and Mayo Clinic and up to 41% of the 57 Finnish hereditary prostate cancer families. In Study IV, concentrating on the Finnish families, stronger evidence for HPCX linkage was observed by sib-pair analysis than by two-point parametric analyses. The peak sib-pair LOD score in Study IV was 3.335 ( $p < 0.00009$ ) (in the no male-to-male transmission group), which suggests that the model used for the parametric analyses may not be optimal. The model for linkage analyses was derived from segregation analyses (Carter et al. 1992, Grönberg et al. 1998, Schaid et al. 1998), which suggested a rare locus with a high penetrance at an early age of onset. Segregation analyses cannot distinguish between different loci or their separate age-dependent penetrance functions. HPCX gene could therefore have a significantly higher population frequency than estimated based on the Study III, where the family material was enriched with extended early-age-of-onset prostate cancer families. Positive linkage results on HPCX region Xq27-q28 have been presented also by Lange et al. (1999). In their study, however, the evidence for HPCX linkage came from families with no male-to-male transmission and early onset (<65 years) disease. This could be due to differences in the family collection criteria.

In contrast to HPCX, HPC1 is not likely to be the major locus contributing to the hereditary prostate cancer in Finland. Our negative results for HPC1 linkage are compatible with recent studies by McIndoe et al. (1997), who analysed 49 families from Seattle, USA, as well as Eeles et al. (1998), who analysed 136 families from the United Kingdom, Quebec, Canada and Texas, USA. The results by Cooney et al. (1997) and by Hsieh et al. (1997) have confirmed the presence of the HPC1 locus by reporting a borderline significant linkage. Grönberg et al. (1999) have reported that almost all the evidence of the linkage in HPC1 families came from families with an early age of onset (<65 years) or with five or more affected cases. Also a large combined analysis of HPC1 linkage with 772 families (Xu and the International Consortium for Prostate Cancer Genetics, 2000), containing also Finnish families, concluded that HPC1 linkage was seen only in a subgroup of families with early age disease and male-to-male transmission. In Study IV, stratification of the families by age and size did not significantly increase the HPC1 LOD scores. Therefore, there are many Finnish prostate cancer families, especially those with many affected cases diagnosed at an early age, where genes other than HPC1 or HPCX are likely to be important. As suggested by genetic heterogeneity, additional susceptibility genes have been localized and probably still remain to be found. Further subgrouping and clinical characterization of prostate cancer families will probably help in further stratification of additional loci. Analyses of additional currently known HPC loci in the Finnish population will further clarify the genetic background of hereditary prostate cancer in Finland. Also a genome-wide linkage study on Finnish prostate cancer families has recently been completed (unpublished observations).

The role of the different hereditary prostate cancer loci and genes in the total prostate cancer burden will be clarified only after the actual genes have been found. It will be interesting to see, whether the contribution of the various HPC genes and other low-penetrance susceptibility genes will be closer to the estimated 5% of all prostate cancers as suggested by population-based epidemiological studies or to the 40% suggested by twin studies.

### **3. Clinical management and counseling of patients with a genetic predisposition to prostate cancer**

Relatives of men with prostate cancer are often concerned about their own risk of cancer. The epidemiological data acquired in this study may help to estimate the familial cancer risks of

prostate cancer among Finnish men. The following conclusions can be drawn of the risks at the population level. First, with the exception of the elevated risk of stomach cancer in the relatives of early-onset prostate cancer patients, the excess risk is focussing on cancer of the prostate. Second, no excess risk of any cancer was seen for female relatives of prostate cancer patients. Third, the cancer risk in relatives of the late age of onset patients (>65 years) is negligible, at least at the population level. It is possible that the risks in strong familial clusters will be different.

Based on our observations, efforts in genetic counseling should be focussing only at the relatives of early-onset (<65 years) prostate cancer patients. The risk of prostate cancer in these relatives also remains relatively low, only about two fold over the population average. Therefore, potential screening, follow-up and intervention efforts would not be justified unless the family history of prostate cancer is very prominent, or unless a specific genetic test was available, enabling one to diagnose and gauge inherited risks more accurately at the level of an individual.

The majority of the men belonging to the prostate cancer families would be interested in presymptomatic genetic testing, if such a method was available (Bratt et al., 2000). Despite the substantial progress in dissecting the molecular basis of the prostate cancer predisposition, it will probably take many years before specific genetic tests will be commonly available. In contrast to most hereditary diseases, prostate cancer develops quite late, usually after 50 years of age, which makes the application of genetic testing more complicated. Finally, it is unclear as to how much benefit could be derived from the identification of genetically predisposed individuals. Could cancer be diagnosed earlier in these cases? Would this earlier diagnosis decrease mortality? Would such an earlier diagnosis improve the quality of life of the men who are diagnosed with prostate cancer? Would the quality of life improve for gene carriers who never end up developing the disease? What are the consequences of such information for other relatives who do not choose genetic testing? Are there preventive measures that can be taken to reduce the risk of cancer in genetically predisposed patients? Would the screening, genetic counseling, early diagnosis, treatment, clinical monitoring be cost-effective? Who would be responsible for such measures and their expenses? Answers for these questions, as well as many others remain unknown for the time being. Answering these questions will require large-scale, long-term clinical studies. However, regardless of the proven efficacy of such measures, clinicians will often face concerns of patients and their family members.

At the moment, PSA screening is likely to have an important role in alleviating concerns of cancer in members of the prostate cancer families who seek medical attention and genetic counseling. Several randomized studies of prostate cancer screening in the general population are ongoing as presented earlier in the review of literature. The results of these studies will help to form guidelines for large scale PSA screening procedures. Men from the families with multiple prostate cancer cases are, however, a special group with an increased risk of prostate cancer. Study VI supported the concept that serum PSA screening is likely to be particularly useful for men with a family history of early-onset prostate cancer. Long-term clinical follow-up procedures should probably be limited to the men of those families where the age of onset of prostate cancer is under 60 or where three or more prostate cancers have been previously already diagnosed. PSA screening of high-risk groups should be started quite early, 5-10 years before the age that the first prostate cancer was diagnosed in the family, perhaps at the age of 45 years, as suggested by Walsh and Partin (1997). For men with continuously normal PSA value during the follow-up, screening should be terminated at approximately 70 years of

age, because the risk of dying of prostate cancer for men with a normal PSA at that age is very small, at least in the general population (Stenman et al., 1994).

There are some experimental data suggesting that chemoprevention may decrease the risk of prostate cancer (Tsukamoto et al., 1998). Also studies on the use of 5-alpha reductase treatment in chemoprevention of prostate cancer are ongoing (Brawley and Parnes, 2000). Dietary manipulations could be designed, but currently the role of diet as an etiological agent is too unclear to offer any guidance (Denis et al., 1999). At the moment there are therefore no methods available for reducing the risk of prostate cancer for genetically predisposed men. Radical alternatives that could only be applied in extreme cases of genetic predisposition (preferably only after a specific genetic test) could include hormonal treatments, or prophylactic radical prostatectomy. However, the side-effects and risks of these measures far outweigh their demonstrated or hypothetical benefits. Therefore, for the foreseeable future, early detection of prostate cancer by serum PSA screening will probably be the only method available for men belonging to the families with hereditary prostate cancer.

#### **4. Future prospects**

Several chromosomal regions that may harbor susceptibility genes for hereditary prostate cancer have already been identified in linkage studies (Table 5, page 29). However, at the moment only one gene associated with hereditary prostate cancer, HPC2/ELAC2 (Tavtigian et al., 2000), has been identified. The role of this gene is also still quite unclear. Furthermore, it is thought to contribute to only a small fraction of prostate cancer families. Based on the large number of loci implicated in linkage studies, several other genes are likely to be found in a near future. Identification of the HPCX gene is one of the major goals of our ongoing studies in Finland. Furthermore, additional genes and genetic alterations may be found through association analyses, and candidate gene studies using high-throughput screening and genotyping. Development of high-throughput genotyping using single nucleotide polymorphism (SNP) markers and DNA microarray technology (Wang et al., 1998) combined with accurate genetic data produced by Human Genome Project will further facilitate the dissection of molecular basis of hereditary prostate cancer. Such studies will require large family, patient and population control materials that are often the limiting steps in studies. Population-based association studies in genetically homogeneous populations will offer an alternative method for these studies (Jorde, 1995). The combination of large-scale nationwide cancer registry data with modern genetic analyses and next-generation SNP-based genome-scale scanning could provide substantial power to identify determinants of genetic predisposition to cancer.

The characterization of the hereditary prostate cancer genes will open insights to prostate cancer etiology and biology and also enable specific diagnostics of hereditary prostate cancer with genetic testing, and in the future possibly even a treatment of the disease. Due to the genetic heterogeneity and complexity of prostate cancer, different genes will have different impact in different populations. When the major genes of hereditary prostate cancer will be identified, the population-based studies will be needed to investigate the frequency and penetrance of these genes in different populations, as well as the effects of environmental and life-style risk factors. In genetically homogeneous populations, like that of Finland, the number of disease causing genes is limited. Thus, the development of genetic testing for research and future clinical diagnostics, may be easier than in genetically more heterogeneous populations.

## SUMMARY AND CONCLUSIONS

Family history is one of the strongest risk factors for prostate cancer. Five to ten percent of the prostate cancers may be strongly influenced by inherited genetic defects. The aim of this study was to search for genetic risk factors and susceptibility genes for human prostate cancer in Finland using epidemiological and molecular genetic methods.

The cancer registry -based Study I suggested a substantial contribution of hereditary factors in prostate cancer development among both older and younger men. The risk of prostate cancer was approximately two-fold in first-degree relatives of prostate cancer patients. We also observed an association between gastric cancer and early-onset prostate cancer. This association could reflect the role of a novel locus predisposing to both of these cancer types.

In Study II we described and validated a new “population array” method for ascertainment of cancer families using population-based cancer registry data. This registry-based method is based on the sorting of the cancer registry data by family name and place of birth, as well as the determination of a higher than expected number of cancer cases (elevated standardized prevalence ratio, SPR) associated with such combinations. Using population array method we identified 468 “candidate prostate cancer families”, men sharing the same family name and the place of birth and having a significantly higher number of prostate cancer cases than expected based on the frequency of the family name in the population of that particular municipality. The population array strategy provided us a comprehensive nation-wide screening for candidate prostate cancer families. We also found geographical clusters of elevated SPRs, which, even if they have a different family name, may reflect ancient founder effects contributing to an elevated prostate cancer prevalence in that region.

Based on the prostate cancer families identified and collected from Finland, linkage analyses were performed. In Study III, as a part of the international consortium, we presented evidence for the linkage of a subset of prostate cancer families to a locus on chromosome region Xq27-q28 (HPCX). The linkage with a maximum two-point LOD score of 4.60 was observed in 360 families from Finland, Sweden and the USA. Classification of families according to the occurrence of male-to-male and no male-to-male transmission within HPC linkage families provided an opportunity to evaluate the evidence for an X-linked HPC locus.

Two prostate cancer loci HPC1 (1q24-q25) and HPCX (Xq27-q28) were further evaluated in the Study IV. Significant evidence of genetic heterogeneity, suggesting that multiple loci are likely to contribute to prostate cancer even in the homogeneous Finnish population, was observed. The HPCX locus on Xq27-q28 seems to explain a particularly large fraction of the Finnish hereditary prostate cancer cases, especially among the families with no male-to-male transmission and late age of diagnosis. In contrast, we found no evidence of the involvement of the HPC1 locus at 1q24-q25 in Finnish families. Thus, most of the large Finnish HPC families, with the early onset prostate cancer still remain unaccounted for these two loci.

As a candidate gene approach, based on Study I, we studied the role of E-cadherin gene in families with both prostate and gastric cancers. The results suggested that individual rare mutations and polymorphisms in the *CDHI* gene, such as the novel S270A mismatch mutation observed in the study, may contribute to the onset of prostate cancer. It seems that *CDHI* gene does not, however, explain the link between prostate and gastric cancers on a population level. Taken together with the lack of *MLHI* gene mutations, and the lack of



phenotypic features of the HNPCC syndrome, the results suggested that currently unknown genes may contribute to the observed link between prostate and gastric cancers.

The results of PSA measurement based screening of prostate cancer among asymptomatic men from prostate cancer families in the Study VI suggest that prostate cancer development in genetically predisposed individuals is preceded by a subclinical period when PSA detection is possible. Serum PSA screening may be particularly useful in the men with a family history of the early-onset prostate cancer.

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