SANNA PAKKANEN

Profiling of High-risk Prostate Cancer Families in Finland

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ACADEMIC DISSERTATION
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the Faculty of Medicine of the University of Tampere,
for public discussion in the Main Auditorium of Building M,
Pirkanmaa Hospital District, Teiskontie 35,
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Contents

List of original communications

This thesis is based on the following communications, referred to in the text by Roman numerals (I-IV). In addition, some unpublished results are presented.

I. **Pakkanen S***, Baffoe-Bonnie AB*, Matikainen MP, Koivisto PA, Tammela TL, Deshmukh S, Ou L, Bailey-Wilson JE, Schleutker J. Segregation analysis of 1,546 prostate cancer families in Finland shows recessive inheritance. Hum Genet. 2007;121(2):257-67.

II. **Pakkanen S**, Matikainen MP, Ha N, Kujala P, Schleutker J, Tammela TLJ. Clinical and histopathological characteristics of familial prostate cancer in Finland. Submitted.

III. **Pakkanen S**, Pukkala E, Kainulainen H, Matikainen MP, Koivisto PA, Tammela TLJ, Schleutker J. Incidence of cancer in Finnish families with clinically aggressive and nonaggressive prostate cancer. Cancer Epidemiol Biomarkers Prev. 2009;18(11):3049-56, 2009.

IV. **Pakkanen S***, Wahlfors T*, Siltanen S, Patrikainen M, Matikainen MP, Tammela TL, Schleutker J. P*ALB2* variants in hereditary and unselected Finnish prostate cancer cases. J Negat Results Biomed. 2009; 5;8:12.

*These authors contributed equally.

Abbreviations

APC	Adenomatous polyposis coli
ALK3	Activin receptor-like3
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
CABP	Prostate cancer/brain cancer susceptibility locus
CDH1	Cadherin 1, type 1, E-cadherin (epithelial)
CHECK2	Cell cycle checkpoint kinase
DNA	Deoxyribonucleic acid
DHT	Dihydrotestosterone
ELAC2	elaC homolog 2 (*E. coli*)
ERSPC	European prostate cancer screening study
FCR	Finnish Cancer Registry
GWAS	genome wide association studies
HNPCC	hereditary non-polyposis colorectal cancer syndrome
HPC	Hereditary prostate cancer
HPC1	Hereditary prostate cancer 1
HPC2	Hereditary prostate cancer 2
HPC20	Hereditary prostate cancer 20
HPCX	Hereditary prostate cancer X
ICPCG	International consortium for prostate cancer genetics
LKB1	Serine/threonine protein kinase
MLH1	MutL homolog L
MSH2	MutS homolog 2, colon cancer, (nonpolyposis) type 1
MSH6	Mut S homolog 6
MSR1	Macrophage scavenger receptor 1
MYH	mutY homolog (*E. coli*)
PCAP	Predisposing for prostate cancer
PIN	Prostatic intraepithelial neoplasia
PALB2	Partner and localiser of *BRCA2*
PMS2	PMS2 postmeiotic segregation increased 2 (*S. cerevisiae*)
PSA	Prostate specific antigen
PTEN	Phosphatase and tensin homologue
RNASEL	Ribonuclease L (2'5'-oligoisoadenylate synthetase-dependent)
SIR	standardised incidence ratio
SNP	single nucleotide polymorphisms
SMAD4	SMAD family member 4
TSG	tumour suppressor gene
TURP	transurethral resection of the prostate

ABSTRACT

Prostate cancer is the most common cancer among males in Finland. Familial aggregation of prostate cancer was first observed in the 1950s, though age and ethnic background are now recognised as additional risk factors. Five to ten percent of prostate cancer cases can be attributed to inherited defects. The aim of this study was to further investigate familial prostate cancer in Finland using epidemiological and molecular genetic methods.

In this thesis, I aimed to further investigate the mode of familial aggregation of prostate cancer, whether there are other cancers present in these families and how these familial cancers act as a clinical disease. I performed segregation analyses for two population-based cohorts of 557 early onset and 989 late onset familial prostate cancer cases. This analysis confirmed the existence of hereditary prostate cancer in the Finnish population under a complex model that included a major susceptibility locus with Mendelian recessive inheritance as well as a polygenic/multifactorial component.

I collected detailed clinical and histopathological data for 617 males affected by prostate cancer from 202 families in Finland. I confirmed observations suggesting that familial prostate cancers show a higher grade, a higher amount of metastases and a trend toward higher PSA levels as compared to sporadic cancers. However, there was no difference in the cancer-specific survival between the cohorts.

The incidence of other cancers was investigated among familial prostate cancer patients and their relatives to define the association of prostate cancer with a cancer syndrome or second-site cancers. I found that the incidence of non-prostate cancer is not increased in either clinically aggressive or nonaggressive familial prostate cancer cases in Finland, with the exception of stomach cancer among female relatives.

I also investigated the role of *PALB2* variants in hereditary and unselected Finnish prostate cancer cases. A total of six variants in *PALB2* were identified. Though none of the detected *PALB2* variants were associated with prostate cancer at the population

level in Finland, it is still possible that some of these variants contribute to cancer susceptibility at the individual level.

In summary, this thesis confirms previous observations of familial aggregation of prostate cancer for the Finnish population and indicates that the inheritance of this disease is best explained by a recessive mode. In addition, the clinical and histopathological characteristics of familial prostate cancer are described, and the incidence of other cancers in these families are investigated. I also determined that *PALB2* variants are not associated with prostate cancer at the population level in Finland.

TIIVISTELMÄ

Eturauhassyöpä on miesten yleisin syöpä Suomessa. Eturauhassyövän seulonnasta johtuen viimeiset 20 vuotta eturauhassyövän ilmaantuvuus oli voimakkaassa kasvussa, mutta saavutti huippunsa vuonna 2006 (115/100 000). Vuonna 2008 Suomessa oli 4235 uutta eturauhassyöpätapausta. Huolimatta eturauhassyövän nopeasta lisääntymisestä ja suuresta kansanterveydellisestä merkityksestä, sen etiologia ja riskitekijät ovat hyvin huonosti tunnettuja. Eturauhassyövän perheitäinen esiintyminen on havaittu jo 1950-luvulla. Tämänhetkinen konsensus on että perinnöllisen eturauhassyövän osuus on noin 5-10%. Tämän väitöskirjatyön tavoitteena oli tutkia perheittäin esiintyvää eturauhassyöpää Suomessa käyttäen epidemiologisia ja molekyyligeneettisiä tutkimusmenetelmiä.

Eturauhassyövän perheittäistä esiintymistä ja periytymismallia selvitettiin tässä väitöskirjatyössä kahdessa laajassa väestöpohjaisessa aineistoissa, joiden koot olivat 8008 ja 12196 henkilöä. Segregaatioanalyysin tuloksena sain, että perheittäinen eturauhassyöpä periytyy resessiivisen mendeliaalisen periytymismallin mukaan ja että tässä on havaittavissa merkittävä polygeeninen/monitekijäinen vaikutus. Tutkimustulos tukee havaintoa, että eturauhassyövän periytymistä säädellään geenitasolla. Tämä julkaisu on ensimmäinen missä resessiivinen periytymismalli sopii sekä nuorena, että vanhempana sairastuneisiin eturauhassyöpä miehiin.

Tässä väitöskirjatyössä on kuvattu perheittäin esiintyvän eturauhassyövän kliiniset ja histopatologiset ominaisuudet Suomessa. Havaitsin, että eturauhassyöpään sairastuneilla miehillä, joiden perheessä esiintyi eturauhassyöpää, on korkeampi kasvaimen erilaistumisaste, useammin alueellisia imusolmukemetastaaseja, korkeampi primaari PSA arvo kuin miehillä, joilla oli sporadinen syöpä. Mutta syöpäspesifisessä elinajassa ei ollut eroa.

On todettu, että eturauhassyöpä on melko itsenäinen sairaus ja ettei muita pahanlaatuisia syöpätauteja esiinny eturauhassyöpäperheissä normaalia enempää. Myöhemmin on havaittu, että Suomessa eturauhasyöpää sairastavien potilaiden sukulaisilla olevan lisääntynyt riski eturauhas- ja mahasyöpiin. Tässä väitöskirjatutkimuksessa

selvitettiin 202 eturauhassyöpäperheen muita syöpiä ja tuloksena saimme, ettei yleinen syöpäilmaantuvuus ole korkeampi kuin verrokkiväestössä, paitsi eturauhassyöpä perheiden naisilla, joilla oli suurempi sairastuvuus mahasyöpään.

Hiljattain on tunnistettu uusi proteiini, PALB2, joka sitoutuu BRCA2:een. Väitöskirjatyössä selvitettiin *PALB2* muutosten esiintyminen suomalaisilla eturauhassyöpäpotilailla. Tuloksena sain, etteivät havaitut *PALB2* variantit selitä eturauhassyövän ilmaantumista populaatiotasolla Suomessa, mutta todennäköisesti osa varianteista saattaa altistaa syövälle yksilötasolla.

Yhteenvetona tämä väitöskirja tukee aikaisempia havaintoja siitä, että eturauhassyöpä on geneettinen tauti ja omaa väestökohtaisia erityispiirteitä. Perheittäinen esiintyminen Suomessa selittyy parhaiten resessiivisellä periytymismallilla ja periytymisalttius johtunee monen eri tekijän yhteisvaikutuksesta. Tässä väitöskirjatutkimuksessa on kuvattu perheittäisen eturauhasyövän kliiniset ja histopatologiset ominaisuudet sekä muiden syöpien esiintyminen. Perheittäin esiintyvä syöpä saattaa olla kliinisenä tautina aggressiivisempi, kuin sporadinen syöpä.

INTRODUCTION

Prostate cancer is the most frequently diagnosed cancer among Caucasian men and the second most common cause of cancer-related deaths, after lung cancer (Finnish Cancer Registry, 2008). The incidence of prostate cancer has increased rapidly in the last few decades, especially in the early 1990s, due to the widespread use of serum prostate-specific antigen (PSA) as a diagnostic tool. Despite the high incidence and mortality of this malignancy, the underlying aetiology of prostate cancer remains poorly understood.

Besides age and ethnicity, the strongest risk factor for prostate cancer is family history. Through numerous epidemiological and molecular biological studies, considerable evidence has accumulated in favour of a significant but heterogeneous hereditary component in prostate cancer susceptibility. Twin studies provide the most straightforward tool for evaluating the significance of genetic and environmental aetiological factors. Several twin studies (Grönberg et al. 1994, Page et al. 1997, Lichtenstein et al. 2000) have suggested that shared genetic factors cause the accumulation of prostate cancer cases within single specific families. In linkage analyses, several loci have been found to show evidence of linkage, but only three genes, with an estimated collective contribution of about 5%, have been identified in hereditary prostate cancer patients to date (Grönberg et al. 1994, Page et al. 1997, Rebbeck et al. 2000a, Tavtigian et al. 2001, Carpten et al. 2002, Xu et al. 2002, Schaid 2004). Because of the heterogeneous nature of the disease, the genes that promote prostate cancer have been extremely hard to identify; no high-risk genes underlying hereditary prostate cancer have yet been discovered.

Prostate cancer is likely caused by multiple relatively common interacting loci. Many questions need to be answered before the genetic susceptibility of prostate cancer is fully understood, but there is still clinical importance within the present genetic knowledge. On average, hereditary prostate cancer is diagnosed six to seven years earlier than sporadic cancer, but does not otherwise clinically differ from the sporadic form.

However, a greater proportion of men with the hereditary form die of the disease than those with non-hereditary prostate cancer. Currently, PSA screening is the only available clinical tool to reduce prostate cancer mortality (Matikainen et al. 1999, Neal and Donavan 2000) and detect the disease when it is still at a curable stage.

In this thesis, I have focused on familial prostate cancer in Finland. I have used unique prostate cancer family data collected across Finland in addition to the Finnish Cancer Registry, Population Registry and parish records.

REVIEW OF THE LITERATURE

1. Epidemiology of prostate cancer

1.1 Incidence and mortality

Prostate cancer is the most commonly diagnosed cancer in men in the USA, accounting for an estimated 186 320 cases in 2008 and 25% of all cancer diagnoses in men. The incidence of prostate cancer has changed notably over the last 20 years, rapidly increasing from 1988-1992, declining sharply from 1992-1995 and levelling off after 1995 (American Cancer Society, 2009). In Finland, there were 4235 diagnosed prostate cancer cases in 2008, accounting for 31% of all cancer cases (Finnish Cancer Registry, 2008). Figure 1 shows the most common cancers among men in Finland in 2008.

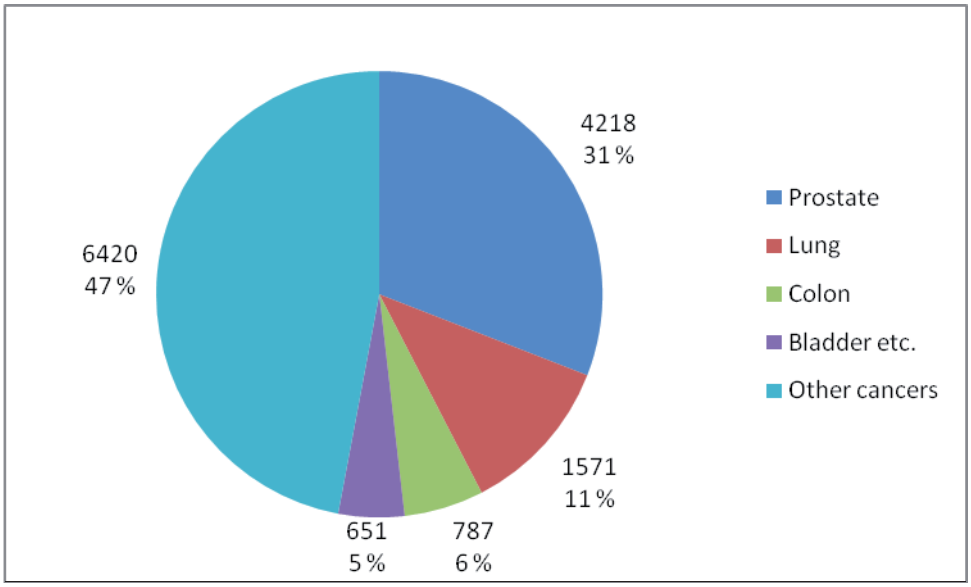

***Figure 1**. The most common cancers among men (all ages, 13647 cases in total) in Finland in 2008.*

Cancer incidence rates in Finland have changed in a similar manner as the rates in the USA, but the rapid increase and decrease became apparent five to ten years after the corresponding changes in the USA. An exceptionally irregular trend in prostate cancer incidence has been observed in recent years due to the increased frequency of PSA testing. The large-scale European prostate cancer screening study (ERSPC) showed a 3.2-fold increase in the diagnosis of prostate cancer for men undergoing systematic PSA screening (Schröder et al. 2009). However, after a rapid increase prior to 2005, there has been an even sharper decrease. The decrease in incidence is expected to level off (as has been seen in the USA), but the exact timing of this levelling off is difficult to predict (Finnish Cancer Registry, 2008).

Although the incidence of prostate cancer has increased substantially, the mortality rate has stayed constant. In the USA, prostate cancer was a leading cause of cancer death, with an estimated 28 660 deaths, in 2008. In Finland, 817 men died of prostate cancer in 2008, indicating an age-adjusted mortality rate of 14.1 per 100 000 men. The ERSPC study, completed in 2008, included 162 000 men and presented the first mortality analysis (Schröder et al. 2009). During the nine years of follow-up, the ERSPC showed an approximately 30% reduction of prostate cancer mortality in those who were actually screened. Figure 2 compares prostate cancer incidence and mortality in Finland, where the incidence was highest across Europe in 2005 (Finnish Cancer Registry, 2008).

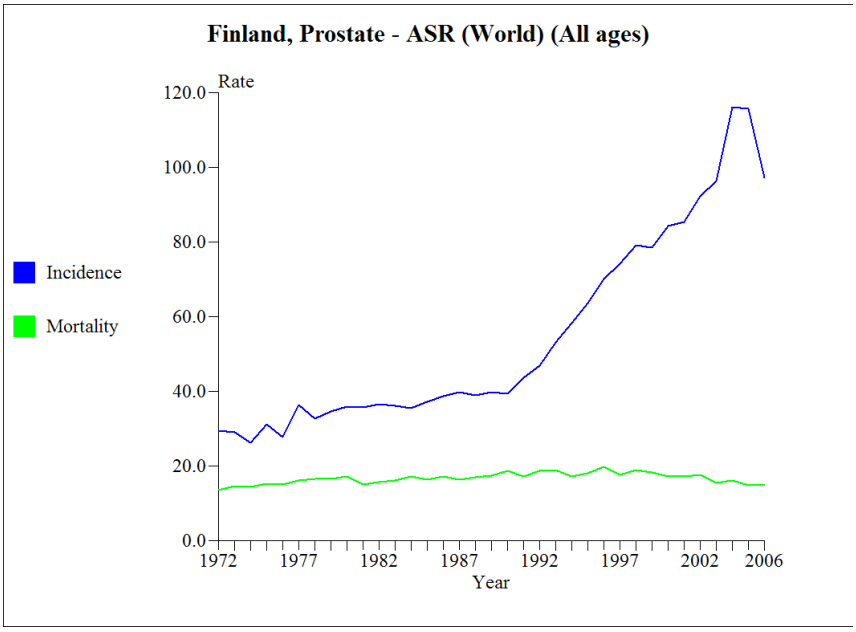

***Figure 2**. Annual age-standardised incidence and mortality rates for prostate cancer in Finland, 1972-2008 (Rate per 100 000). Graph from NORDCAN (Engholm et al. 2010).*

1.2 Risk factors and aetiology

The aetiology of prostate cancer has evolved coincidentally with our deepening understanding of the complex interplay between the human genome and the environment. Genetic determinants, the endocrine milieu and environmental exposures all need to be considered when resolving the aetiology of prostate cancer. The well-established risk factors for prostate cancer are age, ethnicity and family history of the disease.

1.2.1 Age of onset

As typical for all cancers, the risk of developing prostate cancer increases with age. In the USA, the median age at diagnosis of prostate cancer was 67 years of age 2003-2007, and more than 65% of all prostate cancers are diagnosed in men over the age

of 65 (Altekruse et al. 2009). In Finland, the age-specific highest annual number of new cancer cases was 871 prostate cancers in men aged 70-74 years, and the highest age-specific and age-adjusted incidence rate is 1154 per 100 000 in 80- to 84-year-old men in Finland (Finnish Cancer Registry, 2008). Figure 3 shows the incidence rates across age groups from 1972 to 2006 in Finland.

1.2.2 Ethnic origin

The incidence of prostate cancer varies among different countries and is highest in Australia/New Zealand (104.2 per 100 000 person-years), Western and Northern Europe and North America. The lowest age-standardised incidence rate was calculated for South-Central Asia (4.1 cases per 100 000 person-years) (International Agency for Research on Cancer, 2008). As shown in Figure 4, there are substantial ethnic differences in both prostate cancer incidence and mortality in the USA. African Americans have the highest incidence and mortality rates, whereas the lowest is found in Alaskan natives. An interesting issue is that Inuit have low rates of prostate cancer (Dewailly et al. 2003). Figure 5 shows regional incidence rates in Nordic countries, illustrating the variability present even in homogenous countries like Finland.

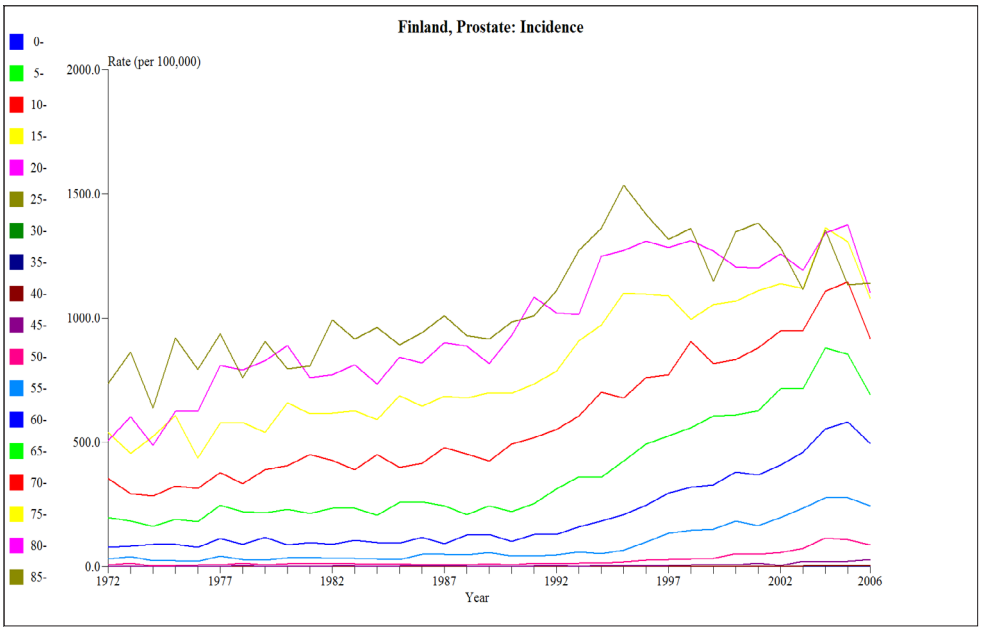

***Figure 3**. Age-standardised prostate cancer incidence rates in Finland. Graph from NORDCAN (Engholm et al. 2010).*

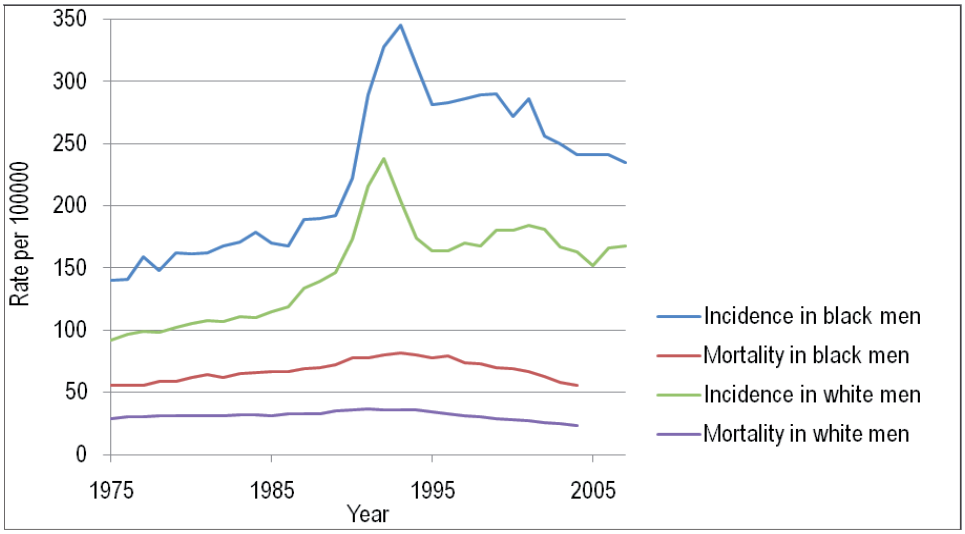

Figure 4. *Age-adjusted incidence and mortality rates of prostate cancer in SEER areas (San Francisco, Connecticut, Detroit, Hawaii, Iowa, New Mexico, Seattle, Utah and Atlanta) arranged by year and race in the USA. Data modified from the U.S. National Cancer Institute (Altekruse et al. 2009).*

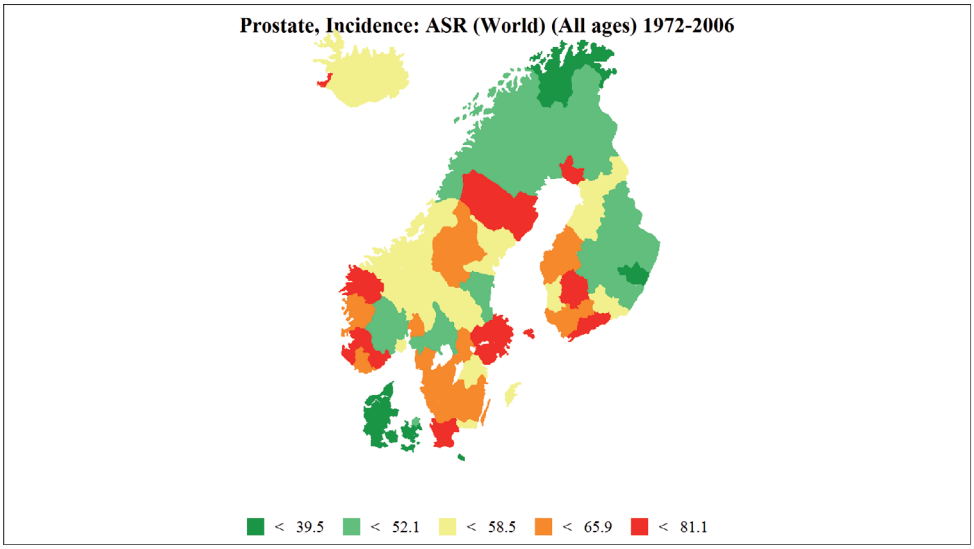

Figure 5. *Age-standardised prostate cancer incidence rates in Nordic countries. Graph from NORDCAN (Engholm, 2010).*

1.2.3 Family history

A positive family history is the most consistently identified risk factor for prostate cancer. Familial clustering of prostate cancer was first observed in the 1950s (Morganti et al. 1956), and there is a clear positive family history of the disease in about 5-10% of all cases. Subsequent studies have shown that a high risk of prostate cancer is associated with having multiple affected relatives: there is a two- to ten-fold increase in the risk of prostate cancer in brothers and sons of men with prostate cancer. Particularly high risk has been associated with men having multiple affected relatives or relatives diagnosed at an early age, where the proportion of familial clustering is also much higher, up to 40-50% (Figure 6) (Grönberg et al. 1999, Bruner et al. 2003). This familial risk has been observed in all ethnic groups and populations studied (Whittemore et al. 1995, Hayes et al. 1995, Ohtake et al. 1998, Glover et al. 1998). In a population-based study in Finland, an approximately two-fold increased risk was observed for men with affected first-degree relatives (Matikainen et al. 2001). The risk of developing prostate cancer increases as the age of probands decreases, as the closeness and number of affected members in the family increases, or when both factors are considered together (Eeles 1999).

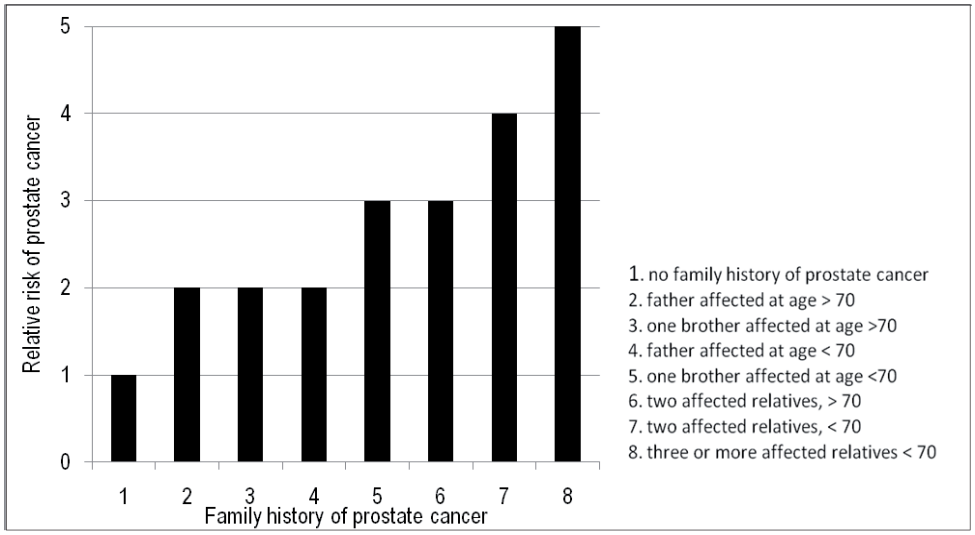

Figure 6. *Relative risk of prostate cancer with respect to affected family members (Eeles 1999, Matikainen et al. 2001). The relative risks are approximations based on a synthesis of published epidemiological studies.*

1.2.4 Aetiology

The observed differences in prostate cancer risk exhibited by different ethnic groups suggest that genetic factors are at least partially responsible. The changes in incidence rates over time also imply that differences in environment or lifestyle are important. Indeed, the observations that prostate cancer risk increases when Japanese migrate to Hawaii (Maskarinec and Noh 2004) or to Los Angeles (Shimizu et al. 1991) suggest that diet and environmental factors also play a major role. Despite extensive research, the environmental risk factors for prostate cancer are not well understood.

Hormonal influences

It is clear that male sex hormones play an important role in the development and growth of prostate cancer. They are needed for prostate growth, development, function and maintenance. In 1941, Huggins and Hodges demonstrated that prostatic cancers are androgen dependent (Huggins 1941). Testosterone diffuses into the prostate gland, where it is converted to the more metabolically active dihydrotestosterone (DHT) by the enzyme 5α-reductase. Both DHT and testosterone bind to the androgen receptor, which then translocates to the nucleus, binds to deoxyribonucleic acid (DNA) and transactivates genes that, for example, control cell division. Prostate cancer does not develop in men who are castrated at an early age (Smith et al. 1991). Men who have higher levels of DHT appear to have a higher risk of developing prostate cancer (Eaton et al. 1999). Accordingly, reduction of DHT formation by 5αreductase inhibitors decreases the incidence of prostate cancer (Andriole et al. 2010).

Diet and nutrition

Diet and nutrition are interesting issues with respect to prostate cancer risk. A wide variety of dietary factors have been implicated in the development of prostate cancer in prospective intervention. However, a true consensus has not been reached. Selenium, vitamin E and lycopene seem to have protective effects against prostate cancer in epidemiological studies (Giovannucci et al. 1995, Clark et al. 1998, Yoshizawa et al. 1998, Heinonen et al. 1998, Schuurman et al. 2002, van den Brandt et al. 2003). Many studies have reported a positive association between milk consumption and prostate cancer risk (Bostwick et al. 2004). The present view states that low fibre consumption, intake of red meat and an imbalance of omega-3 and omega-6 fatty acids may all

increase the risk of prostate cancer. On the other hand, the consumption of fruit and vegetables may lower the risk. A healthy lifestyle and diet drawn up according to the proposed guidelines could decrease the incidence of this disease (Divisi et al. 2006).

Inflammation

The complex relationship between inflammation and cancer has been well described since the late 1800s (Balkwill and Mantovani 2001). Epidemiological studies have convincingly demonstrated that inflammatory diseases increase the risk of developing cancer. For example, gastric infection with *Helicobacter pylori* (Parsonnet et al. 1991), inflammatory bowel disease (Rhodes and Campbell 2002) and chronic hepatitis (Imperial 1999) have been linked to malignancies of the affected organs. Numerous reports have revealed a potential link between chronic prostatic inflammation and prostate cancer (Dennis and Dawson 2002, Nelson et al. 2004). Chronic inflammation of the prostate is common, and epidemiological data suggest that 2-10% of adult men suffer symptoms from prostatitis at some point in their life (Krieger and Riley 2004). An increase in prostate cancer incidence has been correlated with symptoms of prostatitis and with sexually transmitted infections, independent of the specific pathogen (Dennis and Dawson 2002). Further studies are needed to establish a definitive epidemiological link between prostatitis and prostate cancer.

2. Natural history of cancer

Cancer comprises a class of diseases in which a group of cells show uncontrolled growth. Usually, the timing of cell division is strictly controlled by complex signalling mechanisms. Mutations in one or more genes in the nodes of this network can trigger cancer through genetic predisposition and/or exposure to certain environmental factors (e.g. tobacco and radiation). Therefore, cancers are caused by abnormalities in the genetic material of the transformed cells. Besides environmental factors, cancer-promoting genetic abnormalities can randomly occur through errors in DNA replication or may be inherited and thus present in all cells of the body. The inheritance of cancer predisposition is usually affected by complex interactions between carcinogens and the host's genome (Stratton et al. 2009).

Genetic abnormalities found in cancer typically affect two general classes of genes. Cancer-promoting oncogenes are often activated in cancer cells, whereas tumour suppressor genes are inactivated. Briefly, the predominant mechanisms underlying the development of cancer include 1) impairment of DNA repair pathways, 2) transformation of a normal gene into an oncogene, 3) the malfunction of a tumour suppressor gene or 4) changes in gene regulation (e.g. epigenetic changes or non-coding-RNA regulation) (Kirby et al. 2006).

2.1 Knudson's cancer development model

Almost forty years ago, Knudson proposed a two-hit hypothesis of cancer development, showing that tumour formation requires recessive loss of function mutations in certain genes called tumour suppressor genes (Knudson 1971). According to Knudson's

hypothesis, inherited susceptibility to cancer can be traced to germline mutations that lead to malfunctions in one of the copies of these genes. He studied a rare eye tumour, retinoblastoma, and proposed the existence of regulatory genes (tumour suppressor genes) that control the cell cycle, apoptosis and proliferation. Normal human cells have two copies of each gene, one inherited from each parent. Knudson's model suggests that two hits are required to inactivate both alleles of a tumour suppressor gene, leading the cell to undergo malignant transformation (Figure 7). Knudson's hypothesis was verified 20 years later when germline mutations in the retinoblastoma gene were detected in patients with hereditary retinoblastoma (Hogg et al. 1993).

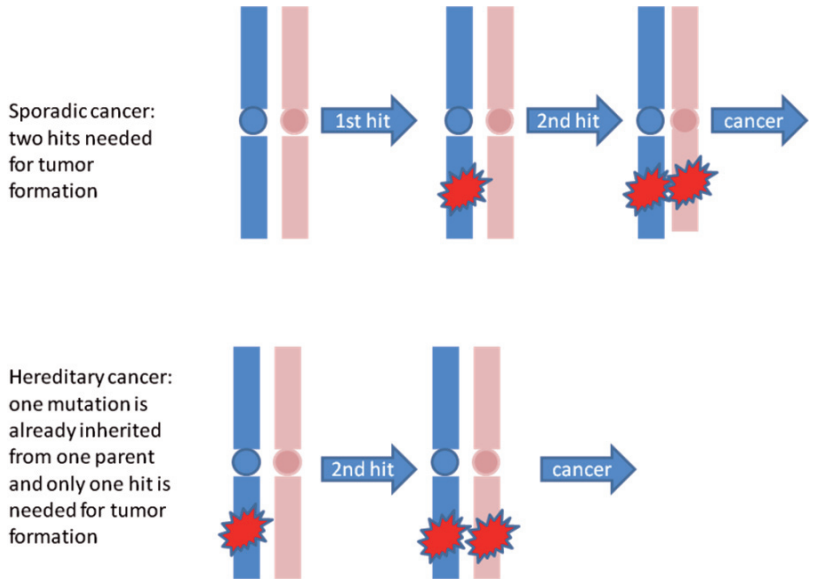

***Figure 7**. Knudson's model for inactivation of a tumour suppressor gene. The first hit is usually a rare mutation in the primary DNA sequence of the gene (red star). This mutation can be transmitted through the germline, giving rise to an inherited form of cancer. The second hit leads to physical loss of genetic material in the gene region (Knudson 1971).*

2.2 Cancer syndromes and association with other cancers

2.2.1 Cancer Syndromes

Most forms of cancers are sporadic, meaning that there is no obvious inherited cause. However, researchers have identified more than 20 inherited cancer syndromes in which there is an inherited predisposition for cancer, often due to a defect in a gene that protects against tumour formation. Finding evidence for hereditary cancer is more likely when the cancer has an early age of onset, when there is a range of cancer types and when there are multiple cases in a family, affecting several generations. Several hereditary cancer syndromes have been identified; most of them are rare, affecting only about 1% of all cancer patients (Fearon 1997). The known cancer syndromes are listed in Table 1, as previously reviewed by Garber and Offit (2005).

Table 1. *Syndromes of inherited cancer predispositions as reviewed by Garber and Offif (2005).*

Syndrome	Component tumours	Mode of inheritance	Genes
Hereditary prostate cancer	prostate cancer	Dominant	*HPC1, HPCX, HPC2, PCAP, PCBC, PRCA, GPC3*
Hereditary breast and ovarian cancer	breast, ovarian, prostate and pancreatic cancers	Dominant	*BRCA1, BRCA2*
Li-Fraumeni Syndrome	soft tissue sarcoma, breast cancer, osteosarcoma, leukaemia, brain tumours, adrenocortical carcinoma	Dominant	*p53, CHECK2*
Cowen Syndrome	breast, thyroid and endometrial cancer	Dominant	*PTEN*
Bannayan-Riley-Ruval-caba syndrome	breast cancer, meningioma, thyroid follicular cell tumours	Dominant	*PTEN*
Ataxia teleangiectasia	leukaemia, lymphoma	Recessive	*ATM*
Hereditary non polyposis colorectal cancer (HNPCC) Lynch syndrome	colon, endometrial, ovarian, renal pelvis, urethral, pancreatic, stomach and small bowel cancers	Dominant	*MLH1, MSH2, MSH6*
Familial polyposis	colon cancer	Dominant	*APC*
Hereditary gastric cancer	stomach cancers	Dominant	*CDH1*
Juvenile polyposis	gastrointestinal and pancreatic cancers	Dominant	*SMAD4/DPC4, BMPR1A*
Peutz-Jeghers syndrome	colon, small bowel, breast, ovarian and pancreatic cancers	Dominant	*STK11*
Hereditary melanoma pancreatic cancer	pancreatic cancer, melanoma	Dominant	*CDKN2A/p16*

Hereditary pancreatitis	pancreatic cancer	Dominant	*PRSS1*
Turcot syndrome	colon and basal cell cancer and ependymoma, medulloblastoma, glioblastoma	Dominant	*APC, MLH1, PMS2*
Familial gastrointestinal stromal tumour	gastrointestinal stromal tumours	Dominant	*KIT*
Melanoma syndromes	malignant melanoma	Dominant	*CDKN2, CDK4, CMM*
Basal cell cancers, Gorlin syndrome	basal cell cancers, brain tumours	Dominant	*PTCH, PTEN*
Neurofibromatosis 1 and 2	neurofibrosarcomas, phaeochromocytomas, optic gliomas, meningiomas	Dominant	*NF1, NF2*
Tuberous sclerosis	myocardial rhabdomyoma, bilateral renal angiomyolipoma, ependymoma	Dominant	*TSC1, TSC2*
Carney complex	myxoid subcutaneous tumours, adrenocortical nodular hyperplasia, testicular Sertoli cell tumour	Dominant	*PRKAR1A*
Rothmund Thomson syndrome	basal cell carcinoma, squamous cell carcinoma, osteogenic sarcoma	Recessive	*RECQL4*
Bloom syndrome	leukaemia, carcinoma of the tongue, squamous cancers, Wilm's tumour, colon cancer	Recessive	*BLM*
Fanconi Anaemia	leukaemia, squamous cancers, skin carcinoma, hepatoma	Recessive	*FANCA-F*
Shwachman-Diamond syndrome	myelodysplasia, acute myelogenous leukaemia	Recessive	*SBDS*
Nijmegan breakage syndrome	lymphoma, glioma, medulloblastoma, rhabdomyosarcoma	Recessive	*NBS1*
Canale-Smith syndrome	lymphoma	Dominant	*FAS, FASL*
Wiskott-Aldrich	hematopoietic malignancies	X-linked recessive	*WAS*
Severe combined immune deficiency	B-cell lymphoma	X-linked recessive	*IL2RG*
X-linked lymphoproliferative syndrome	lymphoma	X-linked recessive	*SH2D1A*
Simpson-Golabi-Behmel syndrome	embryonal tumours, Wilm's tumour	X-linked recessive	*GPC3*
von Hippel-Lindau syndrome	hemangioblastomas of retina and central nervous system	Dominant	*VHL*
Beckwith-Wiedeman syndrome	Wilm's tumour, hepatoblastoma, adrenal carcinoma, gonadoblastoma	Dominant	*CDKN1C, NSD1*
Wilm's tumour syndrome	Wilm's tumour	Dominant	*WT1*
Birt-Hogg-Dube syndrome	renal tumours	Dominant	*FLCL*
Papillary renal cancer syndrome	papillary renal cancer	Dominant	*MET, PRCC*
Rhabdoid predisposition syndrome	rhabdoid tumours	Dominant	*SNF5/INI1*
Hereditary paraganglioma	paraganglioma, phaeocromocytoma	Dominant	*SDHD, SDHC, SDHB*
Retinoblastoma	retinoblastoma, osteosarcoma	Dominant	*RB1*
Multiple exostoses	chondrosarcoma	Dominant	*EXT1, EXT2*
Leiomyoma/renal cancer syndrome	papillary renal cell carcinoma, uterine leiomyosarcomas	Dominant	*FH*
Werner syndrome	sarcoma/osteosarcoma, meningioma	Recessive	*WRN*
MEN1	pancreatic islet cell tumours, pituitary adenomas, parathyroid adenomas	Dominant	*MEN1*
MEN2	medullary thyroid cancers, phaeochromocytoma, parathyroid hyperplasia	Dominant	*RET*
Familial papillary thyroid cancer	papillary thyroid cancer	Dominant	*Multiple loci*

2.2.2 Prostate cancer syndromes

Extensive efforts have been made to reveal the genetics behind prostate cancer susceptibility, but our knowledge of crucial genes and variations predisposing to prostate cancer is still insufficient. To date, only three putative susceptibility genes have been identified. The first, elaC *E. coli* homolog 2 (*HPC2/ELAC2*) at 17p11, was identified from extended high-risk HPC families studied in Utah (Tavtigian et al. 2001). The second reported prostate cancer gene, Ribonuclease L (*HPC1/RNASEL*) at the 1q24-25 locus, was identified among prostate cancer families at Johns Hopkins Hospital (Carpten et al. 2002). Macrophage scavenger receptor 1 (*MSR1*) is the third reported prostate cancer gene and is positioned at 8p22-23 (Xu et al. 2002). The evidence that these genes underlie prostate cancer predisposition in Finnish prostate cancer-prone families and patients is weak (Rökman 2002 et al. 2002, Rökman et al. 2001, Seppälä et al. 2003). This is likely because of the heterogeneous nature of this disease. Genetic risk factors seem to vary between populations and ethnic groups. It remains a significant challenge to find genetic variations in prostate cancer that could distinguish between different types of the disease, from marginal clinical relevance to lethal prostate cancer, in different populations.

Other susceptibility genes with an associated risk for prostate cancer include *BRCA1* and *BRCA2* (Easton et al. 1997, Friedenson et al. 2005). The estimated relative risk of developing prostate cancer was calculated to be 3.3 for male carriers of *BRCA1* mutations compared with the general population (Ford et al. 2004). Alarmingly, mutations in *BRCA2* are associated with a 23-fold increased risk of prostate cancer (Edwards et al. 2003, Friedenson 2005).

2.2.3. Prostate cancer and other cancers

In epidemiological studies, an increased risk of prostate cancer has been observed and confirmed among the first-degree relatives of men with the disease. Nonetheless, studies of associations between prostate cancer and cancers at other sites are conflicting. Some studies have indicated that hereditary prostate cancer is site-specific and that no other malignancy occurs at a higher than expected rate (Isaacs et al. 1995). However, there seems to be a connection between susceptibility to prostate cancer and brain tumours (Isaacs et al. 1995, Gibbs et al. 1999), gastric cancer (Grönberg et al. 2000, Matikainen et al. 2001) and breast cancer (Valeri et al. 2000, Grönberg et al. 2001).

In population-based studies, associations have been reported to exist between prostate cancer and gastric, colon, rectal, kidney, breast, ovarian, bladder, thyroid and brain cancers as well as melanoma and non-Hodgkin's lymphoma, but such findings have been inconsistent between studies, and only a few of these neoplasms have been reported even twice (Goldgar et al. 1994, Matikainen et al. 2001, Grönberg et al. 2001, Eldon et al. 2003, Verhage et al. 2004, Amundadottir et al. 2004, Negri et al. 2005). A study by Hemminki et al. (2005), performed with 170 000 men with prostate cancer, revealed an excess of breast, ovarian and liver cancers as well as Hodgkin's disease, leukaemia and melanoma (Hemminki and Chen 2005). Although differences in study populations (i.e. genetic heterogeneity) may account for these diverse findings, the available data suggest that the inherited susceptibility to prostate cancer is relatively "site-specific" and not part of an inherited cancer syndrome like the *BRCA1*-associated breast and ovary cancer syndrome or the mismatch repair gene-related hereditary non-polyposis colorectal cancer syndrome (HNPCC) (Verhage et al. 2004).

2.3 Familial clustering of prostate cancer

Based on family history, three prostate cancer patient groups can be identified: hereditary, familial and sporadic. Hereditary prostate cancer was first described by Carter et al. (1993) and includes nuclear families with three or more prostate cancer cases, the occurrence of prostate cancer in each of three generations in the maternal or paternal lineage or a cluster of two first-degree relatives (father, brothers or sons) diagnosed with prostate cancer at the age of 55 or younger (Carter et al. 1993). As no high-risk mutations causing hereditary prostate cancer have yet been identified, the definition of this disease is solely based on family history and pedigree. Approximately 3 to 5% of prostate cancer cases can be classified as hereditary based on these criteria (Carter et al. 1992). However, because of the difficulties inherent in identifying three generations of disease carriers or the female carriers within the pedigree, these criteria has a poor sensitivity for detecting families with hereditary susceptibility to prostate cancer. The true proportion of prostate cancer caused by mutations in dominantly inherited susceptibility genes with high penetrance is more likely 5 to 10% (Bratt 2002). Among men with early onset prostate cancer, inherited susceptibility is much more common and may account for more than 40% of those diagnosed before the age of 55 (Bratt

et al. 1999). Familial prostate cancer does not meet these strict criteria, but it represents families in which there are two first-degree or one first-degree and two or more second-degree relatives with prostate cancer. Familial prostate cancer is estimated to account for 10 to 20% of all prostate cancer cases (Carter et al. 1993, Stanford and Ostrander. 2001). Sporadic prostate cancer signifies that only one man in a family has been diagnosed with prostate cancer, with no known affected relatives. However, as prostate cancer is a late onset disease, it is important to determine the disease status of the index parents, brothers and children to be sure that the patient indeed represents a sporadic case of prostate cancer.

2.4 Histology and histopathology of prostate cancer

Prostate cancer develops from the secretory epithelial cells, most often in the peripheral zone of the prostate. Prostatic intraepithelial neoplasia (PIN) is also often found in the peripheral zone and is believed to be a premalignant stage of prostate carcinoma (De Marzo et al. 2003).

Prostate cancer progression is a multistep process in which an organ-confined tumour eventually invades though the capsule of the prostate into the surrounding environment and metastasises to local lymph nodes and distant organs, mainly bones. Growth of the vast majority of prostate cancers is androgen dependent. However, during androgen withdrawal, a hormone-refractory tumour clone eventually emerges. Like PIN, commonly observed latent microscopic prostate cancer may also represent a preliminary stage of clinical cancer. However, the potential of the latent cancer to progress into a theoretically life-threatening clinical cancer is not definitively known. Microscopic cancer lesions have been found in autopsies of more than 50% of men between 70 and 80 years old (Sheldon et al. 1980). Like PIN, these common latent microscopic prostate cancers may also represent a preliminary stage of clinical cancer. Most often, only a minority of these lesions would develop into clinical cancer (Gittes 1991). However, it is unclear whether these incidental small carcinomas represent the same disease as clinically relevant life-threatening tumours (Selman 2000). Most clinically detected prostate carcinomas are adenocarcinomas and can be further classified by the Gleason grading system introduced over 40 years ago. Gleason grading remains one of the most powerful prognostic factors for prostate cancer and defines

five histological patterns or grades with decreasing differentiation (Gleason 1966). In addition to Gleason grading, histological staging can be used to classify prostate cancers. Three different characteristics are considered: primary tumour (T), regional lymph nodes (N) and distant metastases (M) (Eble et al. 2004).

2.5 Diagnosis of prostate cancer

Even before the PSA test came into common usage twenty years ago, most prostate cancers were asymptomatic and detected by digital rectal examination. The diagnosis is typically established by histopathologic examination of needle biopsy tissue. Clinical symptoms are usually a manifestation of more advanced disease. Only 8% of prostate cancers cause bladder outlet obstruction, and only rarely does urinary obstruction result from large-volume periurethral tumours (Mai et al. 2000). Locally extensive tumours may present with pelvic pain, rectal bleeding or obstruction (Scott et al. 1969). Metastatic prostatic adenocarcinomas can present as bone pain (Huddart et al. 1997). Stamey and coworkers reported that serum PSA levels were proportional to the size of the palpable cancer; subsequently, the PSA test and the Gleason histologic grading system for tumour samples were recognised as the key diagnostic tools for prostate cancer (Stamey et al. 1987). Because the clinical phenotype of prostate cancer is complex and heterogeneous, the arrival of the PSA era has further complicated analysis of prostate cancer by allowing the early diagnosis of a disease that might remain latent or clinically unimportant. In addition, other prostatic diseases, like hyperplasia or inflammation, can cause increases in PSA levels, making it important to exclude cancer in certain patients.

The use of PSA as a screening test has been studied in two large randomised trials: ERSPC and the US-based Prostate, Lung, Colorectal and Ovarian cancer screening (PLCO) (Grubb et al. 2008, Schröder et al. 2009). The ERSPC study showed that PSA screening led to a significant but relatively small decline in mortality rate, but pointed out the associated risk of overdiagnosis and overtreatment. In addition, quality of life issues need to be further studied before conclusions from PSA screening can be made (Eckersberger et al. 2009).

Serum PSA is widely used in the diagnosis and surveillance of prostate cancer, although its non-specificity limits its usefulness. Other novel markers have been

studied (e.g. *the prostate cancer gene 3*), but they have not overtaken PSA in specificity and sensitivity of diagnosis (Teahan et al. 2010).

2.6 Treatment of prostate cancer

When choosing a treatment plan for a patient diagnosed with prostate cancer, the selection strategy depends upon the risk posed by the newly diagnosed cancer. There is no universally accepted definition of clinically significant or insignificant disease, but studies have clearly shown that cancer volume, clinical stage and tumour histology are important predictors of long-term outcomes (Epstein 1994). Tumour size and stage are estimated by serum PSA levels and digital rectal examination, and tumour histology is assessed by the Gleason scoring system. In addition, the patient's age, general condition and presence of other diseases must be considered when selecting a treatment (Epstein 1994). Treatment options can be divided into two categories according to whether the disease appears to be local or of an advanced type (Kirby et al. 2006).

2.7 Clinical characteristics of hereditary prostate cancer

Several studies have noted the earlier age of onset observed in familial prostate cancer as compared to sporadic prostate cancer (Carter et al. 1993, Keetch et al. 1996, Grönberg et al. 1997b, Norrish et al. 1999, Bratt et al. 1999, Valeri et al. 2000, Ahaghotu et al. 2004). In a nationwide Swedish study of 201 patients from 62 Swedish hereditary prostate cancer families and 402 matched controls, the age of onset was six years earlier in hereditary prostate cancer, with a median age at diagnosis of 68 years. Studies that did not find support for an earlier age of onset in hereditary prostate cancer most often used prostatectomy patients as their selected study populations. Roehl et al. (2006) found an age of onset of 61-63 years among the various groups studied, but the low age can be explained by the study setting in which only patients who underwent radical prostatectomy were included. The small difference in age at onset between hereditary and sporadic prostate cancer (six to seven years compared with 20 years for breast,

ovarian and colorectal cancers) implies that environmental factors may be important in many families with hereditary prostate cancer. However, a greater proportion of men with hereditary prostate cancer die of the disease than those with nonhereditary prostate cancer (Grönberg et al. 1997). Currently, PSA screening is the only available clinical tool to reduce prostate cancer mortality and detect the disease when it is still at a curable stage in hereditary prostate cancer families.

An increased number of precursor lesions and tumour multifocality are commonly associated with hereditary cancers. However, multifocality and multiple precursor lesions are not specifically associated with familial types of prostate cancer (Bastacky et al. 1995). Tumour grade and pathological stage at diagnosis do not differ between patients with hereditary prostate cancer and those with sporadic prostate cancer (Bastacky et al. 1995, Valeri et al. 2000, Bratt 2002).

Many studies have reported that *BRCA1*-associated breast cancers show particular tumour characteristics such as a basal-like phenotype and high histological grade (Phillips 2000, Narod and Foulkes 2004). In HNPCC, tumours are usually located in the proximal colon, but in sporadic colon cancer they are instead found in the distal colon (Lindblom 2001). However, differences in the histological characteristics of hereditary prostate cancer and sporadic prostate cancer have not been found (Bratt 2007).

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

Overall, no substantial clinical or pathological differences seem to exist between clinically defined hereditary, familial or sporadic prostate cancer cases. However, this does not exclude the possibility that certain hereditary prostate cancer genes are associated with specific biological characteristics. Indeed, given the unique features of other hereditary cancers (HNPCC, BRCA), it is surprising that differences between sporadic and hereditary prostate cancer have not been found. PSA screening allows the detection of latent prostate cancers that would not have been found otherwise. In addition, this screening may eventually reveal distinct types of prostate cancer and alter the established clinical paradigm of prostate cancer.

3. Genetic epidemiology of prostate cancer

Accumulating evidence has shown that prostate cancer results from interactions between genes, hormones and environmental factors (Ross and Henderson 1994, Kolonel 1996, Bosland 2000). Through numerous epidemiological and molecular biological studies, a considerable amount of data has been gathered in favour of a significant but heterogeneous genetic component in prostate cancer susceptibility. The field of genetic epidemiology studies the genetic contribution to disease along with the environmental factors affecting families and inherited causes of disease in populations (Morton and Chung 1978, Thomas 2004). Traditionally, genetic epidemiology study designs have included familial aggregation, segregation, linkage and association studies. Familial aggregation studies investigate whether the disease clusters in families. Segregation studies determine the mode of inheritance of the disease. Linkage studies resolve the chromosomal locus where the disease gene is located, and association studies determine the particular allele related to the disease (Khoury et al. 1993, McCarthy et al. 2008).

Twin studies provide the most straightforward tool for evaluating the significance of genetic and environmental aetiological factors. Twin studies compare disease concordance rates for a given disease for identical (monozygotic) versus non-identical (dizygotic) twins and allow researchers to test for evidence of genetic aetiology. 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 in the development of prostate cancer. In the largest of these studies, using data for 44,788 pairs of Nordic twins, Lichtenstein et al. (2000) reported a heritability of 42% for prostate cancer, which was the highest ever reported for a

common malignancy. Interestingly, this fraction was even higher than that estimated for either breast or colon cancers, which have been clearly linked to high-penetrance risk genes. Figure 9 illustrates the interacting effects of high and low as well as rare and common allele frequencies in common diseases such as prostate cancer.

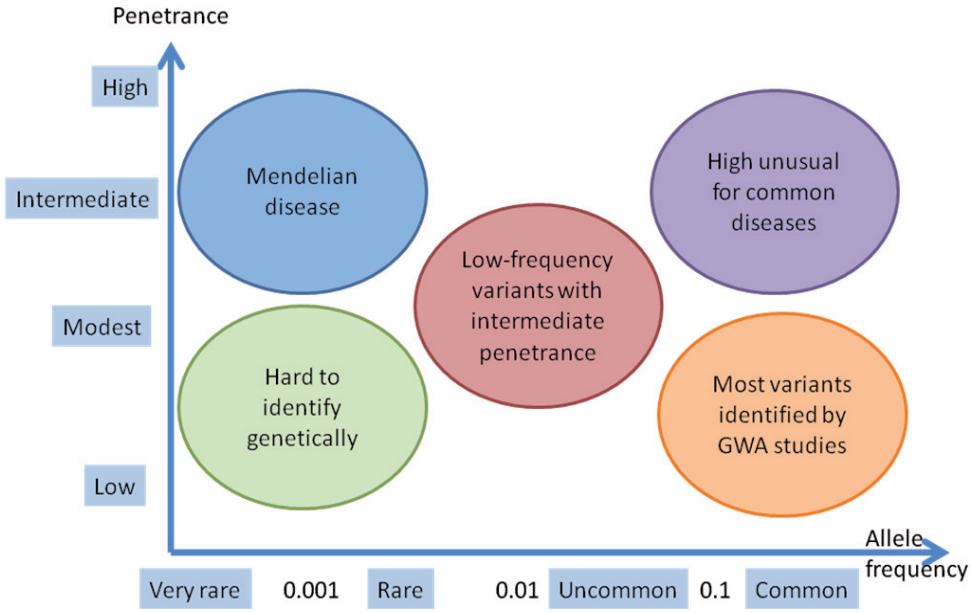

Figure 9. *A graph describing different allele frequencies and penetrance levels. Linkage analyses mainly identify rare alleles with high penetrance. Genome-wide association studies identify common variants. With DNA sequencing, low-frequency variants can be detected. Picture modified from McCarthy et al. (2010).*

3.1 Mode of inheritance, segregation analysis

Segregation analysis is a statistical method used to test compatibility with Mendelian expectations by estimating the parameters of a given model of inheritance from family data. The results of these analyses are difficult to interpret for complex diseases that are likely to be caused by multiple predisposing genes. However, such estimates are required for carrying out parametric linkage analyses for cancer.

When testing for genetic control, three types of individuals are considered: AA, AB and BB, where the disease allele is A and B represents the unaffected allele(s). Figure 8 explains the differences between dominant and recessive inheritance models. Previous segregation analyses in diverse populations have suggested that familial aggregation of prostate cancer follows autosomal dominance, multifactorial, recessive or X-linked inheritance, but remain inconclusive. Six reports suggested a dominant inheritance mode (Table 2) (Carter et al. 1992, Grönberg et al. 1997a, Schaid et al. 1998, Verhage et al. 2001, Valeri et al. 2003, Conlon et al. 2003). Cui (2001) reported a mixture of models, including autosomal dominant inheritance in younger onset families and recessive or X-linked inheritance in older-onset families (Cui et al. 2001). A multifactorial model has been suggested by Gong et al. (2002).

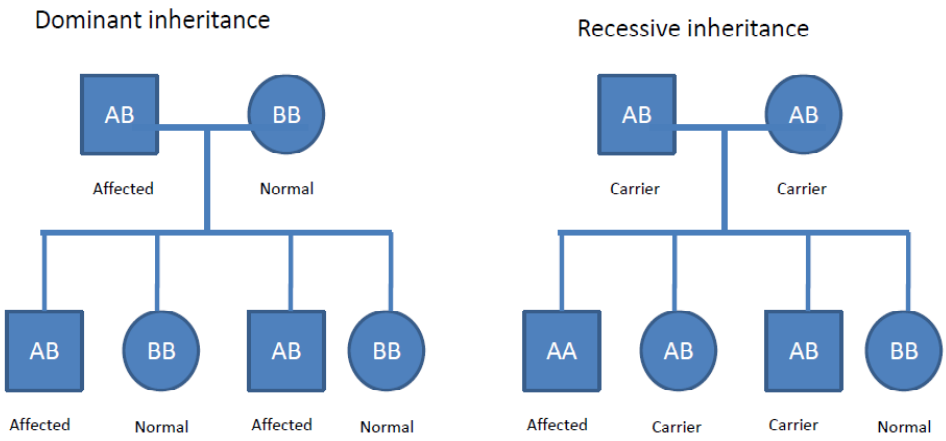

***Figure 8**. The disease locus is assumed to have a gene with two forms, A and B. A causes disease, but B does not. Each child has two alleles, one from the father and one from the mother. In dominant inheritance, those who receive A from the father have the disease. People with the BB genotype do not have the disease. If the disease is recessive, a child also has to receive A from the mother because the homozygous AA genotype is required to inherit the disease. In the recessive case, the AB and BB genotypes are not affected. However, the AB genotype is a disease carrier.*

In a recent study by MacInnis et al. (2009), genetic susceptibility to prostate cancer was proposed to be mostly explained by one or more genes having a strong recessively inherited risk as well as a number of genes with variants having small, multiplicative risks (MacInnis et al. 2010).

Table 2. *Main characteristics of previous segregation analyses for prostate cancer*

Study	Publication year	Study period	Population	No. of families	Suggested inheritance model
Carter et al.	1992	1982-1989	American	691	rare dominant gene with high lifetime risk
Grönberg et al.	1997	1959-1963	Swedish	2857	relatively frequent dominant gene with moderate lifetime risk
Schaid et al.	1998	1966-1995	American	4288	rare dominant gene with high lifetime risk
Verhage et al.	2001	1991-1993	American	1199	rare dominant gene with high lifetime risk
Cui et al.	2001	1994-1997	Australian	1476	relatively frequent dominant component with intermediate lifetime risk and recessive or X-linked component with high lifetime risk
Gong et al.	2002	1989-1995	American, Canadian	1719	multifactorial
Baffoe-Bonnie et al.	2002	_	Icelandic	389	codominant
Valeri et al.	2003	1994-1997	French	691	rare dominant gene with high lifetime risk and residual brother-brother dependence
Conlon et al	2003	–	American	263	autosomal dominant
MacInnis et al.	2009	1960-2004	Australian and UK	4390	mixed recessive model

3.2 Linkage studies

Results of segregation analyses have strongly indicated that a subset of prostate cancers can likely be attributed to the action of one or more major genes. These findings have led to large efforts worldwide to identify and carry out linkage studies on families with multiple prostate cancer cases. This method aims to map markers and diseases on chromosomes to identify the genetic basis of disease. Neighbouring genes have a tendency to stick together during meiotic recombination, enabling the localisation of cancer genes by their close proximity to a specific marker gene (Altshuler et al. 2008). Linkage analyses mainly identify tumour suppressor genes. In hereditary cancer syndromes, one abnormal copy of the tumour suppressor gene is inherited in the germline from one parent, whereas the other copy of the gene is normal. To develop cancer, the normal allele needs to acquire a mutation in somatic cells. These inherited mutated

tumour suppressor genes are associated with a high risk of cancer, but they are usually relatively rare (Hofstra et al. 2008).

Prostate cancer linkage studies have been used to localise rare and highly penetrant susceptibility genes. The linkage approach has been successful in identifying major susceptibility genes for other common cancers, such as those of the breast (*BRCA1* and *BRCA2*) and colon (*APC, MLH1, MSH2, MSH6, PMS2, MYH, LKB1, SMAD4, ALK3, PTEN*) (Miki et al. 1994, Wooster et al. 1995). The International Consortium for Prostate Cancer Genetics (ICPCG) was founded to improve the mapping of prostate cancer genes and has emphasised that one of the major difficulties in studying prostate cancer is genetic heterogeneity, possibly due to multiple genes with incomplete penetrance (Xu et al. 2005). Over a dozen linkage analyses have been performed, but despite the strong evidence for genetic predisposition to prostate cancer, the findings from these studies have been inconclusive Several susceptibility loci, including 1p36 (*CABP*), 1q24-25 (*HPC1*), 1q42.2-43 *(PCAP*), 8p22-23, 8q24, 16q23, 17p11, 19q13, 20q13 (*HPC20*) and Xq27-28 (*HPCX*) have been reported, but most of these findings could not be confirmed in other independent studies. Because of the heterogeneous nature of the disease, the genes that promote prostate cancer have been extremely hard to identify; no high-risk genes underlying hereditary prostate cancer have yet been discovered.

In Finland, the *HPC1/RNASEL*, *HPC2/ELAC2* and *MSR1* loci have been shown to explain only a small fraction of prostate cancer cases (Rökman et al. 2001, Rökman et al. 2002, Seppälä et al. 2003a). Instead, four additional major susceptibility loci have been identified in Finnish families, including the *HPCX* (Xq27-28), 3p25-26, 11q14 (Xu et al. 2005, Chang et al. 2006) and 17q21 regions (Cropp et al. submitted). Linkage to loci on 3p, 11q and 17q has been found in other populations as well (Lange et al. 2003, Xu et al. 2005, Chang et al. 2006). The *HPCX* locus on Xq27-28 seems to explain a particularly large fraction of 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 for the involvement of the *HPC1* locus at 1q24-q25 in Finnish families. A recent genome-wide linkage analysis of 69 Finnish families confirmed the presence of a signal at 17q21-22 (unpublished data). Even so, a large proportion of Finnish hereditary prostate cancer cases remain unexplained.

Overall, confirmation of the involvement of these genes has been inconsistent across different study populations (Schaid 2004, Kirby et al. 2006). Despite extensive efforts using linkage analysis, no genes that reproducibly account for more than a small percentage of familial prostate cancer cases have been found. When one considers

the heterogeneity of prostate cancer, this is perhaps not unexpected, but does suggest that new strategies, such as meta-analyses, pooled analyses and larger sample sizes, are needed and that the patients being analysed should be more strictly defined according to, for example, a clinical outcome such as aggressive disease.

3.3 Association studies

After the aforementioned linkage analyses indicated that major genes are unlikely to underlie prostate cancer susceptibility, more interest has been given to association studies seeking to identify genes that may have more common, albeit weaker, risk alleles. These studies are performed with case-control approaches and typically detect more common, lower-risk variants with lower penetrance compared to linkage analyses (McCarthy et al. 2008). With respect to prostate cancer, these studies are typically performed in study populations of men with and without prostate cancer, often without regard to family history (Houlston and Peto 2004, Kirby et al. 2006). The selection and validation of the control group is essential, otherwise false associations may be drawn. These case-control studies have been greatly aided by the increased understanding of the variability within the human genome sequence among different individuals and the concept that common diseases can be attributed to common, possibly population-specific, genetic variants (Lohmueller et al. 2003). For example, *CHECK2* variants have been shown to associate with sporadic prostate cancer in the USA, but in analyses done in Finland and Poland, the association was with hereditary prostate cancer (Seppälä et al. 2003b, Cybulski et al. 2004). These associations between genes and disease risk can be rapidly assessed by simply examining the frequency of polymorphic alleles, typically single nucleotide polymorphisms (SNPs), among cases and controls. A large number of genes involved in the critical processes of prostate cancer, e.g. androgen action and metabolism, growth factor signalling, carcinogen detoxification, DNA repair and inflammation, have been systematically evaluated in this fashion (Simard et al. 2002, Rennert et al. 2005, Kirby et al. 2006). Additionally, genome-wide association studies (GWAS) of prostate cancer involving hundreds of thousands of genetic markers have been conducted; these studies implicate a large number of genes in prostate cancer risk (Gudmundsson et al. 2008, Eeles et al. 2008, Thomas et al. 2008).

A recent example of the power of GWASs can be found in the discovery that common genetic variations in 8q24 influence the risk of developing prostate cancer. Interestingly, none of the prostate cancer risk variants align to a known gene or alter the coding sequence of an encoded protein (Freedman et al. 2006, Gudmundsson et al. 2007, Haiman et al. 2007). In light of this new information, prostate cancer may not be caused by mutations in genes but instead by mutations in regulatory regions. The original reports did not divide material into familial versus sporadic cases. However, in a study by Wang et al. (2007), a stronger association with 8q24 was found in familial cases of prostate cancer (Wang et al. 2007). In a study by Suuriniemi et al. (2007), a genetic variant at locus 8q24 is a strong risk factor for aggressive prostate cancer (Suuriniemi et al. 2007). In addition, a meta-analysis by Cheng et al. (2008) suggested that 10 tested SNPs in 8q24 would increase the risk of prostate cancer up to 50% (Cheng et al. 2008). The 8q24 association has been recently verified in a meta-analysis of genome-wide and replication association studies (Liu et al. 2010).

3.4 Candidate gene studies in prostate cancer

The candidate gene approach can be used to confirm prostate cancer susceptibility genes. In general, these studies rely on previous evidence obtained from other studies. Candidate gene studies may include genes that have been linked to other cancers or cancer syndromes or even genes involved in oestrogen or androgen metabolism, inflammation or DNA repair pathways. Analyses of DNA repair pathway genes are based on the assumption that cancer arises from problems in a cell's ability to repair DNA damage. Examples of such genes previously investigated in Finnish prostate cancer patients are *MSR1*, *CHECK2*, *KLF6* and *ARLTS1* (Seppälä et al. 2003a, Seppälä et al. 2003b, Seppälä et al. 2007, Siltanen et al. 2008).

3.4.1 PALB2 partner and localiser of BRCA2

DNA repair pathway genes play an undisputed role in cancer progression, and inherited mutations in these genes have been strongly associated with different cancers. DNA

repair mechanisms are critical to prevent accumulation of DNA damage and maintain stability (Agalliu et al. 2007). *BRCA1* and *BRCA2* are both tumour suppressor genes involved in DNA repair. Mutations in these genes predominantly predispose carriers to breast and ovarian cancers, though potential links to prostate cancer have also been studied for both genes. Multiple studies have shown that mutations in *BRCA2* lead to an increased risk of developing prostate cancer (Friedenson et al. 2005, Sigurdsson et al. 1997). However, Finnish *BRCA1* and *BRCA2* founder mutations are not associated with prostate cancer predisposition among Finnish prostate cancer patients (Ikonen et al. 2003), but an increased risk of prostate cancer has been observed in Finnish breast cancer families carrying *BRCA2* mutations (Eerola et al. 2001).

PALB2 is a recently discovered BRCA2-binding protein. The BRCA2-PALB2 interaction is essential for BRCA2-mediated DNA repair. Two independent studies have recently reported that biallelic truncating mutations in *PALB2* cause a new type of Fanconi anaemia (Xia et al. 2006, Reid et al. 2007). Fanconi anaemia is an autosomal or X-linked recessive genetic disorder characterised by developmental defects, susceptibility to cancer at young age, bone marrow failure and early mortality. Family histories of Fanconi anaemia patients also indicate an increased risk for adulthood cancers among monoallelic mutation carriers. This observation has been confirmed in several studies showing that truncating monoallelic *PALB2* mutations are associated with an increased risk of breast cancer (Erkko et al. 2007, Xia et al. 2007, Rahman et al. 2007, Foulkes et al. 2007, Tischkowitz et al. 2007). However, incomplete segregation of *PALB2* mutations among affected relatives is an indication of a low-risk susceptibility allele, and the risk association is clearly more modest than that of *BRCA2* monoallelic mutations (Rahman et al. 2007).

In the Finnish study by Erkko and colleagues (Erkko et al. 2009), a novel *PALB2* founder mutation (1592delT) was identified among Finnish breast cancer families. In addition, a truncation mutation was detected in one family with multiple cases of prostate cancer, indicating that it could also contribute to prostate cancer development in the Finnish population. Functional studies revealed decreased DNA repair function in all of the cell lines where the mutation was introduced. However, in the original study by Erkko and colleagues (Erkko et al. 2007), the prostate cancer cases were only screened for the c.1592delT mutation, and involvement of other cancer-related mutations in *PALB2* was not ruled out.

AIMS OF THE STUDY

The aim of the study was to investigate the genetic epidemiology of prostate cancer in Finland. The specific aims were:

I. To determine the most parsimonious model that explains the familial segregation of prostate cancer in the Finnish population (I).

II. To investigate clinical and histopathological characteristics in Finnish familial prostate cancer (II).

III. To assess whether primary non-prostate cancer tumours are associated with Finnish families with either clinically aggressive or nonaggressive prostate cancer (III).

IV. To assess whether any other *PALB2* variants besides the 1592delT mutation are associated with increased risk of prostate cancer and provide a clinical description of patients with the *PALB2* delT mutation (IV).

MATERIALS AND METHODS

1. Data sources

The data for this thesis were ascertained from the following sources: the Finnish Cancer Registry, the Population Register Centre and local population registries. Clinical information was collected from the regional hospitals where patients had undergone treatment and follow-up.

Population registries have a long history in Finland, where population information has been registered since the 1530s. Currently, the Finnish population information system is a computerised national register that contains accurate information about Finnish citizens based on electronic personal identity codes. This database is maintained by the Population Register Centre and local register offices. Finland's population data registry is internationally considered to be of high quality.

The population-based Finnish Cancer Registry was founded in 1952 and covers virtually all histologically confirmed cancer diagnoses made in the past six decades. Cancer notification to the Finnish Cancer Registry was made obligatory by law in 1961. Reports to the cancer registry are made by three independent sources: physicians, hospitals and pathology laboratories. In addition, cancer-related information from death certificates is sent to the Finnish Cancer Registry. The coverage of the cancer registry is considered to be of excellent quality, over 99% in Finland (Teppo et al. 1994).

The Finnish population of five million inhabitants represents a genetically homogenous population with a unique gene pool useful for the study of genetic susceptibility to cancer and other complex diseases (de la Chapelle 1993, Peltonen 1997). However, even among homogenous Finns, substantial differences can be seen between the eastern and western parts of the country (Jakkula et al. 2008, Palo et al. 2009).

Another advantage to studying the Finnish population is that information from different registries can be linked to unique personal identity codes. Moreover, the public has a favourable attitude towards medical research, and registries of the national health

care system are well organised and reliable. In addition, the training of clinicians is considered excellent, and the quality of medicine is similar throughout all of Finland. Therefore, clinical information on diseases is comparable nationwide.

2. Study subjects

2.1 Probands and relatives for segregation analysis (Study I)

I identified 9142 men who were newly diagnosed with prostate cancer between 1 January 1988 and 31 December 1993, before the PSA test became available. Two non-overlapping cohorts were established according to the age of onset of prostate cancer. A group of 557 men diagnosed before the age of 61 were chosen as index patients and designated as the early onset cohort (Cohort 1). In addition, 989 men diagnosed at or after the age of 61 (Cohort 2) were collected from three hospital regions in Mid-Finland (Pirkanmaa) and Eastern Finland (North-Karelia and Kainuu). Details regarding the collection of population-based prostate cancer families and cancers analyses among first-degree relatives were published previously (Matikainen et al. 2001). Briefly, information on the birthplaces of probands was obtained from the Central Population Registry. The local registries (church parishes and local authorities) were contacted to obtain the names and birthplaces of their parents, siblings, spouses and children. The parents and siblings were followed up from parish records until death or until they obtained personal identification codes in 1967. Tracing of family members was successful for 94% of the index patients. Altogether, 10650 first-degree relatives were identified from the registries. Table 3 summarises the analysed cohorts.

***Table 3**. Descriptive statistics for prostate cancer cohorts in Finland. More detailed descriptions of the data can be found in the original publication (I).*

Description	Cohort 1	Cohort 2	Combined cohort
Probands	557	989	1546
Non-probands	3631	7019	10650
Number of affected individuals	608	1098	1706
Individuals in cohort	4188	8008	12196
Number of males	2418 (58%)	4664 (53%)	7082 (58%)
Number of females	1770 (42%)	3344 (47%)	5114 (42%)
Mean age of probands (years)	56.6 ± 3.4	74.4 ± 7.4	68.2 ±10.7
Range of age at diagnosis (years)	42 to 60.9	61 to 96	42 to 96
Mean age of affected non-probands (years)	70 ± 8.0	72 ± 7.8	71 ±7.9

2.2 Finnish prostate cancer families (Studies II, III, IV)

Since January 1995, data on prostate cancer families with two or more affected cases have been collected in the Laboratory of Cancer Genetics at Tampere University and Tampere University Hospital, Finland. Methods for collecting data on such families included referrals to physicians, family questionnaires sent to patients, a nationwide registry-based search and advertisements in newspapers, radio and television. A detailed description of the Finnish prostate cancer family collection process was previously described (Matikainen et al. 2000, Matikainen et al. 2001). Since then, additional families were found between 1999 and 2008 and integrated into the database using the same methods. For studies II and III, the relatives of 202 families with prostate cancer were collected in the following manner: parents of the index patients, siblings of the index person, children of the siblings and children of the index person. Follow-up for relatives was done from parish records and local authorities. Altogether, there were 617 affected males in these families. Genealogical information on family members was confirmed from records kept by the Finnish Population Registry Centre, parish records and local authorities. Medical information regarding prostate cancer was obtained from regional hospital records where the patient received treatment and follow-up. The following information was gathered: date at diagnosis, age at diagnosis, the reason for diagnosis, primary PSA value, follow-up PSA values, PSA progression date, clinical progression, histology, WHO grading of tumour samples (biopsy, TURP, prostatectomy), Gleason grading of tumour samples, clinical and pathological TNM stage, primary treatment and follow-up.

For study III, families were sorted into two groups according to the following clinical characteristics: age of diagnosis, primary PSA value, WHO grade, Gleason score and TNM stage at diagnosis.

For study IV, we utilised a group of 178 families with two or more affected first-degree relatives. A blood sample was analysed from the youngest affected male in each family.

2.3 Pirkanmaa hospital control group (Studies II and IV)

Beginning in 1995, detailed clinical information has been collected for a population-based group of prostate cancer patients at the Department of Urology at Tampere University Hospital, the Tampere City Hospital and the Valkeakoski district hospital. For study II, a control group of 3011 men from 1996-2009 was used (1995 was left out because the data were not extensive).

From 1999 onwards, a blood sample has been taken from each patient in this group. Patients visiting the clinic give informed consent, fill out a family questionnaire and donate blood samples for DNA extraction. Altogether, the coverage is over 85% of all prostate cancer patients treated in the Pirkanmaa Hospital District since 1996. For study IV, we used 285 unselected patients. Potentially interesting variants were verified in an additional set of 463 unselected cases.

2.4 Control samples

The population controls were blood donors from the Finnish Red Cross in the cities of Tampere, Turku and Kuopio. Blood donors in Finland are 18- to 65-year-old healthy volunteers. The number of controls used in this study (IV) was 470, whereas 760 were assessed for potentially interesting variants.

3. Methods

3.1 Dividing families into clinically aggressive and nonaggressive prostate cancer groups (Study III)

Prostate cancer is a common disease. Even the strict criteria for identifying hereditary cancer families are carefully followed, a family with sporadic disease aggregation can be falsely classified as a hereditary prostate cancer family. Therefore, we wanted to divide families into two cohorts according to the clinical characteristics of the prostate cancer cases in each family. We collected detailed clinical characteristics from hospital records, including age at diagnosis, primary PSA value, WHO grade and tumour-node-metastasis stage at diagnosis. In addition, we calculated the proportion of prostate cancer cases in the family by dividing the number of prostate cancer cases by the number of males in the family. Using the collected clinical data, patients were sorted with cluster analysis into two groups: group 1 (clinically nonaggressive prostate cancer) and group 2 (clinically aggressive prostate cancer). From a total of 202 families, 59 were classified as clinically aggressive and 143 as clinically nonaggressive.

Hierarchical cluster analysis by the agglomerative method and average distance was used to classify families with clinically aggressive disease into a separate cohort. Diagnostic age, primary PSA and the proportion of prostate cancer cases in the family were standardised to equalise variables.

3.2 Mutation analysis of PALB2 (Study IV)

DNA samples from the youngest man in each family (n = 178), 285 unselected cases and 470 control samples were initially used for direct sequencing of the entire coding region and splice sites of *PALB2*. The gene was screened using the ABI PRISM BigDye

Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems) from unselected cases with Gleason scores over seven and an early age of onset. For five variants showing a trend toward prostate cancer association, additional direct sequencing was performed (*PALB2* 1592delT, 1674A>G, 2993G>A and 3300T>G and *BRCA2* 353A>G). This additional analysis was done for 95 early onset aggressive cases and 368 nonaggressive cases with an average age of onset of 67 years. Mutation detection and loss of heterozygosity analysis are described in detail in the original publication (IV).

The associations of different variants with prostate cancer were calculated using odds ratios and 95% confidence intervals, and association analyses were made using Fisher's exact test. Pairwise linkage disequilibrium between the three most frequently detected variants (1674A>G, 2293G>A and 3300T>G) in *PALB2* were analysed with Haploview 4.1.

4. Data analysis and statistical methods

4.1 Segregation analysis (Study I)

Complex segregation analysis was used to specifically test Mendelian inheritance of prostate cancer in Finnish pedigrees. The REGTL program refers to the logistic version of the regressive module in the Statistical Analysis for Genetic Epidemiology (Release 3.1). REGTL is used for segregation analysis of a censored trait such as age of onset of a disease. Censoring means that not all individuals have experienced the disease. The basis of REGTL is a regressive model described by Bonney (Bonney 1986). This model assumes that similarity between siblings is a consequence of having the same parents. For our analysis, the phenotype for prostate cancer is the age of onset. For those with known age, the phenotype is a continuous variable that follows a logistic distribution probability density function, possibly after log transformation. For prostate cancer, only a proportion of the population are susceptible, i.e. males, and therefore are the only individuals to show an age of onset. The disease is binary with variable age of onset. REGTL makes the assumption that log-transformed age of onset follows a logistic distribution, as genetic and non-genetic causes are possible.

REGTL uses five hypotheses of disease transmission (no major gene, dominant, recessive, codominant and environmental model) and a general model for comparison. Altogether, six models are estimated. The general model has no restrictions, and all nine parameters are estimated. For the 'no major gene' model, three parameters are estimated, and for the dominant and recessive models, five parameters are estimated (see original publication (I), Tables 2-4, for details).

All models are compared to the general model. The null hypothesis states that there is no difference between the best model of transmission and the general model except those arising by chance alone. The difference with the smallest chi-squared value and largest p-value is closest to the general model and the most parsimonious

model for disease segregation. If the p value is < 0.0001, then we reject the hypothesis because the model is too different from the general model.

Maximum likelihood segregation analysis was performed on the log-transformed age at diagnosis of prostate cancer data, expressed as a censored trait using the REGTL program SAGE 3.1 (SAGE3.1. 1997).

4.2 Estimated standardised incidence risk ratio (Study III)

The follow-up analysis of cancer incidence in relatives of prostate cancer patients was done using the files of the Finnish Cancer Registry. The follow-up period extended from January 1, 1967, to December 31, 2004. More specifically, the follow-up for index patients began at their first prostate cancer diagnosis. For parents of the index patients, follow-up began at the birth of the index patient. For the other cohort members, follow-up began at their date of birth if it this was later than January 1, 1967. If the person had emigrated or died before December 31, 2004, the calculation of person-years ended at the earlier date.

In an ideal situation, when using family data, we would designate the first man to be diagnosed with prostate cancer as the index person in any given family recruited to the study. Otherwise, we might induce bias in defining a prostate cancer family. However, because the source information was occasionally incomplete and showed a large amount of variability, we calculated familial standardised incidence ratios (SIRs) in two ways. In the single-index data method (method 1), the follow-up from the date of prostate cancer diagnosis was excluded for one prostate case, but all other family members were followed-up as described above. In the multiple-index data method (method 2), all known prostate cancer cases were considered index patients, and follow-up data prior to prostate cancer diagnosis were excluded from the analysis. The SIRs calculated by these two methods provide the lowest and highest possible estimates of the true relative risk for prostate cancer.

The expected number o f malignancies is based on person-years at risk, gender, age and calendar period of the specific incidence rates in the general population. SIRs were calculated by dividing the observed numbers of malignancies by the expected numbers. Exact 95% confidence intervals were defined, assuming that the number of observed cases followed a Poisson distribution.

4.3 Statistical analysis

Comparisons between clinical information were calculated using Student's t-test, the Mann-Whitney U test and the Pearson's chi-squared test.

5. Ethical considerations

Permission to utilise 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). Prostate cancer family data collection was approved by the Ministry of Health and Social Affairs local ethics committees of regional hospitals. Written informed consent was obtained from all patients and relatives who gave blood samples for study IV.

RESULTS

1. Segregation model for prostate cancer in Finland

1.1 Cohort 1: 557 early onset prostate cancer families

In the early onset prostate cancer cohort, the 'no major gene' model gave a poor fit to the data. It was rejected against the general unrestricted model in which all parameters were estimated based on the likelihood ratio test ($p < 0.005$). In addition, the dominant ($p = 0.0014$), codominant ($p = 0.036$) and environmental ($p = 0.0082$) models were all rejected compared to the general model. The final general model reported was almost identical to the recessive Mendelian model ($p = 0.83$). Akaike's information criteria, which take into account the number of parameters estimated, also confirmed that the recessive model was the most parsimonious. The predicted mean age at diagnosis for the high-risk homozygous carriers of the putative risk allele (AA individuals) was 60 years, whereas the age at diagnosis for heterozygous AB and BB non-carriers was estimated to be 65 years. Under this Mendelian recessive model, the cumulative probability that a male in Finland would be affected by prostate cancer by age 70 was 0.92 for carriers and 0.79 for non-carriers. This result implies that if carriers and non-carriers did not die from competing causes, the estimated risk of being diagnosed with prostate cancer at age 70 for homozygous carriers of the deleterious allele ($q = 0.054$) would be 2.7 per 1000 among a hypothetical cohort of 100 000 men.

1.2 Cohort 2: 989 late onset prostate cancer families

For the late onset prostate cancer family cohort, the unrestricted general model, the 'no major gene model', the Mendelian dominant model and the environmental model did not fit the data and were rejected at $p < 0.001$. The Mendelian codominant model was also rejected by a p-value of 0.006. The recessive model was the most parsimonious model according to the likelihood ratio test ($p < 0.15$ for 4df), and it also had the lowest Akaike's information criteria value (1331.10). Under this recessive model, inheritance of a putative high-risk allele A with an allele frequency (± SE) of 0.086 (± 0.006) gave predicted mean ages of onset of 66 years for men with the AA genotype and 72 years for AB/BB males. The lifetime risk of being diagnosed with prostate cancer under this model was 5.0 per 1 000 among a hypothetical cohort of 100 000 men.

1.3 Combined Cohort 1 and Cohort 2: 1 546 prostate cancer families

The combined cohort gave similar parameters as those obtained from cohort 2. All models except the Mendelian recessive model were rejected when compared with the unrestricted general model. The recessive model was the most parsimonious model according to the likelihood ratio test ($p < 0.07$ for 4df), and it also had the lowest Akaike's information criteria value (1795.12). Under this recessive model, inheritance of a putative high-risk allele A with an allele frequency (± SE) of 0.0903 (± 0.005) gave predicted mean ages of onset of 64 years for men with the AA genotype and 71 years for men with the AB/BB genotypes. With a cumulative risk of 0.80 for homozygous carriers of the A allele at age 70 years, the estimated risk of being diagnosed with prostate cancer in the absence of competing causes of death was 6.5 per 1 000 among a hypothetical cohort of 100 000 men.

2. Clinical characteristics of Finnish familial prostate cancer

The mean age at diagnosis of prostate cancer was 68 years (SD 9.0, range 43-98), and the mean year of diagnosis was 1992 (SD 9.3, range 1962-2006). The primary median PSA value was 16 (Quartile deviation (QD) 17; range 0.8-11000), and the mean year in which PSA levels were measured was 1997 (SD 4.2, range 1988-2006). Histologically, 95% (n = 537) of the males had adenocarcinoma, 4.9% (n = 28) had unclassified carcinoma and the histology was unknown in 8.4% (n = 54) of the cases. Table 4 summarises the clinical and histopathological parameters of Finnish familial prostate cancer.

***Table 4**. Clinical and histopathological characteristics of 617 Finnish familial prostate cancer cases. n = the number of data points available for different variables. * = transurethral resection of the prostate.*

Year of Diagnosis (n = 617)	Mean, median (range)	1992, 1994 (1962-2006)
	Standard deviation	9.3
Age at Diagnosis (n = 617)	Mean, median (range)	68, 68 (43-98)
	Standard deviation	9.0
Primary PSA value (n = 416)	Mean, median (range)	134, 16 (0.8-11000)
	Standard deviation	672
Year when primary PSA was taken (n = 416)	Mean, median (range)	1997, 1997 (1988-2006)
	Standard deviation	4.2
Reason for diagnosis (n = 533)	Symptoms	313 (51%)
	Elevated PSA	173 (28%)
	TURP*	47 (8%)

First treatment (n = 531)	Surgical castration	167 (27%)
	Prostatectomy	149 (24%)
	Chemical castration	68 (11%)
	Radiation therapy	60 (10%)
	Active surveillance	41 (7%)
	Antiandrogen treatment	16 (3%)
	Brachytherapy	8 (1%)
PSA Progression	Yes	106 (17%)
	No	299 (48%)
	Unknown	212 (34%)
Clinical progression	Distant progression	77(12%)
	Local progression	66 (11%)
WHO grading in biopsy samples (n = 477)	Well differentiated	158 (33%)
	Moderately differentiated	53 (11%)
	Poorly differentiated	53 (11%)
Gleason grading in biopsy sample (n = 202)	Gleason score 2-5	86 (42%)
	Gleason score 6-7	98 (48%)
	Gleason score 8-10	3 (10%)
WHO grading in prostatectomy samples (n = 135)	Well differentiated	32 (24%)
	Moderately differentiated	94 (70%)
	Poorly differentiated	9 (7%)
Gleason grading in prostatectomy samples (n = 101)	Gleason score 2-5	55 (54%)
	Gleason score 6-7	41 (41%)
	Gleason score 8-10	5 (5%)
TNM stage for prostatectomy patients (n = 142)	Clinically unapparent (T1)	3 (2%)
	Within prostate confined (T2)	96 (68%)
	Over prostate extending (T3)	42 (30%)
	No lymph node metastasis N0	130 (92%)
	Lymph node metastasis N1	6 (4%)
TNM stage provided by the urologist (n = 546)	Clinically unapparent (T1)	158 (29%)
	Within prostate confined (T2)	145 (27%)
	Over prostate extending (T3)	193 (35%)
	Fixed tumour (T4)	50 (9%)
	Metastasis (M1)	103 (17%)

Altogether, 52% (323/617) of the biopsy samples were recollected and reclassified. The reasons for missing information included missing samples (75%, n = 227), diagnosis made from a TURP sample (17%, n = 44), diagnosis made from a cytological sample (5%, n = 15), diagnosis made from a prostatectomy sample (2%, n = 5), diagnosis made from metastasis (1%, n = 2) and diagnosis made during an autopsy (0.4%, n = 1)).

We reanalysed 301 original samples with WHO grading and 160 original samples with Gleason scores. When comparing the old and new biopsy samples, similar trends in WHO and Gleason grading patterns were seen, though the older grading was less aggressive. Statistical analyses were done using the paired T-test. The newly graded WHO values were significantly different from the original graded values, with p = 0.000 (correlation coefficient of 0.74; the difference between original and regraded samples was 0.26, with a 95% confidence interval of 0.31-0.21)). Similarly, the Gleason grading pattern showed a remarkable change, with p = 0.000 (correlation coefficient of 0.90; the difference in mean score was 0.87, with a 95% confidence interval of 0.77-0.97). Figures 11a and b show how the biopsy sample grading pattern shifted to more aggressive values.

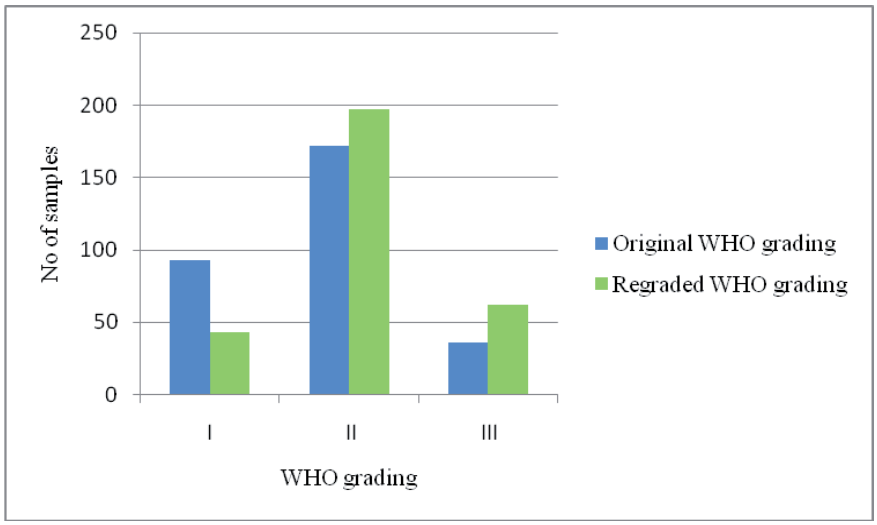

***Figure 11a**. Original and regraded WHO grades from 323 men in Finnish prostate cancer families.*

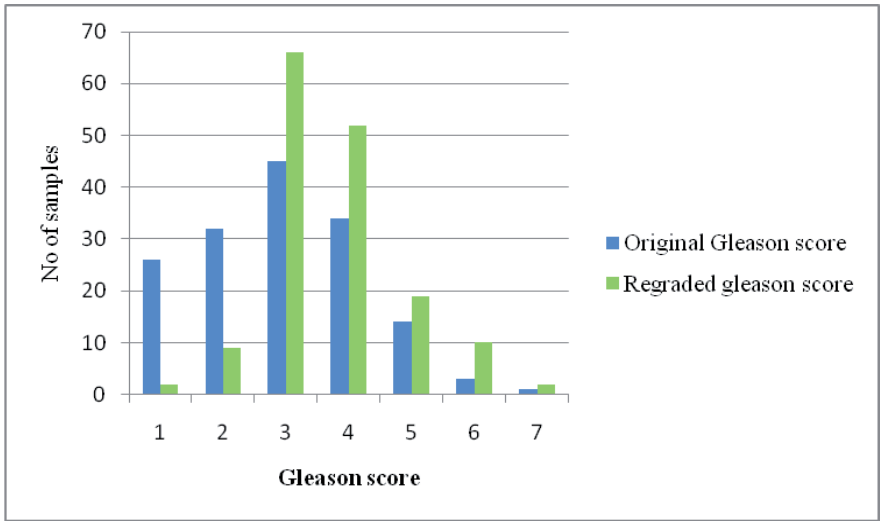

***Figure 11b**. Original and regraded Gleason scores from 160 men in Finnish prostate cancer families.*

When comparing the regraded WHO and Gleason scores to the population-based Pirkanmaa hospital cohort of 3011 prostate cancer patients (excluding the males belonging to prostate cancer families), the family samples showed a lower grade in diagnostic biopsy samples. This difference was statistically significant, with a p-value of 0.007 for the WHO grade and 1.0×10^{-5} for the Gleason score. Figures 12 a and b show the differences in WHO and Gleason grades between these two groups.

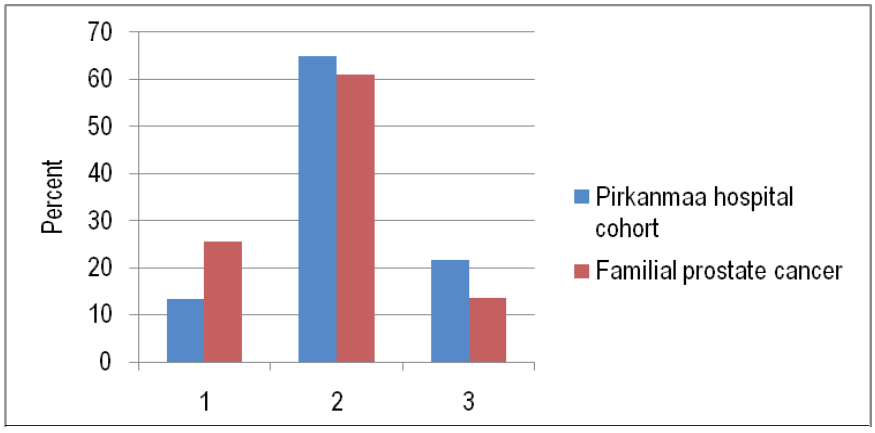

***Figure 12a.** Distribution of WHO grades between cases of familial prostate cancer and the population-based Pirkanmaa hospital cohort of 3011 men.*

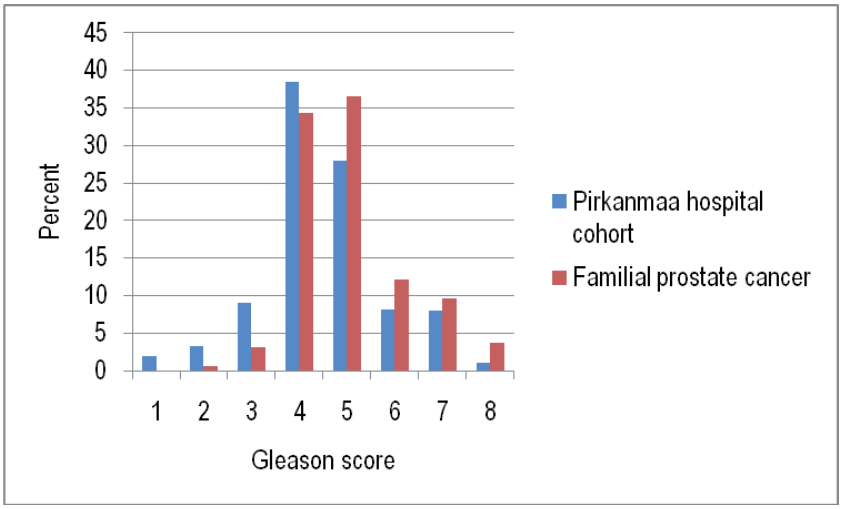

Figure 12b. *Distribution of Gleason scores between cases of familial prostate cancer and the population-based Pirkanmaa hospital cohort of 3011 men.*

Men with familial prostate cancer had a significantly higher PSA level, with a p-value of 9.9 x 10^{-6} (Student's t-test). In addition, the tumour (T) and nodal (N) stages of men who underwent radical prostatectomy were compared between men with familial prostate cancer (n = 139) and the population-based Pirkanmaa hospital cohort (n = 1133). Tumour stage was compared using combined groups of T1, T2 and T3. Familial samples had a larger proportion of T2-stage diseases (familial 69% vs. PIR 65%), whereas the population-based Pirkanmaa cohort had more T3-stage diseases (familial 29% vs. PIR 34%) (Figure 12c). Differences in T or N stages between these two groups were not statistically significant (Mann-Whitney U test). There was no difference in cancer-specific survival rates between the two cohorts.

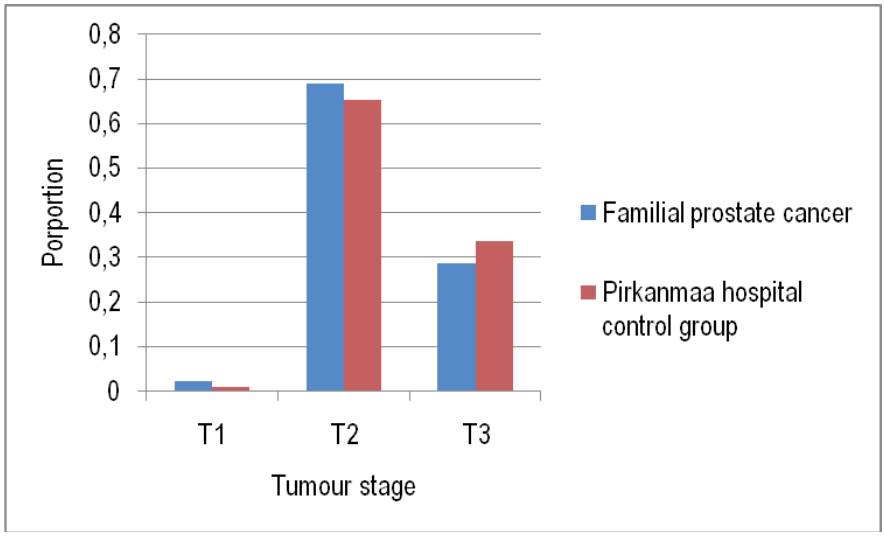

Figure 12c. *Tumour stages in men with familial prostate cancer and men in the Pirkanmaan hospital control group.*

3. Cancer risks in clinically aggressive and nonaggressive prostate cancer families

There were a total of 497 malignancies among the 3,902 members of the clinically nonaggressive prostate cancer families and the 1,621 members of the 59 clinically aggressive families. Table 5 describes the numbers of observed and expected cancer cases obtained using methods 1 and 2.

***Table 5**. Numbers of observed and expected cancer cases with methods 1 and 2 in clinically nonaggressive and aggressive prostate cancer families.*

Clinically nonaggressive prostate cancer families				
	observed cancer cases	expected cancer cases	SIR	95% CI
Men, method 1	364	211	1.7	1.6-1.9
Men, method 2	151	180	0.8	0.7-1.0
Women	133	140	1.0	0.8-1.1
Clinically aggressive prostate cancer families				
	observed cancer cases	expected cancer cases	SIR	95% CI
Men, method 1	188	97	1.9	1.7-2.2
Men, method 2	83	79	1.1	0.8-1.3
Women	72	96	1.1	0.8-1.3

We observed a significantly increased risk of gastric cancer among women in clinically nonaggressive prostate cancer families (SIR 1.9, CI 1.0-3.2). Among men, there was less lung cancer than expected (SIR 0.4, 0.2-0.6, method 1 and SIR 0.5, 0.4-1.1, method 2). When analysing males and females separately, males presented more cases of gall bladder cancer (obs 3, SIR 4.1, CI 0.8-11.8 method 1, obs 3, SIR 5.1, CI 1.0-14.9), while females had more cases of liver cancer (obs 3, SIR 4.8, CI 1.0-14.0).

Altogether, there were 479 cases of cancer in the 202 families. Some people had more than one malignancy. Sixty individuals had two malignancies, and six individuals had three. Forty-one individuals had prostate cancer as one of the cancers.

4. PALB2 variants in hereditary and unselected Finnish prostate cancer cases

Probands from 178 Finnish hereditary prostate cancer families and 285 unselected cases were screened for *PALB2* mutations. Six variants were found among the coding regions and exon-intron boundaries of *PALB2*. All of the variants have been previously described (Erkko 2007), and only the truncating mutation (c.1592delT) has been shown to have functional consequences for the DNA damage response. The association of four detected variants (c.1592delT, 1674A>G, 2993G>A and 3300T>G) with prostate cancer was assessed in more detail in a larger set of unselected cases (n = 463). The truncating c.1592delT mutation was detected in one control sample and two sporadic cases in addition to the previously described family number 310. Interestingly, family 310 has lately been found to also harbour predispositions to stomach and skin cancer in addition to earlier described breast cancer predisposition. Three of the detected variants, 1674A>G in exon 4, 2993G>A in exon 9 and 3300T>G in exon 12, co-existed in 6/178 familial samples (OR 1.52 CI 95% 0.6-3.9), 20/748 unselected cases (OR 1.20 CI 95% 0.6-2.3) and in 17/760 of the controls, showing no statistically significant difference between the sample sets.

DISCUSSION

Prostate cancer is a major health problem in the Western world, and the economic burden of the disease is substantial and growing. The number of newly diagnosed cases has been increasing since 1990. Demographic trends in the next 20 years will exacerbate the effects of the disease epidemiology by increasing the population of older men at risk for prostate cancer. New diagnostics and therapeutic procedures are needed, though these will put additional demands on our research and health care resources.

This thesis studied the mode of inheritance of prostate cancer and the clinical and histopathological characteristics of familial prostate cancer in Finland. The special characteristics that made this study possible relate to the fairly homogenous Finnish population, the standardised health care system and various comprehensive registries. These features have made it possible to collect family history data that are registry-based and genealogically confirmed. In addition, the excellent health care services in Finland provide detailed patient registries, enabling the use of clinical and histopathological information on diseases and a registry-based approach rather than needing to rely on solely patient interviews.

1. The mode of inheritance

The familial clustering of prostate cancer has been well documented (Morganti et al. 1956). Previous segregation analyses suggested different inheritance patterns, from Mendelian inheritance (dominant, recessive with X-linked components, codominant) to multifactorial inheritance (Carter et al. 1992, Grönberg et al. 1997, Cui et al. 2001, Cong et al. 2002). In most of the previous studies, probands have been selected from hospital-based radical prostatectomy patients presenting with primarily localised disease. This has led to a subgroup of patients not representative of all prostate cancer cases in the population. The aim of our study was to assess the basis for the observed familial aggregation of histologically confirmed familial prostate cancer in a large population-based cohort in Finland.

In our population-based segregation analysis (Study I), the inheritance of prostate cancer in the Finnish population was best explained by a Mendelian recessive model with a significant paternal regressive coefficient indicative of a polygenic multifactorial component. These results suggest that prostate cancer inheritance in multiplex families is controlled by major genes. This is the first time a recessive model has been suggested to fit all of the data, even when divided into early and late onset cohorts. This is in line with our previous genetic epidemiological analyses, where X-linked recessive inheritance was shown to be an important contributor to hereditary prostate cancer, especially in Finland (Schleutker et al. 2000). However, the results of the segregation analysis need to be considered carefully because the analysis was performed with different data and the program can give variable results.

A recent study by MacInnis et al. (2010) investigated genetic models of susceptibility to prostate cancer using segregation analyses of occurrence in families ascertained through a population-based series totalling 4390 cases. Their analyses indicated that one or more genes have a strong recessively inherited effect of risk and that a number

of other genes have variants with small multiplicative effects of risk, confirming our previous results (MacInnis et al. 2010).

Because multiple different modes of inheritance of prostate cancer have been proposed, prostate cancer is most likely caused by a number of genes, each with different modes of inheritance, population frequencies and penetrance. Reliable estimates of gene frequencies and penetrance cannot be made until the genes have been identified and the frequencies of mutations have been screened at the population level.

2. Clinical and histopathological characteristics

Previous studies published on the clinical characteristics of familial prostate cancer have used variable selection criteria for selecting familial prostate cancer families. In addition, the collection of three generations is demanding due to the structure of the health care system and population registration methods. Hereditary cancers are characterised as having an earlier age of onset, a more aggressive form of the disease and aggregation of cancer across several generations in a family. The earlier age of onset of familial prostate cancer has been published in many studies (Carter et al. 1993, Grönberg et al. 1997b, Norrish et al. 1999, Bratt et al. 1999, Valeri et al. 2000, Ahaghotu et al. 2004). More contradictory findings have been published for other clinical and histopathological characteristics.

One challenge inherent in family studies is the long study period, which can exceed a hundred years in three-generation families. Clinical practices have changed remarkably in the past century. The Gleason grading system was introduced over 40 years ago, but it remains one of the most powerful prognostic factors available (Gleason 1966). The system was updated in 2005 to address a trend in practice towards a grading shift that rarely used Gleason patterns 1 and 2 or Gleason scores of 2-4 (Epstein. 2010). In our study (Study II), we wanted to overcome this problem of non-comparable samples. We collected all of the diagnostic biopsy samples and had them re-evaluated by the same experienced uropathologist. As hypothesised, the differences between the original and reanalysed WHO and Gleason grading scores of biopsy samples were large and statistically significant.

However, the year of prostate cancer diagnosis in the control dataset (1996-2009) has a different range than that of the family data (1962-2006). This means that the control patients were more likely to have been exposed to PSA screening, and thus, their cancers were likely detected at an earlier stage of the disease. In addition, we

were not able to reanalyse the biopsy samples in control data; the histological scores for these samples were likely lower than they would have been if regraded.

Prior studies investigating primary differences in PSA levels between familial and sporadic prostate cancer cases most often reported no difference between groups (Keetch et al. 1996, Grönberg et al. 1997b, Bauer et al. 1998, Valeri et al. 2000, Ahaghotu et al. 2004, Spangler et al. 2005, Roehl et al. 2006). However, Siddiqui et al. (2006) observed higher primary PSA levels in cases of familial prostate cancer (Siddiqui et al. 2006). Most of the previous studies were done on prostatectomy patients with more localised disease as opposed to population-based data. In our study (Study II), a significantly higher primary PSA level was observed among the familial prostate cancer patients.

Previously published studies assessing differences between the grades of sporadic and familial prostate cancer cases usually reported no differences (Bastacky et al. 1995, Kupelian et al. 1997, Bauer et al. 1998, Valeri et al. 2000, Bratt 2002, Roehl et al. 2006, Siddiqui et al. 2006). I found that the familial prostate cancers displayed a higher grade and were more likely to have metastasised to lymph nodes and bone compared to the unselected patient series, but there was no difference in cancer-specific survival (Study IV). However, these findings have to be considered carefully due to the differences between the family and control data. The most valuable aspect of this study is the clinical and histopathological description of the Finnish prostate cancer families.

3. Other cancers in Finnish prostate cancer families

In our nationwide prostate cancer family study, the incidence of non-prostate cancer malignancies was not higher than that in the greater Finnish population, with the exception of gastric cancer in women. We tested numerous associations and found increased risks of liver cancer in women and gallbladder cancer in men from clinically aggressive prostate cancer families.

Previously, Isaacs et al. (1995) reported prostate cancer to be relatively site-specific and that prostate cancer families only show an increased risk of developing cancers of the central nervous system (Isaacs et al. 1995). Subsequent studies have published contradictory results, finding aggregation of breast, gastric, colon, rectum, gallbladder or kidney cancer in addition to multiple myeloma, non-Hodgkin's lymphoma and melanoma (Grönberg et al. 2000, Valeri et al. 2000, Albright et al. 2005, Hemminki at al. 2005). However, only the Swedish and Utah studies were performed in a population-based manner (Isaacs et al. 1995).

We observed an increase in gastric cancers among female members of prostate cancer families (Study III). In a previous Finnish population-based study, an increased risk of gastric cancer was detected among male relatives of early onset prostate cancer patients (Matikainen et al. 2001). E-cadherin (*CDH1*) has been suggested as a potential gene underlying prostate and gastric cancer aggregation (Ikonen et al. 2001), but further studies are needed to explain the association between these two cancers.

Previous epidemiological identified a coaggregation of prostate and breast cancers (Thiessen 1974, Tulinius et al. 1992). In our analysis of clinically aggressive prostate cancer families, female relatives under 40 years of age had increased risks of breast and ovarian cancer. This association can be partially explained by the presence of recognised cancer-predisposing genes; for example, *BRCA2* has been implicated in prostate cancer predisposition in some populations (Baffoe-Bonnie et al. 2002, Edwards et al.

2003). It is likely that mutations in *BRCA1/2* pathway genes can cause malignancies at multiple cancer sites (Breast cancer linkage consortium 1999).

Some of the differences between the aforementioned publications can be explained by the difficulty of identifying hereditary prostate cancer populations and all malignancies exhibited by relatives. In addition, differences in cancer aggregation and clinical phenotypes among the populations studied might reflect different genetic backgrounds and heterogeneity of germline mutations among populations.

In summary, we did not detect a general elevation in malignancies in prostate cancer families in Finland. Furthermore, the risk of developing other cancers did not appear to be related to the clinical and histopathological characteristics of familial prostate cancer cases. In addition, the profile of related cancers and risk genes for hereditary prostate cancer families may differ between populations.

4. Genetic loci predisposing to prostate cancer in Finland

Prompted by the previous observation that the truncating founder mutation *PALB2* 1592delT segregated in a Finnish family (family 310) with four prostate cancer cases, we wanted to determine whether other possible *PALB2* mutations contributed to prostate cancer susceptibility. Six previously described variants were identified in the coding region and exon-intron boundaries of *PALB2*, none of which induced a truncation. In addition to its previously described link with breast cancer, family 310 was also found to be predisposed to stomach and skin cancers. Interestingly, three of the detected *PALB2* variants co-existed in six patients. A haplotype analysis was performed to assess whether these three variants had a stronger combined effect, but no association with prostate cancer was observed.

The mitomycin C sensitivity test was previously performed for the c.1592delT homozygous mutation. We wanted to test whether this mutation has functional consequences in a heterozygous form, as was the case in all of our patients. We exposed two heterozygous lymphoplastoid cell lines with c.1592delT to mitomycin C. In both cell lines, mitomycin C-induced growth inhibition was observed, suggesting *PALB2* haploinsufficiency.

Our findings suggest that no other deleterious *PALB2* variants, except the 1592delT mutation, contribute even marginally to prostate cancer risk in Finland. Variant 2993G>A is now reported for the first time in the Finnish population. As reported by Rahman and colleagues (Rahman et al. 2007), the 2993G>A variant might damage protein function, based on SIFT (sorting intolerant from tolerant) analysis. Eleven variants in *PALB2* were found, but none were truncating mutations, and no association with the disease was observed. The small amount of variation seen among Finns likely reflects the known genetic homogeneity and founder effect of the population, though substantial differences between eastern and western Finland do exist (de la

Chapelle 1993, Palo et al. 2009). Although *PALB2* variation does not explain familial aggregation of prostate cancer at a population level, it may still be important at an individual level. It is also possible that other genes from the *BRCA1-PALB2-BRCA2* pathway have prostate cancer-predisposing alleles. *PALB2* has been found to predispose individuals to breast and pancreatic cancer and could thus be considered a common cancer-predisposing gene (Eerola et al. 2001, Slater et al. 2010).

5. Special characteristics of studying hereditary prostate cancer

There are a number of factors that complicate the study of prostate cancer. First, prostate cancer is a common disease in the population. Autopsy studies suggest that most ageing men develop lesions that if detected clinically would be diagnosed as prostate cancer (Xu et al. 2010). Due to the high frequency of prostate cancer, it is likely that some cases considered to be hereditary in pedigree analyses actually represent sporadic cases (phenocopies). Phenocopies are most likely also present in families that segregate a disease-predisposing mutation.

Second, prostate cancer grows relatively slowly, with a doubling time of three to four years (Friberg and Mattson 1997). It takes about 20-25 years for prostate cancer to grow from initiation to the stage at which the phenotype can be clinically detected (Griffiths et al. 2007). However, there is substantial variation in the spectrum of the clinical disease phenotype. In addition, prostate cancer is multifocal, meaning that the cancer might grow in several locations within the gland. Finally, the wide use of the PSA test leads to overdiagnosis of this disease.

Third, prostate cancer has a late age of onset. The mean age of onset in Finland is 71 years. Because of this, the identification of more than two generations for molecular studies is difficult. More than one hundred years need to be covered to complete a three-generation pedigree.

6. Future prospects

Why is the study of familial prostate cancer and prostate cancer susceptibility important? We hope that by extensive study of prostate cancer pedigrees and in-depth analysis and appreciation of other cancers and diseases that co-occur in prostate cancer families, we can more effectively optimise the predictive information of family history. This will be important for providing more clinical detail regarding the prostate cancers present throughout the family history. In addition, defining genes that affect prostate cancer risk can potentially provide otherwise unattainable insight into the mechanisms of prostate cancer carcinogenesis and support the identification of novel therapeutic targets. Moreover, future family studies will make use of new high-throughput sequencing methods and thus become more accurate and useful for gene hunting attempts.

While progress has been made in identifying genes associated with both sporadic and familial cancer, there is still much to be learned. Many findings in this field have been difficult to replicate, possibly because prostate cancer is heterogeneous disease with a complex aetiology involving genetic as well as strong environmental influences and, inevitably, interactions between the two. It is noteworthy that the *APC* gene, which is inactivated in the majority of both familial and sporadic forms of colon cancer, was originally identified via studies of families with familial adenomatous polyposis (FAP), a syndrome that accounts for only a small fraction of colon cancers or familial colorectal cancer (Kinzler and Vogelstein. 1996).

As we foresaw, the genetic mechanisms that promote prostate cancer are likely to be complex, and it is probable that different genes are important in different populations. Despite extensive efforts and a number of promising leads, no major gene that can be used to identify individuals at high risk for prostate cancer has been identified. In the light of the current data, a multigenic model provides a more likely explana-

tion. However, once population-specific and the most relevant prognostic genes have been identified, it will be possible to determine the status of multiple genes in a single assay. It may be worthwhile to screen high-risk families for a particular susceptibility mutation (e.g. *BRCA1* and *BRCA2*), but for prostate cancer, such screening may not be effective at a larger scale. However, a more focused analysis of clinically distinct subsets of prostate cancer might lend important insight into this question. Once patients can be diagnosed at the molecular level, those patients at high risk could be identified earlier and provided with individual treatment and follow-up plans.

Different populations may have their own risk genes, and all nations should determine the impact of these genes in their populations. For example, in Poland, *CHEK2* mutations are associated with a moderate risk for prostate cancer, larger than in any other population analysed (Cybulski et al. 2004, Cybulski et al. 2006). Moreover, in Iceland, a specific *BRCA2* mutation accounts for most of the familial prostate cancer cases observed in families with excess numbers of breast cancer cases (Tulinius et al. 2002).

Future studies will systematically screen regulatory regions and introns as well as exons to determine the genetic code of common diseases. Successful interpretation and application of the data for public health purposes will require close cooperation between different experts, e.g. statisticians, geneticists and basic researchers. Though each malignancy harbours its own genetic structure, the mutations underlying common malignancies should be unravelled in the near future. We hope that this will improve the diagnostics and prognostics of cancer and lead to the development of novel pharmaceutical molecules. The mutation profiles in different malignancies appear to be extensive, thus posing challenges for the development of curative medications. In the future, people will know their own genetic risk profile, how to attend follow-up and how their diseases should be treated (personalised medicine). In addition, the frequency of overdiagnosis caused by excessive use of the PSA test signals the need to distinguish clinically mild from aggressive diseases and decrease unnecessary burdens on patients' quality of life and national economics.

Despite the challenges inherent in studying cancer, the puzzle will be resolved, piece by piece.

CONCLUSIONS

The present study was conducted to provide new information on the mode of inheritance of prostate cancer and clinical and histopathological characteristics of familial prostate cancer.

The major findings of this study were:

1. A recessive mode of inheritance is likely involved in familial prostate cancer in Finland.
2. Patients from Finnish prostate cancer families have been clinically and histopathologically described and do not significantly differ from sporadic cancers.
3. The overall incidence of cancer is not increased in clinically aggressive or non-aggressive prostate cancer families in Finland, with the exception of stomach cancer among female relatives.
4. The detected *PALB2* variants do not explain the aggregation of prostate cancer at the population level, but some of these variants may contribute to cancer susceptibility at the individual level.

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References

Adriole GL, Bostwick DG, Brawley OW, Gomella LG, Marberger M, Montorsi F, Pettaway CA, Tammela TL, Teloken C, Tindall DJ, Somerville MC, Wilson TH, Fowler IL, Rittmaster RS. (2010): Effect of dutasteride on the risk of prostate cancer. N Engl J Med. 362:1192-202

Agalliu I, Kwon EM, Zadory D, McIntosh L, Thompson J, Stanford JL and Ostrander EA (2007): Germline mutations in the BRCA2 gene and susceptibility to hereditary prostate cancer. Clin.Cancer Res. 13:839-843

Ahaghotu C, Baffoe-Bonnie A, Kittles R, Pettaway C, Powell I, Royal C, Wang H, Vijayakumar S, Bennett J, Hoke G, Mason T, Bailey-Wilson J, Boykin W, Berg K, Carpten J, Weinrich S, Trent J, Dunston G and Collins F (2004): Clinical characteristics of African-American men with hereditary prostate cancer: the AAHPC Study Prostate Cancer. Prostatic Dis. 7:165-169

Ahlbom A, Lichtenstein P, Malmstrom H, Feychting M, Hemminki K and Pedersen NL (1997): Cancer in twins: genetic and nongenetic familial risk factors. J.Natl.Cancer Inst. 89:287-293

Albright LA, Schwab A, Camp NJ, Farnham JS and Thomas A (2005): Population-based risk assessment for other cancers in relatives of hereditary prostate cancer (HPC) cases. Prostate 64:347-355

Altshuler D, Daly MJ and Lander ES (2008): Genetic mapping in human disease. Science 322:881-888

Altekruse SF, Kosary CL, Krapcho M, Neyman N, Aminou R, Waldron W, Ruhl J, Howlader N, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Cronin K, Chen HS, Feuer EJ, Stinchcomb DG, Edwards BK (2009): SEER Cancer Statistics Review, 1975-2007, National Cancer Institute. Bethesda, MD, at http://seer.cancer.gov/csr/1975_2007/

American Cancer Society (2009): Cancer Facts and Figures at www.cancer.org

Amundadottir LT, Thorvaldsson S, Gudbjartsson DF, Sulem P, Kristjansson K, Arnason S, Gulcher JR, Bjornsson J, Kong A, Thorsteinsdottir U and Stefansson K (2004): Cancer as a complex phenotype: pattern of cancer distribution within and beyond the nuclear family. PLoS Med. 1:e65

Baffoe-Bonnie AB, Kiemeney LA, Beaty TH, Bailey-Wilson JE, Schnell AH, Sigvaldason H, Olafsdottir G, Tryggvadottir L and Tulinius H (2002): Segregation analysis of 389 Icelandic pedigrees with Breast and prostate cancer. Genet.Epidemiol. 23:349-363

Balkwill F and Mantovani A (2001): Inflammation and cancer: back to Virchow? Lancet 357:539-545

Bastacky SI, Wojno KJ, Walsh PC, Carmichael MJ and Epstein JI (1995): Pathological features of hereditary prostate cancer. J.Urol. 153:987-992

Bauer JJ, Srivastava S, Connelly RR, Sesterhenn IA, Preston DM, McLeod DG and Moul JW (1998): Significance of familial history of prostate cancer to traditional prognostic variables, genetic biomarkers, and recurrence after radical prostatectomy. Urology 51:970-976

Bonney GE (1986): Regressive logistic models for familial disease and other binary traits. Biometrics 42:611-625

Bosland MC (2000): The role of steroid hormones in prostate carcinogenesis. J.Natl.Cancer. Inst.Monogr. 27:39-66

Bostwick DG, Burke HB, Djakiew D, Euling S, Ho SM, Landolph J, Morrison H, Sonawane B, Shifflett T, Waters DJ and Timms B (2004): Human prostate cancer risk factors. Cancer 101:2371-2490

Bratt O (2007): What should a urologist know about hereditary predisposition to prostate cancer? BJU Int. 99:743-748

Bratt O (2002): Hereditary prostate cancer: clinical aspects. J.Urol. 168:906-913

Bratt O, Kristoffersson U, Lundgren R and Olsson H (1999): Familial and hereditary prostate cancer in southern Sweden. A population-based case-control study. Eur.J.Cancer 35:272-277

The Breast Cancer Linkage Consortium (1999): Cancer risks in BRCA2 mutation carriers. J.Natl.Cancer Inst. 91:1310-1316

Bruner DW, Moore D, Parlanti A, Dorgan J and Engstrom P (2003): Relative risk of prostate cancer for men with affected relatives: systematic review and meta-analysis. Int. J.Cancer 107:797-803

Carpten J, Nupponen N, Isaacs S, Sood R, Robbins C, Xu J, Faruque M, Moses T, Ewing C, Gillanders E, Hu P, Bujnovszky P, Makalowska I, Baffoe-Bonnie A, Faith D, Smith J, Stephan D, Wiley K, Brownstein M, Gildea D, Kelly B, Jenkins R, Hostetter G, Matikainen M, Schleutker J, Klinger K, Connors T, Xiang Y, Wang Z, De Marzo A, Papadopoulos N, Kallioniemi OP, Burk R, Meyers D, Gronberg H, Meltzer P, Silverman R, Bailey-Wilson J, Walsh P, Isaacs W and Trent J (2002): Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. Nat. Genet. 30:181-184

Carter BS, Beaty TH, Steinberg GD, Childs B and Walsh PC (1992): Mendelian inheritance of familial prostate cancer. Proc.Natl.Acad.Sci.U.S.A. 89:3367-3371

Carter BS, Bova GS, Beaty TH, Steinberg GD, Childs B, Isaacs WB and Walsh PC (1993): Hereditary prostate cancer: epidemiologic and clinical features. J.Urol. 150:797-802

Chang BL, Lange EM, Dimitrov L, Valis CJ, Gillanders EM, Lange LA, Wiley KE, Isaacs SD, Wiklund F, Baffoe-Bonnie A, Langefeld CD, Zheng SL, Matikainen MP, Ikonen T, Fredriksson H, Tammela T, Walsh PC, Bailey-Wilson JE, Schleutker J, Grönberg H, Cooney KA, Isaacs WB, Suh E, Trent JM and Xu J (2006): Two-locus genome-wide linkage scan for prostate cancer susceptibility genes with an interaction effect. Hum. Genet. 118:716-724

Cheng I, Plummer SJ, Jorgenson E, Liu X, Rybicki BA, Casey G and Witte JS (2008): 8q24 and Prostate Cancer: Association with Advanced Disease and Meta-Analysis. Eur. J.Hum.Genet. 16:496-505

Clark LC, Dalkin B, Krongrad A, Combs GF,Jr, Turnbull BW, Slate EH, Witherington R, Herlong JH, Janosko E, Carpenter D, Borosso C, Falk S and Rounder J (1998): Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. Br.J.Urol. 81:730-734

Conlon EM, Goode EL, Gibbs M, Stanford JL, Badzioch M, Janer M, Kolb S, Hood L, Ostrander EA, Jarvik GP and Wijsman EM (2003): Oligogenic segregation analysis of hereditary prostate cancer pedigrees: evidence for multiple loci affecting age at onset. Int.J.Cancer 105:630-635

Cui J, Staples MP, Hopper JL, English DR, McCredie MR and Giles GG (2001): Segregation analyses of 1,476 population-based Australian families affected by prostate cancer. Am.J.Hum.Genet. 68:1207-1218

Cybulski C, Gorski B, Huzarski T, Masojc B, Mierzejewski M, Debniak T, Teodorczyk U, Byrski T, Gronwald J, Matyjasik J, Zlowocka E, Lenner M, Grabowska E, Nej K, Castaneda J, Medrek K, Szymanska A, Szymanska J, Kurzawski G, Suchy J, Oszurek O, Witek A, Narod SA and Lubinski J (2004): CHEK2 is a multiorgan cancer susceptibility gene. Am.J.Hum.Genet. 75:1131-1135

Cybulski C, Wokolorczyk D, Huzarski T, Byrski T, Gronwald J, Gorski B, Debniak T, Masojc B, Jakubowska A, Gliniewicz B, Sikorski A, Stawicka M, Godlewski D, Kwias Z, Antczak A, Krajka K, Lauer W, Sosnowski M, Sikorska-Radek P, Bar K, Klijer R, Zdrojowy R, Malkiewicz B, Borkowski A, Borkowski T, Narod S and Lubinski J (2006): A large germline deletion in CHEK2 is associated with an increased risk of prostate cancer. J.Med.Genet. 43:863-6

de la Chapelle A (2004): Genetic predisposition to colorectal cancer. Nat.Rev.Cancer. 4:769-780

de la Chapelle A (1993): Disease gene mapping in isolated human populations: the example of Finland. J.Med.Genet. 30:857-865

De Marzo AM, Meeker AK, Zha S, Luo J, Nakayama M, Platz EA, Isaacs WB and Nelson WG (2003): Human prostate cancer precursors and pathobiology. Urology 62:55-62

Dennis LK and Dawson DV (2002): Meta-analysis of measures of sexual activity and prostate cancer. Epidemiology 13:72-79

Dewailly E, Mulvad G, Sloth Pedersen H, Hansen JC, Behrendt N and Hart Hansen JP (2003): Inuit are protected against prostate cancer. Cancer Epidemiol.Biomarkers Prev. 12:926-927

Divisi D, Di Tommaso S, Salvemini S, Garramone M and Crisci R (2006): Diet and cancer. Acta Biomed. 77:118-123

Eaton NE, Reeves GK, Appleby PN and Key TJ (1999): Endogenous sex hormones and prostate cancer: a quantitative review of prospective studies. Br.J.Cancer 80:930-934

Eble JN, Sauter G, Epstein JI and Sesterhenn IA (2004): Tumours of the Urinary System and Male Genital Organs. WHO Press. 159-214

Eckersberger E, Finkelstein J, Sadri H, Margreiter M, Taneja SS, Lepor H and Djavan B (2009): Screening for Prostate Cancer: A Review of the ERSPC and PLCO Trials. Rev.Urol. 11:127-133

Edwards SM, Kote-Jarai Z, Meitz J, Hamoudi R, Hope Q, Osin P, Jackson R, Southgate C, Singh R, Falconer A, Dearnaley DP, Ardern-Jones A, Murkin A, Dowe A, Kelly J, Williams S, Oram R, Stevens M, Teare DM, Ponder BA, Gayther SA, Easton DF, Eeles RA, Cancer Research UK/Bristish Prostate Group UK Familial Prostate Cancer Study Collaborators and British Association of Urological Surgeons Section of Oncology (2003): Two percent of men with early-onset prostate cancer harbor germline mutations in the BRCA2 gene. Am.J.Hum.Genet. 72:1-12

Eeles RA (1999): Genetic predisposition to prostate cancer. Prostatic Dis. 2:9-15

Eeles RA, Kote-Jarai Z, Giles GG, Olama AA, Guy M, Jugurnauth SK, Mulholland S, Leongamornlert DA, Edwards SM, Morrison J, Field HI, Southey MC, Severi G, Donovan JL, Hamdy FC, Dearnaley DP, Muir KR, Smith C, Bagnato M, Ardern-Jones AT, Hall AL, O'Brien LT, Gehr-Swain BN, Wilkinson RA, Cox A, Lewis S, Brown PM, Jhavar SG, Tymrakiewicz M, Lophatananon A, Bryant SL; UK Genetic Prostate Cancer Study Collaborators; British Association of Urological Surgeons' Section of Oncology; UK ProtecT Study Collaborators, Horwich A, Huddart RA, Khoo VS, Parker CC, Woodhouse CJ, Thompson A, Christmas T, Ogden C, Fisher C, Jamieson C, Cooper CS, English DR, Hopper JL, Neal DE, Easton DF (2008): Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet. 40:316-21

Eerola H, Pukkala E, Pyrhonen S, Blomqvist C, Sankila R and Nevanlinna H (2001): Risk of cancer in BRCA1 and BRCA2 mutation-positive and -negative breast cancer families (Finland). Cancer Causes Control 12:739-746

Eldon BJ, Jonsson E, Tomasson J, Tryggvadottir L and Tulinius H (2003): Familial risk of prostate cancer in Iceland. BJU Int. 92:915-919

Engholm G, Ferlay J, Christensen N, Bray F, Gjerstorff ML, Klint Å, Kotlum JE, Olafsdórrir E, Pukkala E, Storm HH (2010): NORDCAN: Cancer incidence, mortality, prevalence, and prediction in the Nordic countries, Version 3.6. Association of Nordic Cancer Registeries. Danish Cancer Society at http://www.ancr.nu.

Epstein JI (2010): An update of the Gleason grading system. J.Urol. 183:433-440

Epstein JI (1994): Pathology of prostatic intraepithelial neoplasia and adenocarcinoma of the prostate: prognostic influences of stage, tumor volume, grade, and margins of resection. Semin.Oncol. 21:527-541

Erkko H, Xia B, Nikkila J, Schleutker J, Syrjakoski K, Mannermaa A, Kallioniemi A, Pylkas K, Karppinen SM, Rapakko K, Miron A, Sheng Q, Li G, Mattila H, Bell DW, Haber DA, Grip M, Reiman M, Jukkola-Vuorinen A, Mustonen A, Kere J, Aaltonen LA, Kosma VM, Kataja V, Soini Y, Drapkin RI, Livingston DM and Winqvist R (2007): A recurrent mutation in *PALB2* in Finnish cancer families. Nature 446:316-319

Fearon ER (1997): Human cancer syndromes: clues to the origin and nature of cancer-Science278:1043-1050

Finnish Cancer Registry (2008): Cancer incidence and mortality in Finland. Cancer Statistics at www.cancerregistry.fi

Foulkes WD, Ghadirian P, Akbari MR, Hamel N, Giroux S, Sabbaghian N, Darnel A, Royer R, Poll A, Fafard E, Robidoux A, Martin G, Bismar TA, Tischkowitz M, Rousseau F and Narod SA (2007): Identification of a novel truncating *PALB2* mutation and analysis of its contribution to early-onset breast cancer in French-Canadian women. Breast Cancer Res. 9:R83

Freedman ML, Haiman CA, Patterson N, McDonald GJ, Tandon A, Waliszewska A, Penney K, Steen RG, Ardlie K, John EM, Oakley-Girvan I, Whittemore AS, Cooney KA, Ingles SA, Altshuler D, Henderson BE and Reich D (2006): Admixture mapping identifies 8q24 as a prostate cancer risk locus in African-American men. Proc.Natl. Acad.Sci.U.S.A. 103:14068-14073

Friberg S and Mattson S (1997): On the growth rates of human malignant tumors: implications for medical decision making. J.Surg.Oncol. 65:284-297

Friedenson B (2005):BRCA1 and BRCA2 pathways and the risk of cancers other than breast or ovarian. MedGenMed 7:60

Garber JE and Offit K (2005): Hereditary cancer predisposition syndromes. J.Clin.Oncol. 23:276-292

Gibbs M, Stanford JL, McIndoe RA, Jarvik GP, Kolb S, Goode EL, Chakrabarti L, Schuster EF, Buckley VA, Miller EL, Brandzel S, Li S, Hood L and Ostrander EA (1999): Evidence for a rare prostate cancer-susceptibility locus at chromosome 1p36. Am.J.Hum.Genet. 64:776-787

Giovannucci E, Ascherio A, Rimm EB, Stampfer MJ, Colditz GA and Willett WC (1995): Intake of carotenoids and retinol in relation to risk of prostate cancer. J.Natl.Cancer Inst. 87:1767-1776

Gittes RF (1991): Carcinoma of the prostate. N.Engl.J.Med. 324:236-245

Gleason DF (1966): Classification of prostatic carcinomas. Cancer Chemother.Rep. 50:125-128

Glover FE,Jr, Coffey DS, Douglas LL, Russell H, Cadigan M, Tulloch T, Wedderburn K, Wan RL, Baker TD and Walsh PC (1998): Familial study of prostate cancer in Jamaica. Urology 52:441-443

Goldgar DE, Easton DF, Cannon-Albright LA and Skolnick MH (1994): Systematic population-based assessment of cancer risk in first-degree relatives of cancer probands. J.Natl.Cancer Inst. 86:1600-1608

Gong G, Oakley-Girvan I, Wu AH, Kolonel LN, John EM, West DW, Felberg A, Gallagher RP and Whittemore AS (2002): Segregation analysis of prostate cancer in 1,719 white, African-American and Asian-American families in the United States and Canada. Cancer Causes Control 13:471-482

Griffiths K, Prezioso D, Turkes A and Denis LJ (2007): The prevention of prostate cancer. Recent Results Cancer Res. 175:33-63

Grönberg H, Ahman AK, Emanuelsson M, Bergh A, Damber JE and Borg A (2001): BRCA2 mutation in a family with hereditary prostate cancer. Genes Chromosomes Cancer 30:299-301

Grönberg H, Bergh A, Damber JE and Emanuelsson M (2000): Cancer risk in families with hereditary prostate carcinoma. Cancer 89:1315-1321

Grönberg H, Damber L, Jonsson H and Damber JE (1997): Prostate cancer mortality in northern Sweden, with special reference to tumor grade and patient age. Urology. 49:374

Grönberg H, Damber L, Damber JE and Iselius L (1997a): Segregation analysis of prostate cancer in Sweden: support for dominant inheritance. Am.J.Epidemiol. 146:552-557

Grönberg H, Damber L and Damber JE (1994): Studies of genetic factors in prostate cancer in a twin population. J.Urol. 152:1484-9

Grönberg H, Isaacs SD, Smith JR, Carpten JD, Bova GS, Freije D, Xu J, Meyers DA, Collins FS, Trent JM, Walsh PC and Isaacs WB (1997b): Characteristics of prostate cancer in families potentially linked to the hereditary prostate cancer 1 (HPC1) locus. JAMA 278:1251-1255

Grönberg H, Wiklund F and Damber JE (1999): Age specific risks of familial prostate carcinoma: a basis for screening recommendations in high risk populations. Cancer 86:477-483

Grubb RL,3rd, Pinsky PF, Greenlee RT, Izmirlian G, Miller AB, Hickey TP, Riley TL, Mabie JE, Levin DL, Chia D, Kramer BS, Reding DJ, Church TR, Yokochi LA, Kvale PA, Weissfeld JL, Urban DA, Buys SS, Gelmann EP, Ragard LR, Crawford ED, Prorok PC, Gohagan JK, Berg CD and Andriole GL (2008): Prostate cancer screening in the Prostate, Lung, Colorectal and Ovarian cancer screening trial: update on findings from the initial four rounds of screening in a randomized trial. BJU Int. 102:1524-1530

Gudmundsson J, Sulem P, Manolescu A, Amundadottir LT, Gudbjartsson D, Helgason A, Rafnar T, Bergthorsson JT, Agnarsson BA, Baker A, Sigurdsson A, Benediktsdottir KR, Jakobsdottir M, Xu J, Blondal T, Kostic J, Sun J, Ghosh S, Stacey SN, Mouy M, Saemundsdottir J, Backman VM, Kristjansson K, Tres A, Partin AW, Albers-Akkers MT, Godino-Ivan Marcos J, Walsh PC, Swinkels DW, Navarrete S, Isaacs SD, Aben KK, Graif T, Cashy J, Ruiz-Echarri M, Wiley KE, Suarez BK, Witjes JA, Frigge M, Ober C, Jonsson E, Einarsson GV, Mayordomo JI, Kiemeney LA, Isaacs WB, Catalona WJ, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A and Stefansson K (2007): Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. Nat.Genet. 39:631-7

Gudmundsson J, Sulem P, Rafnar T, Bergthorsson JT, Manolescu A, Gudbjartsson D, Agnarsson BA, Sigurdsson A, Benediktsdottir KR, Blondal T, Jakobsdottir M, Stacey SN, Kostic J, Kristinsson KT, Birgisdottir B, Ghosh S, Magnusdottir DN, Thorlacius S, Thorleifsson G, Zheng SL, Sun J, Chang BL, Elmore JB, Breyer JP, McReynolds KM, Bradley KM, Yaspan BL, Wiklund F, Stattin P, Lindström S, Adami HO, McDonnell SK, Schaid DJ, Cunningham JM, Wang L, Cerhan JR, St Sauver JL, Isaacs SD, Wiley KE, Partin AW, Walsh PC, Polo S, Ruiz-Echarri M, Navarrete S, Fuertes F, Saez B, Godino J, Weijerman PC, Swinkels DW, Aben KK, Witjes JA, Suarez BK, Helfand BT, Frigge ML, Kristjansson K, Ober C, Jonsson E, Einarsson GV, Xu J, Gronberg H, Smith JR, Thibodeau SN, Isaacs WB, Catalona WJ, Mayordomo JI, Kiemeney LA, Barkardottir RB, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K. (2008) Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. Nat Genet. 40: 281-3

Haiman CA, Patterson N, Freedman ML, Myers SR, Pike MC, Waliszewska A, Neubauer J, Tandon A, Schirmer C, McDonald GJ, Greenway SC, Stram DO, Le Marchand L, Kolonel LN, Frasco M, Wong D, Pooler LC, Ardlie K, Oakley-Girvan I, Whittemore AS, Cooney KA, John EM, Ingles SA, Altshuler D, Henderson BE and Reich D (2007): Multiple regions within 8q24 independently affect risk for prostate cancer. Nat.Genet. 39:638-44

Hayes RB, Liff JM, Pottern LM, Greenberg RS, Schoenberg JB, Schwartz AG, Swanson GM, Silverman DT, Brown LM and Hoover RN (1995): Prostate cancer risk in U.S. blacks and whites with a family history of cancer. Int.J.Cancer 60:361-364

Heinonen OP, Albanes D, Virtamo J, Taylor PR, Huttunen JK, Hartman AM, Haapakoski J, Malila N, Rautalahti M, Ripatti S, Mäenpää H, Teerenhovi L, Koss L, Virolainen M and Edwards BK (1998): Prostate cancer and supplementation with alpha-tocopherol and beta-carotene: incidence and mortality in a controlled trial. J.Natl.Cancer Inst. 90:440-446

Hemminki K and Chen B (2005): Familial association of prostate cancer with other cancers in the Swedish Family-Cancer Database. Prostate 65:188-194

Hofstra RM, Spurdle AB, Eccles D, Foulkes WD, de Wind N, Hoogerbrugge N, Hogervorst FB and IARC Unclassified Genetic Variants Working Group (2008): Tumor characteristics as an analytic tool for classifying genetic variants of uncertain clinical significance. Hum.Mutat. 29:1292-1303

Hogg A, Bia B, Onadim Z and Cowell JK (1993): Molecular mechanisms of oncogenic mutations in tumors from patients with bilateral and unilateral retinoblastoma. Proc. Natl.Acad.Sci.U.S.A. 90:7351-7355

Houlston RS and Peto J (2004): The search for low-penetrance cancer susceptibility alleles. Oncogene 23:6471-6476

Hsing AW, Tsao L and Devesa SS (2000): International trends and patterns of prostate cancer incidence and mortality. Int.J.Cancer 85:60-67

Huddart RA, Rajan B, Law M, Meyer L and Dearnaley DP (1997): Spinal cord compression in prostate cancer: treatment outcome and prognostic factors. Radiother. Oncol. 44:229-236

Huggins ML (1941): A New Society for X-Ray and Electron Diffraction Research Workers. Science 93:489-490

Ikonen T, Matikainen M, Mononen N, Hyytinen ER, Helin HJ, Tommola S, Tammela TL, Pukkala E, Schleutker J, Kallioniemi OP, Koivisto PA (2001) Association of E-Cadherin gemline alterations with prostate cancer. Clin Cancer Res.7:3465-71

Ikonen T, Matikainen MP, Syrjäkoski K, Mononen N, Koivisto PA, Rökman A, Seppälä EH, Kallioniemi OP, Tammela TL and Schleutker J (2003): BRCA1 and BRCA2 mutations have no major role in predisposition to prostate cancer in Finland. J.Med. Genet. 40:e98

Imperial J (1999): Treatment of chronic hepatitis C virus infection: the end of an era. Am.J.Gastroenterol. 94:555-556

International Agency for Research on Cancer (2008): Prostate cancer incidence and mortality in Finland. Cancer statistics at www.iarc.fr

Isaacs SD, Kiemeney LA, Baffoe-Bonnie A, Beaty TH and Walsh PC (1995): Risk of cancer in relatives of prostate cancer probands. J.Natl.Cancer Inst. 87:991-996

Jakkula E, Rehnström K, Varilo T, Pietiläinen OP, Paunio T, Pedersen NL, deFaire U, Järvelin MR, Saharinen J, Freimer N, Ripatti S, Purcell S, Collins A, Daly MJ, Palotie A, Peltonen L (2008): The genome-wide patterns of variation expose significant substructure in a founder population. Am J Hum Genet. 83:787-94

Keetch DW, Humphrey PA, Smith DS, Stahl D and Catalona WJ (1996): Clinical and pathological features of hereditary prostate cancer. J.Urol. 155:1841-1843

Khoury MJ, Beaty TH and Cohen BH (1993): Fundamentals of Genetic Epidemiology. Oxford University Press, Oxford.

Kinzler KW and Vogelstein B (1996): Lessons from hereditary colorectal cancer. Cell 87:159-170

Kirby RS, Partin AW, Feneley M, Parsons JK (2006): Prostate Cancer Principles and Practice. Taylor & Francis, Oxon the United Kingdom.

Knudson AG,Jr (1971): Mutation and cancer: statistical study of retinoblastoma. Proc.Natl. Acad.Sci.U.S.A. 68:820-823

Kolonel LN (1996): Nutrition and prostate cancer. Cancer Causes Control 7:83-44

Krieger JN and Riley DE (2004): Chronic prostatitis: Charlottesville to Seattle. J.Urol. 172:2557-2560

Kupelian PA, Kupelian VA, Witte JS, Macklis R and Klein EA (1997): Family history of prostate cancer in patients with localized prostate cancer: an independent predictor of treatment outcome. J.Clin.Oncol. 15:1478-1480

Lange EM, Gillanders EM, Davis CC, Brown WM, Campbell JK, Jones M, Gildea D, Riedesel E, Albertus J, Freas-Lutz D, Markey C, Giri V, Dimmer JB, Montie Je, Trent JM, Cooney KA (2003): Genome-wide scan for prostate cancer susceptibility genes using families from the University of Michigan prostate cancer genetics project finds evidence for linkage on chromosome 17 near BRCA1. Prostate 57:326-34.

Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A and Hemminki K (2000): Environmental and heritable factors in the causation of cancer--analyses of cohorts of twins from Sweden, Denmark, and Finland. N.Engl.J.Med. 343:78-85

Lindblom A (2001): Different mechanisms in the tumorigenesis of proximal and distal colon cancers. Curr.Opin.Oncol. 13:63-69

Lohmueller KE, Pearce CL, Pike M, Lander ES and Hirschhorn JN (2003): Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. Nat.Genet. 33:177-182

MacInnis RJ, Antoniou AC, Eeles RA, Severi G, Guy M, McGuffog L, Hall AL, O'Brien LT, Wilkinson RA, Dearnaley DP, Ardern-Jones AT, Horwich A, Khoo VS, Parker CC, Huddart RA, McCredie MR, Smith C, Southey MC, Staples MP, English DR, Hopper JL, Giles GG and Easton DF (2010): Prostate cancer segregation analyses using 4390 families from UK and Australian population-based studies. Genet.Epidemiol. 34:42-50

Mai KT, Isotalo PA, Green J, Perkins DG, Morash C and Collins JP (2000): Incidental prostatic adenocarcinomas and putative premalignant lesions in TURP specimens collected before and after the introduction of prostate-specific antigen screening. Arch. Pathol.Lab.Med. 124:1454-1456

Maskarinec G and Noh JJ (2004): The effect of migration on cancer incidence among Japanese in Hawaii. Ethn.Dis. 14:431-439

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

Matikainen MP, Sankila R, Schleutker J, Kallioniemi OP and Pukkala E (2000):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

Matikainen MP, Schleutker J, Morsky P, Kallioniemi OP and Tammela TLJ (1999): Detection of subclinical cancers by prostate-spesific antigen screening in asymptomatic men from high-risk prostate cancer families. Clin. Caner Res. 5:1275-1279

McCarthy MI, Abecasis CR, Cardon LR, Goldstein DB, Littele J, Ioannis JPA and Hirschhorn JN (2010): Genome-wide association studies for complex traits: consensus uncertainty and challenges. Nat Rev Genet 11:415-25

Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM and Ding W (1994): A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266:66-71

Morganti G, Gianferrari L, Cresseri A, Arrigoni G and Lovati G (1956): Clinico-statistical and genetic research on neoplasms of the prostate. Acta Genet.Stat.Med. 6:304-305

Morton NE and Chung CS (1978): In Genetic Epidemiology. Academic Press, NY, USA.

Narod SA and Foulkes WD (2004): BRCA1 and BRCA2: 1994 and beyond. Nat.Rev.Cancer. 4:665-676

Neal DE and Donavan JL (2000): Prostate cancer: to screen or not to screen? Lancet Oncol. 1:17-24

Negri E, Pelucchi C, Talamini R, Montella M, Gallus S, Bosetti C, Franceschi S and La Vecchia C (2005): Family history of cancer and the risk of prostate cancer and benign prostatic hyperplasia. Int.J.Cancer 114:648-652

Nelson WG, De Marzo AM, DeWeese TL and Isaacs WB (2004): The role of inflammation in the pathogenesis of prostate cancer. J.Urol.172:S6-11

Norrish AE, McRae CU, Cohen RJ and Jackson RT (1999): A population-based study of clinical and pathological prognostic characteristics of men with familial and sporadic prostate cancer. BJU Int. 84:311-315

Ohtake N, Hatori M, Yamanaka H, Nakata S, Sada M and Tsuji T (1998): Familial prostate cancer in Japan. Int.J.Urol. 5:138-145

Page WF, Braun MM, Partin AW, Caporaso N and Walsh P (1997): Heredity and prostate cancer: a study of World War II veteran twins. Prostate 33:240-245

Palo J, Ulmanen I, Lukka M, Ellonen P and Sajantila P (2009): Genetic markers and population history; Finland revisited. Eur J of Hum Genet 17: 1336-1346

Parsonnet J, Friedman GD, Vandersteen DP, Chang Y, Vogelman JH, Orentreich N and Sibley RK (1991): Helicobacter pylori infection and the risk of gastric carcinoma. N.Engl.J.Med. 325:1127-1131

Peltonen L (1997): Molecular background of the Finnish disease heritage. Ann.Med. 29:553-556

Phillips KA (2000): Immunophenotypic and pathologic differences between BRCA1 and BRCA2 hereditary breast cancers. J.Clin.Oncol. 18:107S-12S

Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, Reid S, Spanova K, Barfoot R, Chagtai T, Jayatilake H, McGuffog L, Hanks S, Evans DG, Eccles D, Breast Cancer Susceptibility Collaboration (UK), Easton DF and Stratton MR (2007): *PALB2*, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene. Nat.Genet. 39:165-167

Rebbeck TR, Walker AH, Zeigler-Johnson C, Weisburg S, Martin AM, Nathanson KL, Wein AJ and Malkowicz SB (2000a): Association of HPC2/ELAC2 genotypes and prostate cancerAm.J.Hum.Genet.67:1014-1019

Rebbeck TR, Walker AH, Zeigler-Johnson C, Weisburg S, Martin AM, Nathanson KL, Wein AJ and Malkowicz SB (2000b):Association of HPC2/ELAC2 genotypes and prostate cancer. Am.J.Hum.Genet. 67:1014-1019

Reid S, Schindler D, Hanenberg H, Barker K, Hanks S, Kalb R, Neveling K, Kelly P, Seal S, Freund M, Wurm M, Batish SD, Lach FP, Yetgin S, Neitzel H, Ariffin H, Tischkowitz M, Mathew CG, Auerbach AD and Rahman N (2007): Biallelic mutations in *PALB2* cause Fanconi anemia subtype FA-N and predispose to childhood cancer. Nat.Genet. 39:162-164

Rennert H, Zeigler-Johnson CM, Addya K, Finley MJ, Walker AH, Spangler E, Leonard DG, Wein A, Malkowicz SB and Rebbeck TR (2005): Association of susceptibility alleles in ELAC2/HPC2, RNASEL/HPC1, and MSR1 with prostate cancer severity in European American and African American men. Cancer Epidemiol.Biomarkers Prev. 14:949-957

Rhodes JM and Campbell BJ (2002): Inflammation and colorectal cancer: IBD-associated and sporadic cancer compared. Trends Mol.Med. 8:10-16

Roehl KA, Loeb S, Antenor JA, Corbin N and Catalona WJ (2006): Characteristics of patients with familial versus sporadic prostate cancer. J.Urol. 176:2438-42; discussion 2442

Rökman A, Ikonen T, Mononen N, Autio V, Matikainen MP, Koivisto PA, Tammela TL, Kallioniemi OP and Schleutker J (2001): ELAC2/HPC2 involvement in hereditary and sporadic prostate cancer. Cancer Res. 61:6038-6041

Rökman A, Ikonen T, Seppälä EH, Nupponen N, Autio V, Mononen N, Bailey-Wilson J, Trent J, Carpten J, Matikainen MP, Koivisto PA, Tammela TL, Kallioniemi OP and Schleutker J (2002): Germline alterations of the RNASEL gene, a candidate HPC1 gene at 1q25, in patients and families with prostate cancer. Am.J.Hum.Genet. 70:1299-1304

Ross RK and Henderson BE (1994): Do diet and androgens alter prostate cancer risk via a common etiologic pathway? J.Natl.Cancer Inst. 86:252-254

SAGE3.1 (1997): Statistical analysis for genetic epidemiology, release 3.1. Computer program package available from the Department of Epidemiology and Biostatistics. Rammelcamp Centre for Education and Research, Metro Health Campus, Case Western Reserve University, Cleveland, Ohio.

Schaid DJ (2004): The complex genetic epidemiology of prostate cancer. Hum.Mol.Genet. 13 Spec No 1:R103-21

Schaid DJ, McDonnell SK, Blute ML and Thibodeau SN (1998): Evidence for autosomal dominant inheritance of prostate cancer. Am.J.Hum.Genet. 62:1425-1438

Schleutker J, Matikainen M, 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 and Kallioniemi OP (2000): 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

Schroder FH, Hugosson J, Roobol MJ, Tammela TL, Ciatto S, Nelen V, Kwiatkowski M, Lujan M, Lilja H, Zappa M, Denis LJ, Recker F, Berenguer A, Määttänen L, Bangma CH, Aus G, Villers A, Rebillard X, van der Kwast T, Blijenberg BG, Moss SM, de Koning HJ, Auvinen A and ERSPC Investigators (2009): Screening and prostate-cancer mortality in a randomized European study. N.Engl.J.Med. 360:1320-1328

Schuurman AG, Goldbohm RA, Brants HA and van den Brandt PA (2002): A prospective cohort study on intake of retinol, vitamins C and E, and carotenoids and prostate cancer risk (Netherlands). Cancer Causes Control 13:573-582

Scott R,Jr, Mutchnik DL, Laskowski TZ and Schmalhorst WR (1969): Carcinoma of the prostate in elderly men: incidence, growth characteristics and clinical significance. J.Urol. 101:602-607

Selman SH (2000): "Latent" carcinoma of the prostate: a medical misnomer? Urology 56:708-711

Seppälä EH, Ikonen T, Autio V, Rökman A, Mononen N, Matikainen MP, Tammela TLJ and Schleutker J (2003a): Germ-line alterations in MSR1 gene and prostate cancer risk. Clin.Cancer Res. 9:5252-5256

Seppälä EH, Ikonen T, Mononen N, Autio V, Rökman A, Matikainen MP, Tammela TLJ and Schleutker J (2003b): CHEK2 variants associate with hereditary prostate cancer. Br.J.Cancer 89:1966-1970

Seppälä EH, Autio V, Suggai P, Ikonen T, Stenman UH, Auvinen A, Bailey-Wilson JE, Tammela TL, Schleutker J (2007) Eur Urol. 52:1076-81

Sheldon CA, Williams RD and Fraley EE (1980): Incidental carcinoma of the prostate: a review of the literature and critical reappraisal of classification. J.Urol. 124:626-631

Shimizu H, Ross RK, Bernstein L, Yatani R, Henderson BE and Mack TM (1991): Cancers of the prostate and breast among Japanese and white immigrants in Los Angeles County. Br.J.Cancer 63:963-966

Siddiqui SA, Sengupta S, Slezak JM, Bergstralh EJ, Zincke H and Blute ML (2006): Impact of familial and hereditary prostate cancer on cancer specific survival after radical retropubic prostatectomy. J.Urol. 176:1118-1121

Sigurdsson S, Thorlacius S, Tomasson J, Tryggvadottir L, Benediktsdottir K, Eyfjord JE and Jonsson E (1997): BRCA2 mutation in Icelandic prostate cancer patients. J.Mol. Med. 75:758-761

Siltanen S, Syrjäkoski K, Fagerholm R, Ikonen T, Lipman P, Mallott J, Holli K, Tammela TL, Järvinen HJ, Mecklin JP, Aittomäki K, Blomqvist C, Bailey-Wilson JE, Nevalinna H, Aaltonen LA, Schleutker J, Vahteristo P (2008): ARLTS1 germline variants and the risk for breast, prostate and colorectal cancer. Eur J Hum Genet. 16: 983-91

Simard J, Dumont M, Soucy P and Labrie F (2002): Perspective: prostate cancer susceptibility genes. Endocrinology 143:2029-2040

Slater EP, Langer P, Niemczyk E, Strauch K, Butler J, Habbe N, Neoptolemos J, Greenhalf W and Bartsch DK (2010): *PALB2* mutations in European familial pancreatic cancer families. Clin.Genet. Epub ahead of print.

Spangler E, Zeigler-Johnson CM, Malkowicz SB, Wein AJ and Rebbeck TR (2005): Association of prostate cancer family history with histopathological and clinical characteristics of prostate tumors. Int.J.Cancer 113:471-474

Stamey TA, Yang N, Hay AR, McNeal JE, Freiha FS and Redwine E (1987): Prostate-specific antigen as a serum marker for adenocarcinoma of the prostate. N.Engl.J.Med. 317:909-916

Stanford JL and Ostrander EA (2001): Familial prostate cancer. Epidemiol.Rev. 23:19-23

Stratton MR, Campbell PJ and Futreal PA (2009): The cancer genome. Nature 458:719-724

Suuriniemi M, Agalliu I, Schaid DJ, Johanneson B, McDonnell SK, Iwasaki L, Stanford JL and Ostrander EA (2007): Confirmation of a positive association between prostate cancer risk and a locus at chromosome 8q24. Cancer Epidemiol.Biomarkers Prev. 16:809-814

Tavtigian SV, Simard J, Teng DH, Abtin V, Baumgard M, Beck A, Camp NJ, Carillo AR, Chen Y, Dayananth P, Desrochers M, Dumont M, Farnham JM, Frank D, Frye C, Ghaffari S, Gupte JS, Hu R, Iliev D, Janecki T, Kort EN, Laity KE, Leavitt A, Leblanc G, McArthur-Morrison J, Pederson A, Penn B, Peterson KT, Reid JE, Richards S, Schroeder M, Smith R, Snyder SC, Swedlund B, Swensen J, Thomas A, Tranchant M, Woodland AM, Labrie F, Skolnick MH, Neuhausen S, Rommens J and Cannon-Albright LA (2001): A candidate prostate cancer susceptibility gene at chromosome 17p. Nat.Genet. 27:172-180

Teahan O, Beaven C, Waxman J and Keun HC (2010): Metabolic signatures of malignant progression in prostate epithelial cells. Int J Biochem Cell Biol. Epub ahead of print.

Teppo L, Pukkala E and Lehtonen M (1994): Data quality and quality control of a population-based cancer registry. Experience in Finland. Acta Oncol. 33:365-369

Thiessen EU (1974): Concerning a familial association between breast cancer and both prostatic and uterine malignancies. Cancer 34:1102-1107

Thomas C. Duncan (2004) Statistical methods in genetic epidemiology. Oxford University Press.

Thomas G, Jacobs KB, Yeager M, Kraft P, Wacholder S, Orr N, Yu K, Chatterjee N, Welch R, Hutchinson A, Crenshaw A, Cancel-Tassin G, Staats BJ, Wang Z, Gonzalez-Bosquet J, Fang J, Deng X, Berndt SI, Calle EE, Feigelson HS, Thun MJ, Rodriguez C, Albanes D, Virtamo J, Weinstein S, Schumacher FR, Giovannucci E, Willett WC, Cussenot O, Valeri A, Andriole GL, Crawford ED, Tucker M, Gerhard DS, Fraumeni JF Jr, Hoover R, Hayes RB, Hunter DJ, Chanock SJ (2008) Multiple loci identifies in a genome-wide association study of prostate cancer. Nat Genet. 40:310-315

Tischkowitz M, Xia B, Sabbaghian N, Reis-Filho JS, Hamel N, Li G, van Beers EH, Li L, Khalil T, Quenneville LA, Omeroglu A, Poll A, Lepage P, Wong N, Nederlof PM, Ashworth A, Tonin PN, Narod SA, Livingston DM and Foulkes WD (2007):Analysis of *PALB2*/FANCN-associated breast cancer families. Proc.Natl.Acad.Sci.U.S.A. 104:6788-6793

Tulinius H, Egilsson V, Olafsdottir GH and Sigvaldason H (1992): Risk of prostate, ovarian, and endometrial cancer among relatives of women with breast cancer. BMJ 305:855-857

Tulinius H, Olafsdottir GH, Sigvaldason H, Arason A, Barkardottir RB, Egilsson V, Ogmundsdottir HM, Tryggvadottir L, Gudlaugsdottir S and Eyfjord JE (2002): The effect of a single BRCA2 mutation on cancer in Iceland. J.Med.Genet. 39:457-462

Valeri A, Azzouzi R, Drelon E, Delannoy A, Mangin P, Fournier G, Berthon P and Cussenot O (2000): Early-onset hereditary prostate cancer is not associated with specific clinical and biological features. Prostate 45:66-71

Valeri A, Briollais L, Azzouzi R, Fournier G, Mangin P, Berthon P, Cussenot O and Demenais F (2003): Segregation analysis of prostate cancer in France: evidence for autosomal dominant inheritance and residual brother-brother dependence. Ann.Hum.Genet. 67:125-137

van den Brandt PA, Zeegers MP, Bode P and Goldbohm RA (2003): Toenail selenium levels and the subsequent risk of prostate cancer: a prospective cohort study. Cancer Epidemiol.Biomarkers Prev. 12:866-871

Verhage BA, Aben KK, Witjes JA, Straatman H, Schalken JA and Kiemeney LA (2004): Site-specific familial aggregation of prostate cancer. Int.J.Cancer 109:611-617

Verhage BA, Baffoe-Bonnie AB, Baglietto L, Smith DS, Bailey-Wilson JE, Beaty TH, Catalona WJ and Kiemeney LA (2001): Autosomal dominant inheritance of prostate cancer: a confirmatory study. Urology 57:97-101

Verkasalo PK, Kaprio J, Koskenvuo M and Pukkala E (1999): Genetic predisposition, environment and cancer incidence: a nationwide twin study in Finland, 1976-1995. Int. J.Cancer 83:743-749

Wang L, McDonnell SK, Slusser JP, Hebbring SJ, Cunningham JM, Jacobsen SJ, Cerhan JR, Blute ML, Schaid DJ and Thibodeau SN (2007): Two common chromosome 8q24 variants are associated with increased risk for prostate cancer. Cancer Res. 67:2944-2950

Whittemore AS, Wu AH, Kolonel LN, John EM, Gallagher RP, Howe GR, West DW, Teh CZ and Stamey T (1995): Family history and prostate cancer risk in black, white, and Asian men in the United States and Canada. Am.J.Epidemiol. 141:732-740

Wooster R, Bignell G, Lancaster J, Swift S, Seal S, Mangion J, Collins N, Gregory S, Gumbs C and Micklem G (1995): Identification of the breast cancer susceptibility gene BRCA2. Nature 378:789-792

Xia B, Dorsman JC, Ameziane N, de Vries Y, Rooimans MA, Sheng Q, Pals G, Errami A, Gluckman E, Llera J, Wang W, Livingston DM, Joenje H and de Winter JP (2007): Fanconi anemia is associated with a defect in the BRCA2 partner *PALB2*. Nat.Genet. 39:159-161

Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ and Livingston DM (2006): Control of BRCA2 cellular and clinical functions by a nuclear partner, *PALB2*. Mol.Cell 22:719-729

Xu J, Dimitrov L, Chang BL, Adams TS, Turner AR, Meyers DA, Eeles RA, Easton DF, Foulkes WD, Simard J, Giles GG, Hopper JL, Mahle L, Moller P, Bishop T, Evans C, Edwards S, Meitz J, Bullock S, Hope Q, Hsieh CL, Halpern J, Balise RN, Oakley-Girvan I, Whittemore AS, Ewing CM, Gielzak M, Isaacs SD, Walsh PC, Wiley KE, Isaacs WB, Thibodeau SN, McDonnell SK, Cunningham JM, Zarfas KE, Hebbring S, Schaid DJ, Friedrichsen DM, Deutsch K, Kolb S, Badzioch M, Jarvik GP, Janer M, Hood L, Ostrander EA, Stanford JL, Lange EM, Beebe-Dimmer JL, Mohai CE, Cooney KA, Ikonen T, Baffoe-Bonnie A, Fredriksson H, Matikainen MP, Tammela TL, Bailey-Wilson J, Schleutker J, Maier C, Herkommer K, Hoegel JJ, Vogel W, Paiss T, Wiklund F, Emanuelsson M, Stenman E, Jonsson BA, Gronberg H, Camp NJ, Farnham J, Cannon-Albright LA, Seminara D and ACTANE Consortium. (2005): A combined genomewide linkage scan of 1,233 families for prostate cancer-susceptibility genes conducted by the international consortium for prostate cancer genetics. Am.J.Hum.Genet. 77:219-229

Xu J, Zheng SL, Isaacs SD, Wiley KE, Wiklund F, Sun J, Kader AK, Li G, Purcell LD, Kim ST, Hsu FC, Stattin P, Hugosson J, Adolfsson J, Walsh PC, Trent JM, Duggan D, Carpten J, Gronberg H and Isaacs WB (2010): Inherited genetic variant predisposes to aggressive but not indolent prostate cancer. Proc.Natl.Acad.Sci.U.S.A. 107:2136-2140

Xu J, Zheng SL, Komiya A, Mychaleckyj JC, Isaacs SD, Hu JJ, Sterling D, Lange EM, Hawkins GA, Turner A, Ewing CM, Faith DA, Johnson JR, Suzuki H, Bujnovszky P, Wiley KE, DeMarzo AM, Bova GS, Chang B, Hall MC, McCullough DL, Partin AW, Kassabian VS, Carpten JD, Bailey-Wilson JE, Trent JM, Ohar J, Bleecker ER, Walsh PC, Isaacs WB and Meyers DA (2002): Germline mutations and sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. Nat.Genet. 32:321-325

Yoshizawa K, Willett WC, Morris SJ, Stampfer MJ, Spiegelman D, Rimm EB and Giovannucci E (1998): Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer. J.Natl.Cancer Inst. 90:1219-1224

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ORIGINAL INVESTIGATION

Segregation analysis of 1,546 prostate cancer families in Finland shows recessive inheritance

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Abstract Prostate cancer (PCa) is the most frequently diagnosed cancer in men worldwide and is likely to be caused by a number of genes with different modes of inheritance, population frequencies and penetrance. The objective of this study was to assess the familial aggregation of PCa in a sample of 1,546 nuclear families ascertained through an affected father and diagnosed during 1988–1993, from the unique, founder population-based resource of the Finnish Cancer Registry. Segregation analysis was performed for two cohorts of 557 early-onset and 989 late-onset families evaluating residual paternal effects and assuming that age at diagnosis followed a logistic distribution after log-transformation. The results did not support an autosomal dominant inheritance as has been reported in many of the hospital-based prostatectomy series. Instead, it confirmed the existence of hereditary PCa in the Finnish population under a complex model that included a major susceptibility locus with Mendelian recessive inheritance and a significant paternal regressive coefficient that is indicative of a polygenic/multifactorial component. The strengths of our study are the homogenous Finnish population, large epidemiological population-based data, histologically confirmed cancer diagnosis done before the PSA-era in Finland and registry based approach. Our results support the evidence that the inheritance of PCa is controlled by major genes and are in line with the previous linkage studies. Moreover, this is the first time a recessive inheritance is suggested to fit PCa in all data even when divided to early and late-onset cohorts.

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Introduction

Prostate cancer (PCa) is the most frequently diagnosed cancer among men in the western world (World Health Organization 2003). In Finland, where the incidence of PCa has been rising in the last decade, it is estimated that in the year 2006, there will be 5,485 newly diagnosed PCa cases with the age-adjusted incidence rate of 115.4/100,000 inhabitants (Finnish Cancer Registry 2006).

Familial clustering of PCa was observed as early as the 1950s (Gianferrari et al. 1956), and in about 10% of all cases there is a clear positive family history of the disease. Carter et al. (1992) reported that for 40–50% of PCa cases, familial clustering was associated with multiple affected relatives, especially in families of

early-onset probands. Hereditary prostate cancer (HPC), which accounts for 5–10% of all PCa, is an etiologically complex disease with several genes implicated in determining risk (Grönberg et al. 1997). In a large Nordic consortium study of twins, Lichtenstein et al. (2000) reported an unusually high heritability of 42% for PCa. The clinical phenotype of PCa is complex and heterogeneous, and the arrival of the prostate specific antigen (PSA) era has further complicated the genetic analysis of PCa by allowing the early diagnosis of disease that might remain latent or clinically unimportant. The International Consortium for Prostate Cancer Genetics (ICPCG), which seeks to improve the mapping of PCa genes, has emphasized that one of the major difficulties in studying PCa is genetic heterogeneity, possibly due to multiple, incompletely penetrant PCa-susceptibility genes (Xu et al. (2005). Using parametric (dominant and recessive) and nonparametric analyses on 1,233 families, Xu et al. (2005) identified five distinct chromosomal regions with ''suggestive'' linkage (LOD score > 1.86) to PCa, namely 5q12, 8p21, 15q11, 17q21, and 22q12. Subsets of the analyzed group of families characterized by large numbers of early-onset (≤65 years) PCa, which are more likely to segregate highly penetrant mutations, provided stronger evidence of linkage in several regions (including the 22q12 locus, with a LOD score of 3.57). Additional PCa susceptibility loci reported to date (Schaid 2004) also include the three cloned genes: *HPC1/RNASEL, HPC2/ELAC2* and *MSR1* (Rebbeck et al. 2000; Tavtigian et al. 2001; Carpten et al. 2002; Xu et al. 2002).

The Finnish population of 5 million inhabitants represents a genetically isolated population with a unique gene pool useful for the study of genetic susceptibility to cancer and other complex diseases (de la Chapelle 1993; Peltonen 1997). Reliable population data are obtainable from various linked registries and the population-based Finnish Cancer Registry (FCR) covers virtually all histologically confirmed cancer diagnoses over almost 50 years. In addition, church and parish records enable the identification of familial relationships for individuals over several centuries. In Finland, *HPC1/RNASEL, HPC2/ELAC2* and *MSR1* loci explain only a small fraction of PCa cases (Rökman et al. 2001, 2002; Seppälä et al. 2003). Instead, three additional major susceptibility loci have been mapped in Finnish families including the *HPCX* (Xq27–28), 3p25–26 and 11q14 regions (Xu et al. 1998; Schleutker et al. 2003). Even so, a large proportion of Finnish HPC remains unexplained.

The purpose of this study was to assess the nature of familial aggregation of PCa in a sample of 1,546 Finnish nuclear families using regressive models as employed in complex segregation analysis. Segregation analysis is a statistical method for testing compatibility with Mendelian expectations by estimating the parameters of a given model of inheritance from family data. Previous segregation analyses in diverse populations have suggested that familial aggregation of PCa follows autosomal dominance, multifactorial, recessive or X-linked inheritance, but remain inconclusive. Six reports suggest a dominant inheritance mode (Carter et al. 1992; Grönberg et al. 1997; Schaid et al. 1998; Verhage et al. 2001; Conlon et al. 2003; Valeri et al. 2003). Cui (2001) reported a mixture of models including autosomal dominant inheritance in younger onset families with recessive or X-linked inheritance in older-onset families. A multifactorial model has been suggested by Gong et al. (2002). Families of Icelandic breast cancer probands with PCa-affected men yielded a codominant model (Baffoe-Bonnie et al. 2002). To account for the possibility of different modes of inheritance in families of early-onset probands (<61 years) versus late-onset families (≥61 years), we performed segregation analyses on these two separate cohorts and also analyzed the complete, combined dataset to determine the most parsimonious model for explanation of the familial aggregation of the disease in Finland.

Subjects and methods

Data sources

The nation-wide population based Finnish Cancer Registry (FCR) was founded in 1952 and reporting of cancer to the FCR was made obligatory in 1961. Currently physicians, hospitals and pathology laboratories send their reports to the registry independently. In addition, the FCR receives information from every death certificate in which cancer is mentioned, registering over 99% of all solid tumors diagnosed in Finland (Teppo et al. 1994). The FCR files can be linked to the registry of deaths and of immigrants issued by the Population Register Center in Finland. Population registration in Finland has traditions dating back to the sixteenth century and is considered to be of excellent quality. Since 1964 a centralized, nation-wide, computer-based population registry has been maintained by the National Population Registry Center and is based on unique personal identifiers, which are now used as main keys in every major person registry including the Finnish Cancer Registry.

Probands and relatives

We chose the pre-PSA time period between 1 January 1988 and 31 December 1993 and identified 9,142 men with newly diagnosed PCa nationwide from the Finnish Cancer Registry. Two non-overlapping cohorts were identified with 557 early-onset probands (diagnosed at <61 years of age) and 989 late-onset probands (diagnosed at ≥61 years of age). The cut-off of 61 years was selected so that the cohort of early-onset PCa would be informative, i.e., has enough cases. Although all cut-offs are arbitrary, ours is in line with the one used by previous PSA screening trial in Finland (Mäkinen et al. 2002). Details of the collection of population-based PCa families and the analyses for other cancers among first-degree relatives have been published elsewhere (Matikainen et al. 2001). Briefly, information on the birthplaces of probands was obtained from the Central Population Registry. The local registries (parishes and local authorities) of the communities where probands were born were contacted to obtain the names and birth dates of their parents, siblings, spouses and children. Family members were successfully traced for 94% of the probands, giving a total of 10,650 first-degree relatives out of the 11,427 identified. Descriptive statistics of these two non-overlapping cohorts and after combining them are shown in Table 1.

Segregation analysis

To test specifically for Mendelian inheritance of PCa in these Finnish pedigrees, maximum likelihood segregation analyses were performed on the age at diagnosis expressed as a censored trait using the REGTL module of the Statistical Analysis for Genetic Epidemiology program (SAGE 3.1. 1997). Under model 1 of this program, employing class A regressive models (Bonney 1986), the ''type'' or ''ousiotype'' (Cannings et al. 1978) influences age at diagnosis of PCa through the location and scale parameters of the logistic distribution, but does not influence susceptibility. Specifically, some constant proportion (γ) of the male population is assumed to be at a risk of PCa. The PCa phenotype is defined as a dichotomous variable (Y), where $Y = 1$ if affected and $Y = 0$ if unaffected (censored). Parameters estimated in the analysis include: q_{A}, the frequency of the putative high-risk allele 'A', β_i baseline parameters, where i represents an individual's type (AA, AB, BB); α_i the age coefficients and γ_i the susceptibilities (Elston and George 1989). The logistic function describing the probability that an individual is affected by age ''a'' is given as $\gamma_i[1/(1 + e^{-\Phi}]$, where

$$\Phi = \beta_{\mathrm{i}} + \alpha_i(\mathrm{a}) + \delta_{\mathrm{F}}(Y_{\mathrm{F}}) \quad (1)$$

The coefficient δ_{F} reflects familial influence on risk corresponding to having an affected father.

Table 1 Descriptive statistics for prostate cancer cohorts in Finland

Description	Cohort-1	Cohort-2	Combined cohorts
Probands	557	989	1,546
Non-probands	3,631	7,019	10,650
Affected non-probands	51	109	160
Number of affected	608	1,098	1,706
Individuals in cohort	4,188	8,008	12,196
Number of males	2,418 (57.7%)	4,664 (58.2%)	7,082 (58.1%)
Number of females	1,770 (42.3%)	3,344 (46.9%)	5,114 (41.9%)
Number of fathers	390	673	1,063
Number of brothers	846	1,840	2,686
Number of sons	625	1,162	1,787
Number of mothers	388	681	1,069
Number of sisters	794	1,642	2,436
Number of daughters	588	1,021	1,609
Mean age of probands (years)	56.6 ± 3.4	74.4 ± 7.4	68.2 ± 10.7
Range of age at diagnosis of probands (years)	41.8 – 60.9	61 – 96	41.8 – 96
Mean age of affected non-probands (years)	69.6 ± 8.0	71.9 ± 7.8	71.2 ± 7.9
Range of age at diagnosis of affected non-probands (years)	48.3–85.5	48.1–88.7	48.1–88.7
Unaffected men aged ≥48 (years)	909 (18.3%)	2,141 (23.0%)	3,050 (21.4%)
Mean age of unaffected men (years)	65.2 ± 9.8	67.5 ± 12.3	66.6 ± 11.4
Range of ages of unaffected men (years)	48–85.5	48–104.3	48–104.3
Pedigree sizes (average, range)	8.9 (3–23)	9.4 (3–25)	9.2 (3–25)
Percent of pedigrees with ≥10 and ≥20 persons	28% (0.7%)	37.6% (0.5%)	34.2% (0.6%)

Positive values of δ_F mean that the individual with an affected father is more likely to have an earlier age at diagnosis, while negative values mean that the individual with an affected father is more likely to have a later age at diagnosis. Nonzero values of δ_F indicate the effects of polygenic and/or unmeasured shared-familial environmental risk factors on PCa risk.

Age at diagnosis for prostate cancer phenotype is assumed to follow a logistic distribution described by two parameters α and β, with the probability distribution function according to Elston and George (1989)

$$f(age) = [\alpha e^{\beta i+\alpha(age)}]/(1+e^{\beta i+\alpha(age)})^2 \quad (2)$$

This symmetric distribution is similar to a normal distribution and has a mean $-\beta/\alpha$, and variance, $\pi^2/3\alpha^2$, where π has a value of 3.1416. Based on the logistic distribution, the cumulative distribution function (CDF) is given by

$$F(age) = [\alpha e^{\beta i+\alpha(age)}]/[1+e^{\beta i+\alpha(age)}]. \quad (3)$$

The CDF represents the probability that a susceptible person will be affected by a given age. Age-specific penetrances were calculated for each genotype as

$$P(Y|\text{genotype } i,\ \text{age}) = [e^{\beta i+a(age)}]/[1+e^{\beta i+a(age)}]. \quad (4)$$

If the observed sex-specific ages at diagnosis do not follow a logistic distribution, this model may still be appropriate after transformation. A transformation equation equivalent to: aG1 × ln (age) was considered here, where aG1 is the geometric mean age at diagnosis for prostate cancer, computed from the observed ages at diagnosis among the 160 affected non-probands with PCa, 51 for the early-onset and 109 for the late-onset cohorts (Table 1).

Tests for genetic contribution to disease risk were implemented by postulating three types of individuals (AA, AB, BB) with three corresponding transmission parameters (τ_{AA}, τ_{AB}, τ_{BB}) describing the probability that a parent of a given type transmits the disease producing factor 'A' to his/her offspring (Elston and Stewart 1971; Elston and Yelverton 1975; Elston 1981). Under the hypothesis of genetic transmission, these τ parameters are constrained to the Mendelian values of $\tau_{AA} = 1.0$, $\tau_{AB} = 0.5$, $\tau_{BB} = 0.0$. Five sub-models of disease transmission were tested against a general model, where the transmission probabilities are estimated but with the restriction of homogeneity of trait distribution across generations to identify the best model for these data (Elston 1981). The ''no major gene'' model assumes that baseline risk is not influenced by ''type'' therefore all persons would come from a single distribution of age-specific risk for PCa. Single-locus Mendelian models assume that a major locus with two alleles should act in codominant, dominant or recessive fashion. The dominant and recessive models are special cases of the codominant model, where each genotype has a distinct age at diagnosis distribution. An environmental model with potentially distinct types of individuals was also tested, but here the transmission probability was held constant for all individuals.

We present results from the maximum likelihood segregation analyses performed on the log-transformed age at diagnosis of PCa expressed as a censored trait using the REGTL program (SAGE 3.1. 1997). Log-transformation of ages at diagnosis and ages at examination for all individuals with non-zero ages led to a final model that estimated genotypic baseline parameters (β_i) and age coefficient (α_i) and lifetime susceptibility for PCa for males along with the frequency (qA) of the high-risk allele A.

Hypothesis testing

The likelihood ratio test (LRT) was used to test each sub-model against the general model, and was computed as minus twice the natural log likelihood [–2ln(L)] of the general model subtracted from that for a restricted sub-model. This difference is asymptotically distributed as a χ^2 distribution with degrees of freedom equal to the difference in the number of independent parameters estimated in the two models.

Another method to compare models uses Akaike's information criteria (AIC), defined as: AIC = –2ln(L) + 2(number of parameters estimated). The most parsimonious model has the minimum AIC value (Akaike 1974). To correct for ascertainment bias, the likelihood of each pedigree was conditioned on the proband's affection status, using his age at diagnosis as recorded in the Cancer Registry (Cannings and Thompson 1977; Elston and Sobel 1979).

Results

Cohort-1 with 557 early-onset PCa families

As shown in Table 2, the no major gene model gave a very poor fit to the data in the early-onset PCa cohort and was thus rejected against the general unrestricted model in which all parameters were estimated based on the likelihood ratio test (LRT)

Table 2 Parameter estimates from segregation analysis of prostate cancer in 557 early-onset Finnish families ascertained through a single prostate cancer proband aged <61 years

Hypothesis	–2ln L	AIC	*df*	χ^2	*P*	Value of parameter									
						q_A	τ_{AA}	τ_{AB}	τ_{BB}	β_{AA}	β_{AB}	β_{BB}	α	γ	*F*(aff)
No major gene	479.02	487.02	6	18.36	0.005	[1.0]	–	–	–	–53.73 (5.31)	$=\beta_{AA}$	$=\beta_{AA}$	0.20 (0.014)	1.0 (0.0)	3.4 (0.25)
Dominant	473.08	485.08	4	12.42	0.014	0.9421 (0.597)	[1.0]	[0.5]	[0.0]	–62.27 (5.56)	$=\beta_{AA}$	–57.97 (4.32)	0.24 (0.019)	1.0 (0.0)	3.4 (0.24)
Codominant	474.18	488.18	3	13.52	0.036	0.0000007 (0.0)	[1.0]	[0.5]	[0.0]	–41.41 (3.67)	–63.40 (4.48)	–85.39 (7.01)	0.25 (0.017)	1.0 (0.0)	3.4 (0.33)
Recessive	462.20	474.20	4	1.54	0.8266	0.0536 (0.01)	[1.0]	[0.5]	[0.0]	–60.11 (4.11)	–64.63 (5.55)	$=\beta_{AB}$	0.25 (0.021)	1.0 (0.0)	3.7 (0.41)
Environmental	465.76	481.76	2	5.1	0.082	0.4183 (0.24)	1.0 (0.0)	$=\tau_{AA}$	$=\tau_{AA}$	–77.25 (7.01)	–79.69 (8.34)	–68.55 (6.98)	0.27 (0.036)	1.0 (0.0)	3.3 (0.31)
General	460.66	480.66	–	–	–	0.7328 (0.45)	1.0 (0.0)	0 (0.0)	0 (0.0)	–65.08 (6.93)	–67.98 (7.01)	–76.79 (7.86)	0.26 (0.019)	1.0 (0.0)	3.0 (0.30)

χ^2 is defined as (–2ln *L*) of the data under the hypothesis minus (–2ln *L*) of the data under the general model. Numbers in brackets are fixed at the indicated value; q_A the frequency of the putative high-risk allele, τ transmission parameter denoting the probability that a parent of a given type transmits the disease-producing factor A to his or her offspring, β baseline parameter, α age adjustment parameter, γ susceptibility parameter describing the cumulative probability of prostate cancer (assuming infinite lifespan)

(model 1 vs. model 6, $\chi^2 = 18.36$, $P < 0.005$ for 6*df*). The dominant ($P < 0.0014$), codominant ($P < 0.036$) and environmental ($P < 0.0082$) models were all rejected compared to the general model. The final general model reported is almost identical to the recessive Mendelian model (model 4 vs. model 6, $\chi^2 = 1.54$, $P < 0.83$ for 4*df*). The AIC, which takes into account the number of parameters estimated also, confirmed that the recessive model was the most parsimonious model. The estimated frequency ±SE (standard error) for the high-risk allele q_A was 0.054 (±0.01) for the recessive model. Figure 1 presents the predicted cumulative distribution function curves for log-transformed ages at diagnosis for the early-onset families under the recessive model. High-risk homozygous carriers of the putative risk allele AA have predicted age-specific cumulative probabilities greater than the heterozygous AB and BB non-carriers. The predicted mean age at diagnosis (i.e., 50% cumulative risk in Fig. 1) for the AA individuals is 60 and 64.6 years for the non-carriers. The susceptibility parameter γ was estimated at 1.0 for all male carriers of the risk allele, suggesting that 100% of the male population if they lived to infinity and did not die of competing causes, would express PCa if they were homozygous carriers of the allele A. Under this Mendelian recessive model, the cumulative probability that a male in Finland would be affected by PCa by age 70 was 0.92 for carriers and 0.79 for non-carriers, thus implying that if carriers and non-carriers did not die from competing causes, the estimated risk of being diagnosed with PCa at age 70 years for the homozygote carriers of the deleterious allele ($q = 0.054$), would be 2.7 per 1,000 among a hypothetical cohort of 100,000 men.

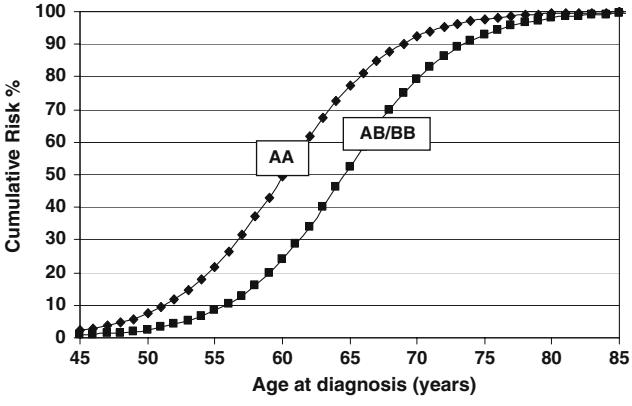

Fig. 1 Predicted cumulative risks for recessive *AA* (carriers) and *AB*/*BB* (non-carriers) with an affected father: 557 early-onset Finnish prostate cancer families

Cohort-2 with 989 late-onset PCa families

Table 3 shows the parameter estimates from the segregation analysis for the 989 families ascertained through late-onset probands diagnosed at ≥61 years of age. Compared to the unrestricted general model, the no major gene, the Mendelian dominant and environmental models did not fit the data and were rejected at $P < 0.001$. The Mendelian codominant model was also rejected by the LRT with a χ^2 of 12.64 and a P value of 0.006. The recessive model was the most parsimonious model according to the LRT (model 4 vs. model 6, $\chi^2 = 6.82$, $P < 0.15$ for $4df$), and it also had the lowest AIC = 1,331.10. Under this recessive model, inheritance of a putative high-risk allele A with an allele frequency (±SE) of 0.086 (±0.006) had predicted mean ages of onset of 65.6 years for men with the AA genotype and 72.2 years for AB/BB males, respectively. The lifetime risk of being diagnosed with PCa under this model was 5.0 per 1,000 among a hypothetical cohort of 100,000 men.

Figure 2 shows the predicted cumulative distribution function curves for log-transformed ages at diagnosis for this late-onset cohort in which the AA genotype has a distinctly different mean age at diagnosis of PCa.

Combined Cohort-1 and Cohort-2 with 1,546 PCa families

From Table 4, the combined cohorts with 1,546 probands gave parameter estimates very similar to those obtained from Cohort-2 with 989 probands. All other models except the Mendelian recessive model were significantly rejected when compared with the unrestricted general model. The recessive model was the most parsimonious model according to the LRT (model 4 vs. model 6, $\chi^2 = 8.78$, $P < 0.07$ for $4df$), and it also had the lowest AIC = 1,795.12. Under this recessive model, inheritance of a putative high-risk allele A with an allele frequency (±SE) of 0.0903 (±0.005) had predicted mean ages of onset of 63.6 years for men with the AA genotype and 71.0 years for AB/BB genotype males, respectively. Figure 3 shows that under the recessive model, the predicted cumulative risks for PCa are distinctly different for the AA compared to the AB/BB genotypes. The estimated mean age at diagnosis for the male homozygous carriers of the putative, high-risk allele A is 63.6 and it is 71.0 years for AB/BB genotype males. With a cumulative risk of 0.80 for homozygote carriers of the A allele at age 70 years, the estimated risk of being diagnosed with PCa in the absence of competing

Table 3 Parameter estimates from segregation analysis of prostate cancer in 989 late-onset Finnish families ascertained through a single prostate cancer proband

Hypothesis	−2ln L	AIC	*df*	χ^2	*P*	Value of parameter									
						q_A	τ_{AA}	τ_{AB}	τ_{BB}	β_{AA}	β_{AB}	β_{BB}	α	γ	*F*(aff)
No major gene	1371.08	1379.08	6	58.8	<0.001	[1]		[0.5]	[0]	−59.92 (4.16)	$=\beta_{AA}$	$=\beta_{AA}$	0.18 (0.013)	0.26 (0.06)	2.02 (0.18)
Dominant	1342.72	1354.72	4	30.44	<0.001	0.00148 (0.001)	[1]	[0.5]	[0]	−57.81 (4.53)	$=\beta_{AA}$	−62.53 (6.17)	0.18 (0.012)	1.0 (0.0)	3.18 (0.29)
Codominant	1324.92	1338.92	3	12.64	0.006	0.03137 (0.005)	[1]	[0.5]	[0]	−70.61 (6.88)	−75.26 (7.46)	−79.91 (8.01)	0.22 (0.016)	1.0 (0.0)	4.17 (0.51)
Recessive	1319.10	1331.10	4	6.82	0.15	0.0855 (0.006)	[1]	[0.5]	[0]	−64.71 (6.12)	−71.30 (6.95)	$=\beta_{AB}$	0.21 (0.015)	1.0 (0.0)	3.99 (0.63)
Environmental	1339.66	1355.66	2	27.38	<0.001	0.04486 (0.003)	0 (0.0)	$=\beta_{AA}$	$=\beta_{AA}$	−64.20 (5.01)	−62.32 (4.99)	−84.39 (8.15)	0.19 (0.012)	1.0 (0.0)	1.91 (0.09)
General	1312.28	1332.28	–	–	–	0.9974 (0.014)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	−66.93 (5.71)	−70.93 (6.34)	−72.21 (6.94)	0.21 (0.014)	0.44 (0.11)	2.85 (0.45)

χ^2 is defined as (−2ln L) of the data under the hypothesis minus (−2ln L) of the data under the general model. Numbers in brackets are fixed at the indicated value; q_A the frequency of the putative high-risk allele, τ transmission parameter denoting the probability that a parent of a given type transmits the disease-producing factor A to his or her offspring, β baseline parameter, α age adjustment parameter, γ susceptibility parameter describing the cumulative probability of prostate cancer (assuming infinite lifespan)

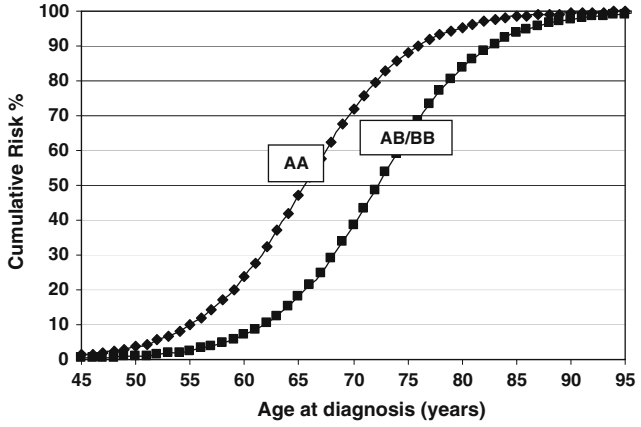

Fig. 2 Predicted cumulative risks for recessive *AA* (carriers) and *AB*/*BB* (non-carriers) of high-risk allele A with an affected father for 989 late-onset Finnish prostate cancer families

causes of death was 6.5 per 1,000 among a hypothetical cohort of 100,000 men.

All of these models (in each cohort and in the combined cohorts) included a residual paternal regressive coefficient, since inclusion of this coefficient significantly improved the fit of these models. The impact of genotype alone versus residual effect of having an affected father can be measured by computing the log odds of various combinations.

In effect, among men of the same age, born in the same cohort, and having the same affected father status, the log odds for being a homozygous carrier of the high risk allele A is computed as the difference between the genotypic baseline coefficients of the homozygous carriers and of the heterozygote and homozygous non-carriers. Using the parameters of the recessive model in the combined cohort as an example:

$$\begin{aligned}&(\beta_{\mathrm{AA}} + \alpha a' + \delta_F(\mathrm{YF})) - (\beta_{\mathrm{AB/BB}} + \alpha a' + \delta_F(Y_F))\\ &= \beta_{\mathrm{AA}} - \beta_{\mathrm{AB/BB}}\\ &= -63.34 - (-71.23) = 7.89.\end{aligned}$$

The odds of PCa in homozygous carriers of the A allele compared to the AB/BB non-carriers is 2,670, i.e., the exp (7.89). The log-odds due to an affected father between two individuals with the same genotype is 3.90 and the corresponding odds ratio is 49.40. The increase in log odds for a homozygote for the high-risk allele A with an affected father compared to the heterozygote carrier of the same age born in the same cohort but having an affected father would therefore be $(\beta_{\mathrm{AA}}-\beta_{\mathrm{AB/BB}}) + \delta_{\mathrm{F}}(Y_{\mathrm{F}})) = 7.89 + 3.90 = 11.79$ leading to high odds ratio.

Table 4 Parameter estimates from segregation analysis of prostate cancer in combined analysis of two cohorts of 1,546 Finnish families ascertained through a single prostate cancer proband

Hypothesis	−2ln L	AIC	*df*	χ^2	*P*	Value of parameter									
						q_A	τ_{AA}	τ_{AB}	τ_{BB}	β_{AA}	β_{AB}	β_{BB}	α	γ	F(aff)
1. No major gene	1884.38	1892.38	6	110.04	<0.001	[1]	[1]	[0.5]	[0]	−54.26 (5.21)	$=\beta_{AA}$	$=\beta_{AA}$	0.17 (0.014)	1.0 (0.0)	3.47 (0.35)
2. Dominant	1830.36	1842.36	4	56.02	<0.001	0.0022 (0.001)	[1]	[0.5]	[0]	−61.57 (5.98)	$=\beta_{AA}$	−65.77 (5.92)	0.21 (0.018)	1.0 (0.0)	3.50 (0.47)
3. Codominant	1806.42	1820.42	3	32.08	<0.001	0.1012 (0.007)	[1]	[0.5]	[0]	−70.16 (6.23)	−74.68 (7.26)	−86.40 (8.11)	0.23 (0.010)	1.0 (0.0)	3.20 (0.45)
4. Recessive	1783.12	1795.12	4	8.78	0.07	0.0903 (0.005)	[1]	[0.5]	[0]	−63.34 (6.01)	−71.23 (6.75)	$=\beta_{AB}$	0.22 (0.013)	1.0 (0.0)	3.90 (0.53)
5. Environmental	1866.34	1882.34	2	92.0	<0.001	0.4868 (0.061)	0 (0.0)	$=\beta_{AA}$	$=\beta_{AA}$	−55.74 (5.64)	−79.41 (8.04)	−56.10 (4.34)	0.18 (0.011)	1.0 (0.0)	3.32 (0.33)
6. General	1774.34	1794.34	–	–	–	0.9542 (0.004)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	−49.77 (4.7)	−53.49 (5.62)	−57.31 (5.89)	0.16 (0.014)	1.0 (0.0)	3.20 (0.29)

χ^2 is defined as (−2ln *L*) of the data under the hypothesis minus (−2ln *L*) of the data under the general model. Numbers in brackets are fixed at the indicated value; q_A the frequency of the putative high-risk allele, τ transmission parameter denoting the probability that a parent of a given type transmits the disease-producing factor A to his or her offspring, β baseline parameter, α age adjustment parameter, γ susceptibility parameter describing the cumulative probability of prostate cancer (assuming infinite lifespan)

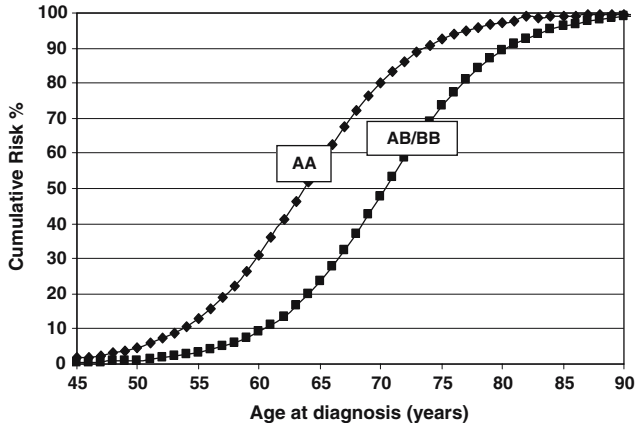

Fig. 3 Predicted cumulative risks for recessive *AA* (carriers) and *AB*/*BB* (non-carriers) of the putative high-risk allele A with an affected father for the 1,546 combined Finnish prostate cancer families

Discussion

In the present segregation analysis of our 1,546 population-based PCa families in Finland with ages at diagnosis of probands ranging between 41.8 and 96 years, a Mendelian recessive model with significant paternal regressive coefficient was shown to fit the homogeneous Finnish population best when the families were not separated into early- and late-onset cohorts. Recessive models with significant paternal regressive coefficients were also the most parsimonious models in both the 557 families of the early-onset cohort and in the 989 late-onset families. Under Hardy–Weinberg equilibrium, the estimated allele frequency of 0.09 for the combined cohort implies that 0.81% of the population in Finland would carry this rare putative high-risk allele. However, being a gender limited disease the susceptibility parameter γ of 1.0 obtained in the analysis suggested that 100% of the homozygous carrier male population at risk would develop PCa if they lived to infinity and did not die of competing causes.

Our results suggest that individuals carrying the risk allele get PCa at younger ages (<66 years) compared to non-carriers, whether they belong to the 557 early-onset or 989 late-onset groups or when the two cohorts are combined. Homozygous carriers of the risk allele (AA genotype) in the three groups above have a mean age at diagnosis of 60.1, 65.6 and 63.6 years, respectively, given that only about 6 years separate the ages at diagnosis of the homozygotes for the risk allele in the early-onset from the late-onset cohorts. Since the residual paternal effect was positive, those with affected fathers were at a higher risk for earlier onset PCa with polygenic and/or unmeasured shared-familial environmental risk factors compared to those with unaffected fathers. These findings are quite different from the previously reported evidence for the segregation of a rare autosomal dominant gene with high penetrance among different populations that included some series of prostatectomy patient families (Carter et al. 1992; Grönberg et al. 1997; Schaid et al. 1998; Verhage et al. 2001; Conlon et al. 2003; Valeri et al. 2003). Likewise a Swedish study by Grönberg et al. (1997) that was carried out on a population-based sample of 2,857 families selected through an affected father diagnosed with PCa in 1959–1963 and identified from the nationwide Cancer Registry, revealed that the observed clustering of PCa was best explained by a high risk allele inherited in a dominant mode, with a high population frequency (1.67%) and a moderate lifetime penetrance (63%). Similarly, the segregation analysis by Valeri et al. (2003) of families identified through 691 PCa patients recruited from three hospitals reported evidence for autosomal dominant gene inheritance of a high risk allele (frequency of 0.03%) with brother–brother dependence.

Our analyses suggest that there are likely to be multiple loci behind PCa and similar results have also been reported previously. A segregation study performed in Australia on 1,476 population-based pedigrees whose probands were diagnosed with PCa before the age of 70 in 1994–1997 suggested that a two-locus model fitted better than single-locus models and included a dominantly inherited risk that was greater at younger ages and a recessively inherited or X-linked increased risk which was greater at older ages (Cui et al. 2001). In a study of 1,719 first degree relatives in American and Canadian families by Gong et al. (2002), it was also observed that the good fit of the multifactorial model suggests that multiple genes, each having low penetrance, may be responsible for most inherited PCa susceptibility, and that the contribution of rare highly penetrant mutations might be small. In a segregation analysis of 389 Icelandic pedigrees that included both breast and prostate cancer, Baffoe-Bonnie et al. (2002), reported that the most parsimonious model was a Mendelian codominant model.

Previously, a recessive mode of inheritance has been reported in only a few studies. Cui et al. (2001) suggested that recessively inherited or X-linked inheritance increased risk at older ages, which was also seen in our linkage analyses of the HPCX locus (Xu et al. 1998; Schleutker et al. 2003). For an adult onset, sex-limited cancer such as PCa, recessive inheritance with incomplete penetrance and sporadic cases is consistent with X-linked PCa, which we previously mapped to the

Xq27–28 region using Finnish families characterized by ''no-male-to-male transmission'' (NMM). A follow-up linkage disequilibrium study utilizing familial/sporadic PCa cases and appropriate healthy controls identified an associated haplotype in the *HPCX* region (Baffoe-Bonnie et al. 2005). The results of this segregation analysis study are therefore consistent with the X-linked PCa transmission described previously (Schleutker et al. 2000). It has also been shown that there is a presence of residual brother–brother dependence (Valeri et al. 2003). In the study, by Narod et al. (1995) the prevalence of PCa was increased in those men with any first-degree relative affected. Most of the increase in relative risk was contributed by affected brothers, thus alluding to recessive or X-linked inheritance of the disease. In the study, by Monroe et al. (1995) an excess risk of PCa in men with affected brothers compared to those with affected fathers was also observed, consistent with the hypothesis of an X-linked, or recessive model of inheritance. Moreover, the prostate cancer risk was higher in probands' brothers than in probands' fathers in the Mayo Clinic Study (Schaid et al. 1998). These observations can be interpreted as evidence of recessive or X-linked effects in the risk of PCa.

Probands in the four American studies (Carter et al. 1992; Schaid et al. 1998; Verhage et al. 2001; Conlon et al. 2003) were part of a radical prostatectomy series for primary clinically localized PCa, and thus corresponded to a subgroup of patients not representative of all prostate cancer cases. This, as conceded by Schaid et al. (1998), could limit the power to assess heterogeneity of transmission across different age groups and represent a selection bias due to phenotypic characteristics. The particular strength of our study is the large population-based data composed of a homogenous Finnish population with registry-based approaches that provide unbiased information of malignancies in families. Prostate cancer was histologically confirmed in all cases in our families, and did not rely on PSA screening. The homogeneity of the Finnish population increases our chances of identifying loci, which may be less, pronounced in ethnically more diverse populations. Thus, linkage and association analyses of HPC conducted on Finnish families have found loci that are different from those reported in studies from other countries and populations (Schleutker et al. 2003; Seppälä et al. 2003a, b). Also, the present study was based on all prostate cancer cases in a population in a certain time window, not just known prostate cancer families, where genetic components may contribute to other cancer types and also be biased by specific family collection criteria.

The International Consortium for Prostate Cancer Genetics (ICPCG) recently announced that even though evidence of the existence of major PCa-susceptibility genes has been provided by multiple segregation analyses, genome-wide screens have not yielded conclusive chromosomal regions due to major difficulties that include genetic heterogeneity (Xu et al. 2005). The ICPCG employed parametric (dominant and recessive) and nonparametric analyses of 1,233 families world wide to identify several regions ''indicative'' of linkage. The main subsets of families likely to segregate highly penetrant mutations include families with large numbers of affected individuals or early age at diagnosis, leading to stronger evidence of linkage in several regions. Linkage and the association analysis of HPC conducted on Finnish families have found loci that are different from those reported in studies from other countries and populations (Schleutker et al. 2003; Seppälä et al. 2003a, b). We therefore believe that the results of these segregation analyses will be applied to defining a new model(s) for improving linkage analyses of the multiplex PCa families collected in Finland.

In conclusion, our findings suggest that the inheritance of PCa in the Finnish population is best explained by a Mendelian recessive model with a significant paternal regressive coefficient that is indicative of a polygenic multifactorial component. The rising incidence of PCa in Finland is possibly due to a combination of factors that include socio-cultural and lifestyle changes, environmental factors and the ongoing PSA screening (Finnish Cancer Registry, Mäkinen et al. 2003).

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References

Akaike H (1974) A new look at the statistical model identification. IEEE Trans 19:716–723

Baffoe-Bonnie AB, Kiemeney LA, Beaty TH, Bailey-Wilson JE, Schnell AH, Sigvaldsson H, Olafsdottir G, Tryggvadottir L,

Tulinius H (2002) Segregation analysis of 389 Icelandic pedigrees with breast and prostate cancer. Genet Epidemiol 23:349–363

Baffoe-Bonnie AB, Smith JR, Stephan DA, Schleutker J, Carpten JD, Kainu T, Gillanders EM, Matikainen M, Teslovich TM, Tammela T, Sood R, Balshem AM, Scarborough SD, Xu J, Isaacs WB, Trent JM, Kallioniemi OP, Bailey-Wilson E (2005). A major locus for hereditary prostate cancer in Finland: localization by linkage disequilibrium of a haplotype in the HPCX region. Hum Genet 117(4):307–316

Bonney GE (1986) Regressive logistic models for familial disease and other binary traits. Biometrics 42:611–625

Cannings C, Thompson EA (1977) Ascertainment in the sequential sampling of pedigrees. Clin Genet 12:208–212

Cannings C, Thompson E, Skolnick M (1978) Probability functions on complex pedigrees. Adv Appl Probl 10:26–26–61

Carpten J, Nupponen N, Isaacs S, Sood R, Robbins C, Xu J, Faruque M et al (2002) Germline mutations in the ribonuclease L gene in families showing linkage with HPC1. Nat Genet 30:181–184

Carter BS, Beaty TH, Steinberg GD, Childs B, Walsh PC (1992) Mendelian inheritance of familial prostate cancer. Proc Natl Acad Sci USA 89:3367–3371

Conlon EM, Goode EL, Gibbs M, Stanford JL, Badzioch M, Janer M, Kolb S, Hood L, Ostrander EA, Jarvik GP, Wijsman EM (2003) Oligogenic segregation analysis of hereditary prostate cancer pedigrees: Evidence for multiple loci affecting age at onset. Int J Cancer 105:630–635

Cui J, Staples MP, Hopper JL, English DR, McCredie MR, Giles GG (2001) Segregation analyses of 1,476 population-based Australian families affected by prostate cancer. Am J Hum Genet 68:1207–1218

De la Chapelle A (1993) Disease gene mapping in isolated human populations: the example of Finland. J Med Genet 30:857–865

Elston RC (1981) Segregation analysis. Adv Hum Genet 11:63–120, 372–373

Elston RC, George VT (1989) Age of onset, age at examination, and other covariates in the analysis of family data. Genet Epidemiol 6:217–220

Elston RC, Sobel E (1979) Sampling considerations in the gathering and analysis of pedigree data. Am J Hum Genet 31:62–69

Elston RC, Stewart J (1971) A general model for the genetic analysis of pedigree data. Hum Hered 21:523–542

Elston RC, Yelverton KC (1975) General models for segregation analysis. Am J Hum Genet 27:31–45

Finnish Cancer Registry (2006) Cancer incidence in Finland 1995 and 2004. Cancer statistics at http://www.cancerregistry.fi last updated on 7 June 2006

Gianferrari L, Arrigoni G, Cresseri A, Lovati G, Morganti G (1956) Genetic and clinico-statistical research on neoplasms of the prostate. Acta Gerontol Milano 5:224–233

Gong G, Oakley-Girvan I, Wu AH, Kolonel LN, John EM, West DW, Felberg A, Gallagher RP, Whittemore AS (2002) Segregation analysis of prostate cancer in 1,719 white, African–American and Asian–American families in the United States and Canada. Cancer Causes Control 13:471–482

Grönberg H, Damber L, Damber JE, Iselius L (1997) Segregation analysis of prostate cancer in Sweden: support for dominant inheritance. Am J Epidemiol 146:552–557

Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K (2000) Environmental and heritable factors in the causation of cancer-analyses of cohorts of twins from Sweden, Denmark, and Finland. N Engl J Med 343:78–85

Mäkinen T, Tammela TLJ, Stenman U-H, Määttänen L, Rannikko S, Aro J, Juusela H, Hakama M, Auvinen A (2002). Family history and prostate cancer screening with prostate-specific antigen. J Clin Oncol 20(11):2658–2663

Mäkinen T, Tammela TLJ, Hakama M, Stenman U-H, Rannikko S, Aro J, Juusela H, Määttänen L, Auvinen A (2003) Tumor characteristics in a population-based prostate cancer screening trial with prostate-specific antigen. Clin Cancer Res 9:2435–2439

Matikainen MP, Pukkala E, Schleutker J, Tammela TL, Koivisto P, Sankila R, Kallioniemi OP (2001) Relatives of prostate cancer patients have an increased risk of prostate and stomach cancers: a population-based, cancer registry study in Finland. Cancer Causes Control 12:223–230

Monroe KR, Yu MC, Kolonel LN, Coetzee GA, Wilkens LR, Ross RK, Henderson BE (1995) Evidence of an X-linked or recessive genetic component to prostate cancer risk. Nat Med 1:827–829

Narod SA, Dupont A, Cusan L, Diamond P, Gomez JL, Suburu R, Labrie F (1995) The impact of family history on early detection of prostate cancer. Nat Med 1:99–101

Peltonen L (1997) Molecular background of the Finnish disease heritage. Ann Med 29:553–556

Rebbeck TR, Walker AH, Zeigler-Johnson C, Weisburg S, Martin AM, Nathanson KL, Wein AJ, Malkowicz SB (2000) Association of HPC2/ELAC2 genotypes and prostate cancer. Am J Hum Genet 67:1014–1019

Rökman A, Ikonen T, Mononen N, Autio V, Matikainen M.P, Koivisto PA, Tammela TL, Kallioniemi OP, Schleutker J (2001) ELAC2/HPC2 involvement in hereditary and sporadic prostate caner. Cancer Res 61:6038–6041

Rökman A, Ikonen T, Seppälä EH, Nupponen N, Autio V, Mononen N, Bailey-Wilson J, Trent J, Carpten J, Matikainen MP, Koivisto PA, Tammela TL, Kallioniemi OP, Schleutker J (2002) Germline alterations of the RNASEL gene, a candidate HPC1 gene at 1q25, in patients and families with prostate cancer. Am J Hum Genet 70 (5):1299–1304

SAGE 3.1. (1997) Statistical analysis for genetic epidemiology, release 3.1. Computer program package available from the Department of Epidemiology and Biostatistics, Rammelcamp Center for Education and Research, MetroHealth campus, Case Western Reserve University, Cleveland

Schaid DJ (2004) The complex genetic epidemiology of prostate cancer. Hum Mol Genet 13 Spec no. 1, ppR103–R121

Schaid DJ, McDonnell SK, Blute ML, Thibodeau SN (1998) Evidence for autosomal dominant inheritance of prostate cancer. Am J Hum Genet 62:1425–1438

Schleutker J, Matikainen M, 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 OP (2000) A genetic epidemiological study of hereditary prostate cancer (HPC) in Finland: frequent HPCX linkage in families with late-onset disease. Clin Cancer Res 6(12):4810–4815

Schleutker J, Baffoe-Bonnie AB, Gillanders E, Kainu T, Jones MP, Freas-Lutz D, Markey C, Gildea D, Riedesel E, Albertus J, Gibbs KD Jr, Matikainen M, Koivisto PA, Tammela T, Bailey-Wilson JE, Trent JM, Kallioniemi OP (2003) Genome-wide scan for linkage in Finnish hereditary prostate cancer (HPC) families identifies novel susceptibility loci at 11q14 and 3p25–26. Prostate 57:280–289

Seppälä EH, Ikonen T, Autio V, Rökman A, Mononen N, Matikainen MP, Tammela TL, Schleutker J (2003a) Germ-

line alterations in MSR1 gene and prostate cancer risk. Clin Cancer Res 9:5252–5256

Seppälä EH, Ikonen T, Mononen N, Autio V, Rökman A, Matikainen MP, Tammela TL, Schleutker J (2003b) CHEK2 variants associate with hereditary prostate cancer. Br J Cancer 89:1966–1970

Smith JR, Freije D, Carpten JD, Gronberg H, Xu J, Isaacs SD, Brownstein MJ, Bova GS, Guo H, Bujnovszky P, Nusskern DR, Damber JE, Bergh A, Emanuelsson M, Kallioniemi OP, Walker-Daniels J, Bailey-Wilson JE, Beaty TH, Meyers DA, Walsh PC, Collins FS, Trent JM, Isaacs WB (1996) Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. Science 274:1371–1374

Tavtigian SV, Simard J, Teng DH, Abtin V, Baumgard M, Beck A, Camp NJ et al (2001) A candidate prostate cancer susceptibility gene at chromosome 17p. Nat Genet 27:172–180

Teppo L, Pukkala E, Lehtonen M (1994) Data quality and quality control of a population-based cancer registry, experience in Finland. Acta Oncol 33:365–369

Valeri A, Briollais L, Azzouzi R, Fournier G, Mangin P, Berthon P, Cussenot O, Demenais F (2003) Segregation analysis of prostate cancer in France: evidence for autosomal dominant inheritance and residual brother–brother dependence. Ann Hum Genet 67:125–137

Verhage BA, Baffoe-Bonnie AB, Baglietto L, Smith DS, Bailey-Wilson JE, Beaty TH, Catalona WJ, Kiemeney LA (2001) Autosomal dominant inheritance of prostate cancer: a confirmatory study. Urology 57:97–101

World Health Organization (2003) Global cancer rates could increase by 50% to 15 million by 2020 http://www.who.int/mediacentre/news/releases/2003/pr27/en/

Xu J, Meyers D, Freije D, Isaacs S, Wiley K, Nusskern D, Ewing C, Wilkens E, Bujnovszky P, Bova GS, Walsh P, Isaacs W, Schleutker J, Matikainen M, Tammela T, Visakorpi T, Kallioniemi OP, Berry R, Schaid D, French A, McDonnell S, Schroeder J, Blute M, Thibodeau S, Trent J (1998) Evidence for a prostate cancer susceptibility locus on the X chromosome. Nat Genet 20:175–179

Xu J, Zheng SL, Komiya A, Mychaleckyj JC, Isaacs SD, Hu JJ, Sterling D et al (2002) Germline mutations and sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. Nat Genet 32:321–325

Xu J, Dimitrov L, Chang BL, Adams TS, Turner AR, Meyers DA, Eeles RA et al (2005) A Combined genome-wide linkage scan of 1,233 families for prostate cancer-susceptibility genes conducted by the International Consortium for Prostate Cancer Genetics. Am J Hum Genet 77:219–229

Incidence of Cancer in Finnish Families with Clinically Aggressive and Nonaggressive Prostate Cancer

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Abstract

Background: **Clinical features of familial prostate cancer (PCa) and other malignancies associated with PCa are poorly described. Using a large family-based data registry of histologically confirmed cancers with a 40-year follow-up, we sought to determine incidence of cancer in Finnish PCa families, separately for clinically aggressive and clinically nonaggressive PCa.**

Methods: **We calculated standardized incidence ratios (SIR) for 5,523 members of 202 families by dividing the number of observed cancers (altogether 497 cases) by the number of expected cancers. The number of expected cancers is based on the national cancer incidence rates.**

Results: **SIR for overall cancer risk, excluding PCa, for male relatives in clinically nonaggressive families was 0.7 [95% confidence interval (95% CI), 0.6-0.8] and in clinically aggressive families 0.8 (95% CI, 0.6-1.0). The respective SIRs for women were 1.0 (95% CI, 0.8-1.1) and 1.1 (95% CI, 0.8-1.3). The incidence of lung cancer among men was significantly lower than in the general population. The SIR for gastric cancer among women was 1.9 in both clinically nonaggressive and clinically aggressive families. In clinically aggressive families, there was borderline significant excess of cancer of the gallbladder in men and liver cancer in women.**

Conclusions: **The incidence of non-PCa cancers is not increased in clinically aggressive or clinically nonaggressive PCa families except for stomach cancer among women.** (Cancer Epidemiol Biomarkers Prev 2009;18(11):3049–56)

Introduction

Prostate cancer (PCa) is the most frequently diagnosed malignancy among men in the Western world.[6] Largely due to the earlier diagnosis provided by the prostate-specific antigen (PSA) test, introduced in the 1990s, the incidence of PCa is no longer increasing. The highest incidence (115/100,000) of PCa in Finland was observed in 2005. In 2007, there were 4,188 newly diagnosed PCa cases in Finland with an age-adjusted incidence rate of 85.7/100,000.[7] Similar patterns of decline in PCa incidence have been observed in most Western industrialized countries (1).

The etiology of PCa has remained poorly understood. Ethnicity, age, and family history are considered major risk factors for PCa. The familial aggregation of PCa has been observed as early as the 1950s (2). Men with a positive family history of PCa have a 2- to 10-fold higher risk of getting PCa compared with those with no family history. The risk of PCa is highest in families with multiple PCa cases and in those with a low age of cancer diagnosis (3, 4).

Extensive efforts have been made to find genetic causes for PCa susceptibility. By linkage analysis, several chromosomal regions have been associated to PCa (5). Fine mapping of promising loci has identified three highly penetrant candidate genes *ELAC2, RNASEL*, and *MSRI* (6-8). However, mutations in these genes seem to be extremely rare, especially in the Finnish population (9-11). Several low penetrant polymorphisms have been found to be associated with PCa; however, recent studies suggest that the detected associations vary in different populations and ethnic groups (12-14), indicating genetic heterogeneity among people with PCa.

Based on the first reports on familial aggregation of PCa, hereditary PCa was considered to be site-specific (15). Hereditary PCa has been associated with a number of cancers, including gastric, breast, central nervous system, colon, multiple myeloma, gallbladder, non–Hodgkins lymphoma, skin melanoma, and kidney cancer (15-17). In population-based study associations between PCa and other cancers, such as gastric, colon, rectal, kidney, breast, ovarian, bladder, thyroid, and brain cancers, melanoma and non–Hodgkin lymphoma, have been reported, but the findings have been inconsistent between studies and only a few of these neoplasms have been reported even twice (4, 18-23). Studies of families with hereditary breast cancer, however, have reported that

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[6] http://www.who.int/

[7] http://www.cancerregistry.fi/

male carriers of *BRCA2* mutations are at increased risk for PCa (24-26).

Being a genetically heterogeneous disease, analysis of PCa families with incidences of similar cancer types could be highly informative in genetic linkage and association analysis toward the identification of PCa predisposition genes. The use of clinically defined phenotypes could simplify locus-heterogeneity problems that confound the search for PCa susceptibility genes (27, 28). Further, alternative phenotypes, such as tumor aggressiveness, may be a solution for overcoming the apparent heterogeneity that has hindered the identification of PCa genes. The odds of finding clinically relevant mutations should be hypotentially greater among clinically aggressive PCa families (29).

We have done the first complete epidemiologic evaluation of cancer risks in Finnish families with two or more cases of PCa across the generations studied. The Finnish genetic population structure is relatively homogenous due to a small founder population some 100 generations ago that has expanded into 5.3 million people today, with immigration contributing little to the genetic variation (30). Moreover, unbiased epidemiologic data on familial cancer clustering can be accessed through nation-wide population and cancer registries. The aim of this study was to assess whether primary non-PCa tumors are associated with Finnish families having two or more PCa cases.

Materials and Methods

Families. A detailed description of the Finnish PCa family data has been previously described (31). Since then, additional families from 1999 to 2008 have been found and integrated into the database using the same methods. In the present study, we used data from 202 families with two or more first-degree relatives with PCa. Genealogic information on family members was confirmed from records kept by the Finnish Population Register Centre, and medical information regarding cancer incidence was obtained from hospital records and the Finnish Cancer Registry. Known cancer syndromes were excluded by pedigree analysis. Previously, *BRCA1* and *BRCA2* Finnish founder mutations and multiple other genes, including *CDH1, CHEK2, MLH1, NBS1*, and *FH* have been screened and found to have only a minor role in predisposition to PCa (32-37).

The genealogic data of 202 families were collected through church and parish registries and the Finnish Population Register Centre. Complete pedigrees were constructed for each family. We traced back the parents of the index person, the earliest person being born at the end of 19th century and then traced all their descendants, through parish registries, until death or until further offspring were considered unlikely (female age, 55 y; male age, 70 y). The 844 persons (13%) who had died before the beginning of follow-up (1967) were excluded.

Statistical Methods. Because PCa is a common disease, it is likely that even when strict hereditary cancer family criteria, the so-called Carter criteria (3), is followed, a family with sporadic disease aggregation can become classified as a hereditary PCa family. To avoid this, we analyzed the clinical characteristics of the PCa cases in each of the families. All familial PCa cases were obtained from hospital records, including age of diagnosis, primary PSA value, WHO grade, Gleason score, and tumor-node-metastasis stage at diagnosis. In addition, the proportion of PCa cases in the family was calculated by dividing the number of PCa cases with the number of males in the family. Hierarchical cluster analysis by the agglomerative method and average distance was used to classify families with clinically aggressive disease into a separate cohort. Diagnostic age, primary PSA and the proportion of PCa cases in the family were standardized to equalize variables. As the incidences of PCa in these families were diagnosed between 1962 and 2006, Gleason grading was introduced in 1990s, Gleason grading was available for only 204 patients. In addition, because the criteria for Gleason grading have changed remarkably from 1990 to 2000, this variable was excluded from our analyses, relying instead on WHO grading.

The mean number of affected men with PCa was 2.9 per family (range, 2-8). The mean year of PCa diagnosis was 1992 (SD, 9.3; range 1962-2006), and the mean age of onset of PCa was 68 y (SD, 9.0; range, 43-98). The primary PSA median value was 16.0 [Quartile deviation (QD), 17; range, 0.8-11000]. The histopathologic WHO grade was 1 for 25%, 2 for 43%, and 3 for 9% (missing 23%).

Primary PSA values were available for 420 patients. To avoid the effect of outliers in hierarchical cluster analysis, the value of primary PSA was downgraded to a value of 101 for the analysis if it was initially measured to be over 100. We assumed that these extreme PSA values over 100 are uninformative. Cluster analysis was done for all variables collected (except Gleason score) and in different combinations. Primary PSA showed significant dependence on WHO grading, tumor size, and metastasis ($P_{\text{Kruskall-Wallis}} < 0.0005$ for all except WHO grading $P_{\text{Mann-Whitney}} < 0.0005$). Overall, the analyses showed that primary PSA alone was sufficient to well group the families compared with the use of all the variables or different combinations of the variables.

Using the collected clinical data, patients were sorted into one of two groups, group 1 (clinically nonaggressive PCa) or group 2 (clinically aggressive PCa). Fifty-nine of the 202 families were classified as a family with clinically aggressive PCa and 143 as nonaggressive PCa. The clinically aggressive PCa men had a primary PSA median value of 230 (QD, 280; range, 76-11,000) compared with 13.3 (QD, 16; range, 1.0-70) of the clinically nonaggressive men. When using the downgraded PSA values in clinically aggressive families the median value was 101 (QD, 101; range, 80-101) and 13.1 (QD, 16.2; range, 0.8-70) in clinically nonaggressive families, respectively. In addition, 71% of the clinically aggressive PCa men had metastases at the time of diagnosis as this was only 10% in clinically nonaggressive PCa men. Eighty-three percent of the clinically aggressive PCa men had extraprostatic tumor growth compared with only 34% in clinically nonaggressive PCa men. Consequently, with clinically aggressive disease, a histologically poorly differentiated and/or more advanced disease is meant showing in either a high tumor grade or stage (38, 39).

Follow-up analysis for cancer incidence among family members was done using medical information obtained by personal identity code–based automatic record linkage from the Finnish Cancer Registry. The follow-up period was from January 1, 1967 to December 31, 2004. However, for index patients, the follow-up started from their first PCa diagnosis. For the parents of the index patient, the

Table 1. Number of relatives of PCa patients in Finland with clinically nonaggressive and aggressive PCa, and person-years at follow-up during 1967 to 2004, by gender

Relative status	Males				Relative status	Females	
	Method 1		Method 2			Method 1 and 2*	
	No	P-years	No	P-years		No	P-years
CNA PCa families							
Index person	143	869	352	2,062			
Father	61	711	32	370	Mother	81	1,429
Brother	429	13,116	266	7,984	Sister	288	8,555
Son	1,185	39,195	1,135	37,956	Daughter	1,067	36,594
Spouse	184	4,054	184	4,054	Spouse	464	12,346
Total	2,002	57,945	1,969	52,426	Total	1,900	58,924
CA PCa families							
Index person	59	315	160	894			
Father	21	287	13	138	Mother	29	488
Brother	160	4,737	90	2,500	Sister	115	3,539
Son	525	19,510	500	18,610	Daughter	434	16,088
Spouse	79	1,908	79	1,908	Spouse	199	5,977
Total	844	26,757	842	24,050	Total	777	26,092

NOTE: The numbers for males are given separately for method 1 (single index PCa) and method 2 (multiple index PCa).
Abbreviations: P-years, person-years; CNA, clinically nonaggressive; CA, clinically aggressive.
*Both methods give the same number of family members who produce person-years at follow-up for females.

follow-up started from the birth of the index. For the other cohort members, the follow-up started from their date of birth, if this was later than January 1, 1967. If the person had emigrated or deceased before December 31, 2004, the calculation of person-years ended at that earlier date.

When calculating family data and trying to avoid the bias inherent in defining a PCa-family, the index person should be the first PCa male from any given family recruited into the study. However, due to the large amount of variability in this information or because the information was simply not available, we calculated familial SIRs in two ways. In the single-index data method (method 1), the follow-up to the date of PCa diagnosis was excluded for one PCa case, but all other family members were followed-up from the beginning. In the multiple index data method (method 2), all known PCa males were considered as index persons and follow-up data before PCa diagnosis was excluded from the analysis. The standardized incidence ratios (SIR) calculated by these two methods provide the lowest and highest possible estimate of the true relative risk for PCa and for overall cancer risk. The number of people analyzed using single index data were 5,523 (169,700 person-years) and 5,504 (161,500 person-years) in the multiple index data (Table 1).

Expected numbers of malignancies is based on person-years at risk, gender, age, and calendar-period of the specific incidence rates in the general population. SIRs were calculated by dividing the observed numbers of malignancies by the expected numbers. Exact 95% confidence

Table 2. Risk of cancer among men in PCa families separately with clinically nonaggressive and aggressive PCa

Cancer site	CNA PCa families				CA PCa families			
	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI
All sites	364	210.8	1.7	1.6-1.9	188	97.0	1.9	1.7-2.2
Prostate	247	44.7	5.5	4.9-6.3	126	21.7	5.8	4.8-6.9
Other	117	166.1	0.7	0.6-0.8	60	75.3	0.8	0.6-1.0
Lip	3	2.8	1.1	0.2-3.2	3	1.2	2.6	0.5-7.6
Stomach	7	13.5	0.5	0.2-1.1	3	5.7	0.5	0.1-1.5
Small intestine	0	1.1	0.0	0.0-3.4	2	0.3	5.9	0.7-21.2
Colon	6	11.7	0.5	0.19-1.1	3	3.0	1.0	0.2-2.9
Rectum	2	8.3	0.2	0.03-0.9	5	3.8	1.3	0.4-3.1
Liver	2	2.9	0.7	0.08-2.5	1	1.4	0.7	0.02-4.1
Gallbladder	1	1.6	0.6	0.02-4.4	3	0.7	4.1	0.8-11.8
Pancreas	9	7.4	1.2	0.6-2.3	1	3.4	0.3	0.01-1.6
Larynx	2	2.9	0.7	0.08-2.5	2	1.3	1.6	0.2-5.6
Lung	16	42.6	0.4	0.2-0.6	10	18.5	0.5	0.3-1.0
Skin melanoma	9	5.5	1.6	0.7-3.1	2	2.7	0.7	0.09-2.7
Kidney	7	7.9	0.9	0.4-1.8	6	3.6	1.7	0.6-3.6
Bladder	15	11.1	1.4	0.8-2.2	5	5.1	1.0	0.3-2.3
Nervous system	5	6.4	0.8	0.3-1.8	2	3.0	0.7	0.08-2.4
Hodgkin lymphoma	4	1.7	2.4	0.6-6.0	0	0.8	0.0	0.0-4.7
Non–Hodgkin lymphoma	3	7.2	0.4	0.09-1.2	3	3.6	0.9	0.2-2.5
Leukemia	2	5.5	0.4	0.04-1.3	3	2.5	1.2	0.2-3.5

NOTE: Observed and expected numbers of cases and SIRs with 95% CIs. Only the first index PCa cases excluded from the observation period (method 1); the other PCa cases counted as observed cases.
Abbreviations: Obs, observed; exp, expected.

Table 3. Risk of cancer among men in PCa families separately with clinically nonaggressive and aggressive PCa

Cancer site	CNA PCa families				CA PCa families			
	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI
All sites	151	179.7	0.8	0.7-1.0	83	79.3	1.1	0.8-1.3
Prostate	45	40.0	1.1	0.8-1.5	27	18.9	1.4	1.0-2.1
Other	106	149.7	0.7	0.6-0.9	56	60.4	0.9	0.7-1.2
Lip	2	2.3	0.9	0.1-3.2	2	0.8	2.4	0.3-8.7
Esophagus	1	2.3	0.4	0.01-2.4	0	1.0	0.0	0.0-3.7
Stomach	5	11.1	0.5	0.1-1.1	2	4.4	0.5	0.06-1.6
Small intestine	0	0.6	0.0	0.0-5.9	2	0.3	6.9	0.8-24.9
Colon	6	8.7	0.7	0.3-1.5	3	3.9	0.8	0.2-2.2
Rectum	2	7.0	0.3	0.03-1.0	4	3.1	1.3	0.4-3.3
Liver	3	2.4	1.2	0.3-3.6	1	1.1	0.9	0.02-5.0
Gallbladder	1	1.4	0.7	0.02-4.0	3	0.6	5.1	1.0-14.9
Pancreas	9	6.3	1.4	0.7-2.7	1	2.7	0.4	0.01-2.0
Larynx	1	2.3	0.4	0.01-2.4	2	0.9	2.1	0.3-7.7
Lung	16	35.1	0.5	0.4-1.1	9	13.3	0.7	0.3-1.3
Skin melanoma	7	4.8	1.5	0.6-3.0	2	2.3	0.9	0.1-3.1
Kidney	7	6.6	1.1	0.4-2.2	6	2.9	2.1	0.6-4.5
Bladder	14	8.6	1.6	0.9-2.7	4	5.0	0.8	0.2-2.1
Nervous system	5	5.5	0.9	0.3-2.1	2	2.6	0.8	0.09-2.8
Hodgkin lymphoma	4	1.5	2.7	0.7-6.8	0	0.7	0.0	0.0-5.4
Non–Hodgkin lymphoma	2	6.2	0.3	0.04-1.2	3	3.0	1.0	0.2-2.9
Leukemia	2	4.7	0.4	0.1-1.5	2	2.0	1.0	0.1-3.5

NOTE: Observed and expected numbers of cases and SIRs with 95% CIs. All index PCa cases excluded from the observation period (method 2).

intervals (CI) were defined, assuming that the numbers of the observed cases followed a Poisson distribution. The SIRs were calculated separately for clinically aggressive and clinically nonaggressive families using method 1 and 2, respectively, as described above.

Cancers belonging to hereditary cancer syndromes tend to have an earlier age of onset compared with sporadic cancers cases in a population; for example, the mean age of onset of breast cancer for *BRCA1* carriers is 35 y (40), so for breast cancer, we looked at the cancer risk for women under the age of 40 y.

Results

Clinically Nonaggressive PCa Families. There were 497 malignancies among 3,902 members of the clinically nonaggressive PCa families. Using analysis method 1, 364 malignancies were observed among males, 211 cases were expected, yielding a SIR of 1.7 (95% CI, 1.6-1.9; Tables 2 and 3). Using analysis method 2, the number of observed malignancies was 151, whereas 180 cases were expected, yielding a SIR of 0.8 (95% CI, 0.7-1.0). The difference in values is mainly due to the different numbers of PCa cases in analysis. Among females, there were 133 observed malignancies compared with 140 expected cases (SIR, 1.0; 95% CI, 0.8-1.1; Table 4). Increased risk for gastric cancer among women in clinically nonaggressive PCa families was statistically significantly (SIR, 1.9; 95% CI, 1.0-3.2; Table 4). Among men, there was less lung cancer than expected (for method 1: SIR, 0.4; 95% CI, 0.2-0.6; and for method 2: SIR, 0.5; 95% CI, 0.4-1.1; Tables 2 and 3).

Table 4. Risk of cancer among women in PCa families separately with clinically nonaggressive and aggressive PCa

Cancer site	CNA PCa families				CA PCa families			
	Obs	Exp	SIR	95% CI	Obs	Exp	SIR	95% CI
All sites	133	139.9	1.0	0.8-1.1	72	68.6	1.1	0.8-1.3
Esophagus	0	1.4	0.0	0.0-2.7	2	0.6	3.1	0.4-11.1
Stomach	13	6.9	1.9	1.0-3.2	6	3.2	1.9	0.7-4.0
Colon	8	8.1	1.0	0.4-2.0	2	3.9	0.5	0.06-1.9
Rectum	5	4.8	1.0	0.3-2.4	3	2.3	1.3	0.3-3.7
Liver	1	1.3	0.8	0.02-4.3	3	0.6	4.8	1.0-14.0
Gallbladder	2	2.4	0.9	0.1-3.0	3	1.1	2.7	0.6-7.9
Pancreas	6	4.8	1.2	0.5-2.7	2	2.3	0.9	0.1-3.1
Lung	6	5.5	1.1	0.4-2.4	1	2.8	0.4	0.01-2.0
Skin melanoma	3	3.9	0.8	0.2-2.2	4	2.0	2.0	0.6-5.2
Breast	42	40.8	1.0	0.7-1.4	22	21.0	1.1	0.7-1.6
Cervix uteri	3	3.4	0.9	0.2-2.6	1	1.5	0.7	0.02-3.6
Corpus uteri	7	8.4	0.8	0.3-1.7	2	4.1	0.5	0.06-1.7
Ovary	5	6.6	0.8	0.2-1.8	4	3.1	1.3	0.3-3.3
Kidney	3	3.8	0.8	0.2-2.3	3	1.8	1.6	0.3-4.8
Bladder	3	2.2	1.4	0.3-4.0	1	1.0	1.0	0.02-5.4
Nervous system	4	6.0	0.7	0.2-1.7	5	2.9	1.7	0.6-4.0
Thyroid gland	3	3.8	0.8	0.2-2.3	2	1.8	1.1	0.1-4.0
Non–Hodgkin lymphoma	6	4.6	1.3	0.5-2.9	0	2.3	0.0	0.0-1.6
Multiple myeloma	1	1.8	0.6	0.01-3.1	3	0.9	3.5	0.7-10.1
Leukemia	3	3.2	0.9	0.2-2.7	0	1.5	0.0	0.0-2.5

NOTE: Observed and expected numbers of cases and SIRs with 95% CIs.

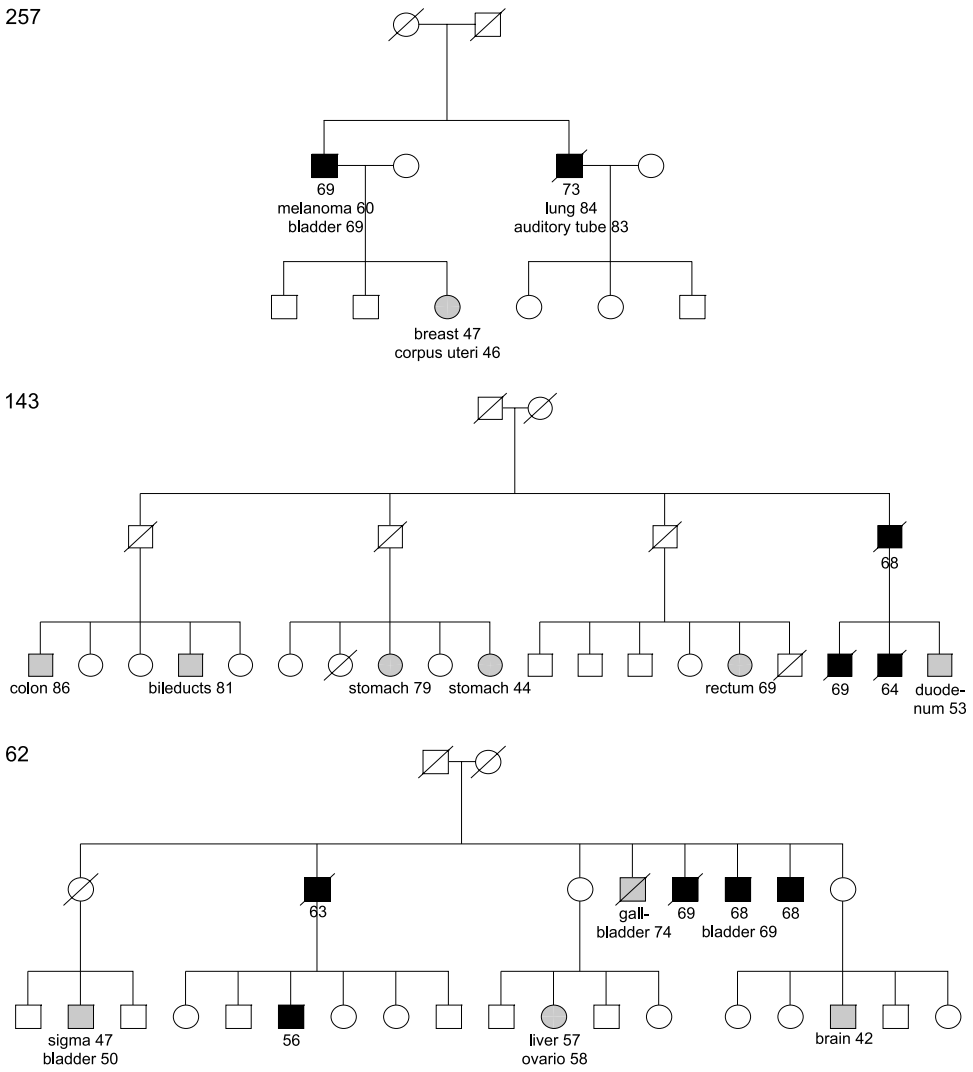

Figure 1. A pedigree of a PCa family with three individuals with multiple malignancies (family 257), with an aggregation of gastric cancer (family 143), and of a clinically aggressive PCa family with gallbladder and small intestine cancer (family 62). The age in years at the time of diagnosis is shown under the symbol for each relative. ■, PCa (male); ■, other cancer (male); ●, cancer (female).

Clinically Aggressive PCa Families. We analyzed 1,621 members of 59 clinically aggressive PCa families using analysis method 1. We observed 188 malignancies (52,849 person-years) among males, 97 malignancies were expected, yielding a SIR of 1.9 (95% CI, 1.7-2.2). Using analysis method 2 to analyze 1,619 members with 50,143 person-years, we observed 83 malignancies, 79 malignancies were expected, yielding a SIR of 1.1 (95% CI, 0.8-1.3). Among females, there were 72 observed cancer cases compared with 68.6 expected cases (SIR, 1.1; 95% CI, 0.8-1.3; Tables 1-4). Analyzing males and females separately, males had more small intestine (observed, 2; SIR, 5.9; 95% CI, 0.7-21.2; method 1: observed, 2: SIR, 6.9; 95% CI, 0.8-24.9) and gallbladder cancer (observed, 3; SIR, 4.1; 95% CI, 0.8-11.8; method 1: observed, 3; SIR, 5.1; 95%, CI 1.0-14.9), whereas females had more liver cancer (observed, 3; SIR, 4.8; 95% CI, 1.0-14.0) and multiple myeloma (observed, 3; SIR, 3.5; 95% CI, 0.7-10.1; Table 4). Women under 40 years had slightly increased risk of breast cancer (observed, 4; SIR, 3.1; 95% CI, 0.8-7.9) and ovarian cancer (observed, 3; SIR, 3.9; 95% CI, 0.8-11.4).

The 479 cancer cases observed during the follow-up were in 425 individuals. Sixty individuals had two malignancies, and six individuals had three. Forty-one had PCa as one of the cancers. Other common cancers found included urinary bladder (9), kidney (4), pancreas (4), gastric (3), and rectal (3). In Fig. 1, we present an example of a family (257) with individuals having multiple malignancies, including two men with three malignancies and a woman with two malignancies. Table 5 lists families with individuals having both PCa and gastric cancer, and Fig. 1 shows a selected sample of a family with gastric cancer (143). Family 62 is an example of a clinically aggressive family showing both gallbladder and small intestine cancers.

Table 5. Individual family data of detected cancers in the PCa families selected on the basis of PCa and stomach cancer aggregation

Family size*	No. of PCa cases	Mean age at PCa diagnosis	Sites of other cancers in the family (ages at diagnosis)
49	3	64.8	Stomach (61, 65, 72), salivary gland (67), pancreas (80), lung (58), breast (61)
31	2	71.7	Stomach (58), vulva (59), skin melanoma (53), leukemia (67),
24	3	74.3	Stomach (75), ovary (58), breast (70), leukemia (81)
22	2	69.7	Stomach (59), breast (56), lung (43), ovary (82), connective tissue (56)
18	2	76.4	Stomach (61), breast (55), multiple myeloma (40)
28	3	67.3	Stomach (44, 79), duodenum (53), colon (86), rectum (69), extra hepatica bile ducts (81)
51	4	67.5	Stomach (69, 76), lip (70), colon (61), lung (66), breast (54), corpus uteri (69), non–Hodgkin lymphoma (72)
17	3	72.9	Stomach (34), breast (53), thyroid gland (55)
19	2	73.1	Stomach (42), rectum (66), breast (53), corpus uteri (71), urinary bladder (52, 65), leukemia (84),
12	2	73.8	Stomach (81), colon (82), lung (69), breast (73), non–Hodgkin lymphoma (58)
20	4	76.5	Stomach (85), kidney (69)
23	3	73.2	Stomach (81), breast (60, 63), ovary (48), urinary bladder (75),
22	3	59.8	Stomach (72), lip (46), corpus uteri (30, 50), urinary bladder (71)
35	2	65.5	Stomach (46), skin melanoma (52)
21	4	73.8	Stomach (83)
52	2	68.7	Stomach (64), pancreas (69), lung (63), breast (51), corpus uteri (36), non–Hodgkin lymphoma (33), leukemia (12)
41	2	73.2	Stomach (66), skin melanoma (63)
15	2	74.0	Stomach (73), breast (53)
25	3	55.0	Stomach (61)

*Number of family members who produced person-years at follow-up.

Discussion

The incidence of non-PCa cancers other than women's gastric cancer was not higher than in the reference population. Out of the numerous associations tested in our study, only the risks of liver cancer in women and gallbladder cancer in men were increased in clinically aggressive PCa families. There was no statistically significant difference in risk of any cancer type studied between clinically aggressive and clinically nonaggressive families.

Unlike this study, to date, there are only a few studies on the overall cancer risk in PCa families where: (*a*) all cancers of the relatives have been identified, (*b*) family pedigrees have been genealogically confirmed, and (*c*) family material is population based and data are registry-based. In a study by Isaacs et al. (15), PCa was suggested to be relatively site specific and only central nervous system tumors were statistically increased. In 2000, Grönberg et al. (17) published a study on 62 hereditary PCa families, which found a significant aggregation of PCa together with breast and/or gastric cancer, suggesting a common germ line mutation in a cancer susceptibility gene. Valeri et al. (41) reported a significant increase in breast cancer risk. Conflicting results were published in 2005, in an American study on 1,238 Utah hereditary prostate cancer cases, supporting the existence of heritable PCa syndromes that included other malignancies. The Utah hereditary prostate cancer families had increased risk for colon, breast, rectum, gallbladder, and kidney cancers and also had increased risk to multiple myeloma, non–Hodgkin lymphoma, and melanoma (16). Of above-mentioned studies, only the study by Albright et al. (16) was done in a population-based manner. However, only 55% to 60% of the Utah Cancer Registry records can be linked to an individual in the Utah Population Data Base genealogy (16).

The different results in the studies done here can be explained in part by the difficulty in identifying hereditary prostate cancer pedigree populations and all malignancies among relatives. In addition, differences in cancer aggregation and clinical phenotype among the studied populations could reflect different genetic backgrounds and heterogeneity of germ line mutations among the different analyzed populations.

In accordance with findings of others, we observed an increase in gastric cancers among female members of the PCa families (42, 43). In a previous population-based Finnish study (18), an increased risk for gastric cancer was only seen among male relatives of early onset PCa patients, whereas in the present analysis, the risk for gastric cancer was increased among female family members. In the study by Grönberg et al. (17), a gender difference was seen in the SIR for gastric cancer; male gastric cancer SIR was 3.7 (1.9-6.2) and female was 1.4 (0.3-4.0). E-cadherin (*CDH1*) had been suggested as a potential gene explaining prostate and gastric cancer association (32), but subsequent studies did not confirm the hypothesis (43). Our findings on the association of familial PCa and gastric cancer support the need for further studies to find a candidate gene.

Our results are to certain extent congruent with the study by Albright et al. (16) showing an increased risk of gallbladder cancer, breast cancers, and multiple myeloma with familial PCa. We also found weak suggestions of increased incidence for liver and small intestine cancers that have not been published previously in any family data. The risk for liver cancer has been found to be elevated among the relatives of PCa patients in population-based settings but not in PCa families (16, 23).

Malignancies in many hereditary cancer syndromes often have an early age of onset and/or a clinically aggressive form of the disease compared with sporadic malignancies of the same cancer type. Due to the heterogeneity of PCa, subgrouping patients based on clinical characteristics will enhance detection of clinically relevant genes and mutations. In comparing clinicopathologic features and progression-free survival among sporadic and familial PCa cases, Roehl et al. (44) observed no clear

differences, but sibling pairs had a trend toward less favorable tumor features and progression-free survival.

In our analysis of clinically aggressive PCa families, females under the age of 40 years had (nonsignificantly) increased risk for breast cancer and ovarian cancer, supporting the epidemiologic findings of coaggregation of prostate and breast cancers (45, 46). Reported associations of PCa and breast cancer may be partially explained by the presence of recognized cancer predisposition genes; *BRCA2* has been implicated in PCa cancer predisposition in some populations (47-49). Significant elevations in PCa risk were found for *BRCA2* mutation carriers (24, 50) in the relatively homogenous Icelandic population. Likewise, in a report from the Breast Cancer Consortium, the risk for PCa in *BRCA2* mutation carriers was RR = 4.65 (51). Although it seems that known Finnish *BRCA1/2* founder mutations do not associate with PCa predisposition among Finnish PCa patients (33), an increased risk for PCa in Finnish breast cancer families carrying *BRCA2* mutations has been observed (52). Other negative findings for an association between *BRCA2* mutations and susceptibility to hereditary PCa in high-risk families have been reported (53). Most likely, mutations in *BRCA1/2* pathway genes can cause malignancies at multiple cancer sites (51). Likewise, several studies of cancer clusters within families have reported cooccurrence of PCa with breast, ovarian and endometrial cancers suggesting a single gene or a limited number of genes could be responsible for association with cancers in hormonal tissue (44, 54, 55).

From our data, male members of PCa families have significantly less lung cancer than men in the general population. One possible explanation is that to get PCa, you have to live to a relatively healthy life-style and old age (the average age of onset is 71 years in Finland). A study by Pukkala et al. (56) revealed that PCa is most common among the males in the highest social class. It is therefore possible that families with PCa are a selected group of people from higher social class with healthier life-style such as lower prevalence of smoking.

The strengths of this study are that it is based on a large family-based data registry (202 families) with total of 5,523 family members with confirmed genealogy within the homogenous Finnish population with a follow-up time of almost 40 years. All malignancies in all family members are verified from the Finnish Cancer Registry with unique linked personal identity codes. Cancer diagnoses were also confirmed from medical records. When calculating SIRs, we compared the observed cases of malignancies to the expected cases in the whole Finnish population and standardized these values with gender, 10-year calendar periods, and age. To our knowledge, this is the first study where clinical data of all PCa cases within families has been collected, enabling separation of families into two groups based on the clinical aggressiveness of the cancers.

In summary, members of the studied 202 Finnish PCa families had no general elevation in non-PCa malignancies. However, females in both clinically nonaggressive and clinically aggressive families had elevated incidence of stomach cancer, indicating that the risk of developing other cancers seems not to be related to the clinical characteristics of familial-PCa cases. The profile of related cancers and risk genes for hereditary PCa families may differ between populations; therefore, further studies of PCa in other populations are warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Kvale R, Auvinen A, Adami HO, et al. Interpreting trends in prostate cancer incidence and mortality in the five Nordic countries. J Natl Cancer Inst 2007;99:1881–7.
2. Morganti G, Gianferrari L, Cresseri A, Arrigoni G, Lovati G. Clinico-statistical and genetic research on neoplasms of the prostate. Acta Genet Stat Med 1956-1957;6:304–5.
3. Carter BS, Bova GS, Beaty TH, et al. Hereditary prostate cancer: epidemiologic and clinical features. J Urol 1993;150:797–802.
4. Goldgar DE, Easton DF, Cannon-Albright LA, Skolnick MH. Systematic population-based assessment of cancer risk in first-degree relatives of cancer probands. J Natl Cancer Inst 1994;2;86:1600–8.
5. Schaid DJ. The complex genetic epidemiology of prostate cancer. Hum Mol Genet 2004;13 Spec No 1:R103–21.
6. Tavtigian SV, Simard J, Teng DHF, et al. A candidate prostate cancer susceptibility gene at chromosome 17p. Nat Genet 2001;27:172–80.
7. Carpten J, Nupponen N, Isaacs S, et al. Germline muations in the ribonuklesase L gene in families showing linkage with HPC1. Nat Genet 2002;30:181–4.
8. Xu J, Zheng SL, Komiya A, et al. Germline mutations and sequence variants of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. Nat Genet 2002;32:321–5.
9. Rökman A, Ikonen T, Mononen N, et al. ELAC2/HPC2 involvement in hereditary and sporadic prostate cancer. Cancer Res 2001; 61:6038–41.
10. Rökman A, Ikonen T, Seppälä EH, et al. Germline alterations of the RNASEL gene, a candidate HPC1 gene at 1q25, in patients and families with prostate cancer. Am J Hum Genet 2002;70:1299–304.
11. Seppälä EH, Ikonen T, Autio V, et al. Germ-line alterations in MSR1 gene and prostate cancer risk. Clin Cancer Res 2003;9:5252–6.
12. Eeles RA, Kote-Jarai Z, Giles GG, et al. Multiple newly identified loci associated with prostate cancer susceptibility. Nat Genet 2008; 40:316–21.
13. Gudmundsson J, Sulem P, Rafnar T, et al. Common sequence variants on 2p15 and Xp11.22 confer susceptibility to prostate cancer. Nat Genet 2008;40:281–3.
14. Thomas G, Jacobs KB, Yeager M, et al. Multiple loci identified in a genome-wide association study of prostate cancer. Nat Genet 2008; 40:310–5.
15. Isaacs SD, Kiemeney LA, Baffoe-Bonnie A, Beaty TH, Walsh PC. Risk of cancer in relatives of prostate cancer probands. J Natl Cancer Inst 1995;87:991–6.
16. Albright LA, Schwab A, Camp NJ, Farnham JS, Thomas A. Population-based risk assessment for other cancers in relatives of hereditary prostate cancer (HPC) cases. Prostate 2005;64:347–55.
17. Grönberg H, Bergh A, Damber JE, Emanuelsson M. Cancer risk in families with hereditary prostate cancer. Cancer 2000;89:1315–21.
18. Matikainen MP, Pukkala E, Schleutker J, et al. Relatives of prostate cancer patients have an increased risk of prostate and stomach cancers: a population-based, cancer registry study in Finland. Cancer Causes Control 2001;12:223–30.
19. Eldon BJ, Jonsson E, Tomasson J, Tryggvadottir L, Tulinius H. Familial risk of prostate cancer in Iceland. BJU Int 2003;92:915–9.
20. Verhage BA, Aben KK, Witjes JA, Straatman H, Schalken JA, Kiemeney LA. Site-specific familial aggregation of prostate cancer. Int J Cancer 2004;109:611–7.
21. Negri E, Pelucchi C, Talamini R, et al. Family history of cancer and the risk of prostate cancer and benign prostatic hyperplasia. Int J Cancer 2005;114:648–52.
22. Amundadottir LT, Thorvaldsson S, Gudbjartsson DF, et al. Cancer as a complex phenotype: pattern of cancer distribution within and beyond the nuclear family. PLoS Med 2004;1:e65.
23. Hemminki K, Chen B. Familial association of prostate cancer with other cancers in the Swedish family-cancer database. Prostate 2005; 65:188–94.
24. Sigurdsson S, Thorlacius S, Tomasson J, et al. BRCA2 mutation in Icelandic prostate cancer patients. J Mol Med 1997;75:758–61.

25. Easton DF, Steele L, Fields P, et al. Cancer risks in two large breast cancer families linked to BRCA2 on chromosome 13q12-13. Am J Hum Genet 1997;61:120–8.
26. Lorenzo Bermejo J, Hemminki K. Risk of cancer at sites other than the breast in Swedish families eligible for BRCA1 or BRCA2 mutation testing. Ann Oncol 2004;15:1834–41.
27. Stanford JL, McDonnell SK, Friedrichsen DM, et al. Prostate cancer and genetic susceptibility: a genome scan incorporating disease aggressiveness. Prostate 2006;66:317–25.
28. Gillanders EM, Xu J, Chang BL, et al. Combined genome-wide scan for prostate cancer susceptibility genes. J Natl Cancer Inst 2004;96: 1240–7.
29. Christensen GB, Cannon-Albright LA, Thomas A, Camp NJ. Extracting disease risk profiles from expression data for linkage analysis: application to prostate cancer. BMC Proc 2007;1 Suppl 1:S82.
30. De la Chapelle A. Disease gene mapping in isolated human populations: the example of Finland. J Med Genet 1993;30:857–865.
31. Matikainen MP, Schleutker J, Morsky P, Kallioniemi OP, Tammela TL. Detection of subclinical cancers by prostate-specific antigen screening in asymptomatic men from high-risk prostate cancer families. Clin Cancer Res 1999;5:1275–9.
32. Ikonen T, Matikainen M, Mononen N, et al. Association of E-cadherin germ-line alterations with prostate cancer. Clin Cancer Res 2001;7: 3465–71.
33. Ikonen T, Matikainen MP, Syrjäkoski K, et al. BRCA1 and BRCA2 mutations have no major role in predisposition to prostate cancer in Finland. J Med Genet 2003;40:e98.
34. Seppälä EH, Ikonen T, Mononen N, et al. CHEK2 variants associate with hereditary prostate cancer. Br J Cancer 2003;89:1966–70.
35. Fredriksson H, Ikonen T, Autio V, et al. Identification of germline MLH1 alterations in familial prostate cancer. Eur J Cancer 2006;42:2802–6.
36. Hebbring SJ, Fredriksson H, White KA, et al. Role of the Nijmegen breakage syndrome 1 gene in familial and sporadic prostate cancer. Cancer Epidemiol Biomarkers Prev 2006;15:935–8.
37. Lehtonen R, Kiuru M, Rökman A, et al. No fumarate hydratase (FH) mutations in hereditary prostate cancer. J Med Genet 2003;40:e19.
38. Schaid DJ, McDonnell SK, Zarfas KE, et al. Pooled genome linkage scan of aggressive prostate cancer: results from the International Consortium for Prostate Cancer Genetics. 2006;120:471–85.
39. Christensen GB, Camp NJ, Farnham JM, Cannon-Albright LA. Genome-wide linkage analysis for aggressive prostate cancer in Utah high-risk pedigrees. The Prostate 2007;67:605–613.
40. Eerola H, Heikkilä P, Tamminen A, Aittomäki K, Blomqvist C, Nevalinna H. Relationship of patients' age to histopathological features of brest tumours in BRCA1 and BRCA2 and mutation-negative brest cancer families. Breast Cancer Res 2005;7:465–9.
41. Valerie A, Fournier G, Morin V, et al. Early onset and familial predisposition to prostate cancer significantly enhance the probability for breast cancer in first degree relatives. Int J Cancer 2000;86:883–7.
42. Hemminki K, Dong C. Familial prostate cancer from the family-cancer database. Eur J Cancer 2000;36:229–34.
43. Jonsson BA, Bergh A, Stattin P, Emmanuelsson M, Grönberg H. Germline mutations in E-cadherin do not explain association of hereditary prostate cancer, gastric cancer and breast cancer. Int J Cancer 2002;98: 838–43.
44. Roehl KA, Loeb S, Antenor JA, Corbin N, Catalona WJ. Characteristics of patients with familial versus sporadic prostate cancer. J Urol 2006; 176:2438–42.
45. Thiessen EU. Concerning a familial association between breast cancer and both prostatic and uterine malignancies. Cancer 1974;34:1102–7.
46. Tulinius H, Egilsson V, Olafsdottir GH, Sigvaldason H. Risk of prostate, ovarian, and endometrial cancer among relatives of women with breast cancer. BMJ 1992;305:855–7.
47. Baffoe-Bonnie AB, Kiemeney LA, Beaty TH, et al. Segregation analysis of 389 Icelandic pedigrees with breast and prostate cancer. Genet Epidemiol 2002;23:349–63.
48. Edwards SM, Kote-Jarai Z, Meitz J, et al. Two percent of men with early-onset prostate cancer harbor germline mutations in the BRCA2 gene. Am J Hum Genet 2003;72:1–12.
49. Arason A, Barkardottir RB, Egilsson V. Linkage analysis of chromosome 17q markers and breast-ovarian cancer in Icelandic families, and possible relationship to prostatic cancer. Am J Hum Genet 1993; 52:711–7.
50. Thorlacius S, Sigurdsson S, Bjarnadottir H, et al. Study of a single BRCA2 mutation with high carrier frequency in a small population. Am J Hum Genet 1997;60:1079–84.
51. The breast cancer linkage consortium. Cancer risks in BRCA2 mutation carriers. J Natl Cancer Inst 1999;91:1310–6.
52. Eerola H, Pukkala E, Pyrhonen S, Blomqvist C, Sankila R, Nevanlinna H. Risk of cancer in BRCA1 and BRCA2 mutation-positive and -negative breast cancer families (Finland). Cancer Causes Control 2001;12: 739–46.
53. Agalliu I, Kwon EM, Zadory D, et al. Germline mutations in the BRCA2 gene and susceptibility to hereditary prostate cancer. Clin Cancer Res 2007;13:839–43.
54. Sellers TA, Potter JD, Rich SS, et al. Familial clustering of breast and prostate cancers and risk of postmenopausal breast cancer. J Natl Cancer Inst 1994;86:1860–5.
55. Tung KH, Goodman MT, Wu AH, et al. Aggregation of ovarian cancer with breast, ovarian, colorectal, and prostate cancer in first-degree relatives. Am J Epidemiol 2004;159:750–8.
56. Pukkala E, Weiderpass E. Socio-economic differences in incidence rates of cancers of the male genital organs in Finland, 1971-95. Int J Cancer 2002;102:643–8.

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PALB2 variants in hereditary and unselected Finnish Prostate cancer cases

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Abstract

Background: *PALB2* 1592delT mutation is associated with increased breast cancer and suggestive prostate cancer (PRCA) risk in Finland. In this study we wanted to assess if any other *PALB2* variants associate to increased PRCA risk and clinically describe patients with formerly found *PALB2* 1592delT mutation.

Methods: Finnish families with two or more PRCA cases (n = 178) and unselected cases (n = 285) with complete clinical data were initially screened for variants in the coding region and splice sites of *PALB2*. Potentially interesting variants were verified in additional set of unselected cases (n = 463).

Results: From our clinically defined sample set we identified total of six variants in *PALB2*. No novel variants among Finnish PRCA cases were found. Clinical characteristics of the variant carriers, including the previously described family carrying *PALB2* 1592delT, revealed a trend towards aggressive disease, which also applied to a few non-familial cases. Hypersensitivity to mitomycin C (MMC) of lymphoblasts from individuals from the family with 1592delT revealed haploinsufficiency among carriers with altered genotype.

Conclusions: Though any of the detected *PALB2* variants do not associate to PRCA in population level in Finland it cannot be ruled out that some of these variants contribute to cancer susceptibility at individual level.

Introduction

Prostate cancer (PRCA) is the most frequently diagnosed cancer among men in the Western world [1]. The incidence of PRCA has been increasing throughout the last decade, partly due to the earlier diagnosis provided by the prostate specific antigen (PSA) test, introduced in the 1990s. In Finland, the highest incidence of 115/100 000 was observed in 2005. In 2007 there were 4188 newly-diagnosed PRCA cases with the age-adjusted incidence rate of 85.9/100,000 [2]. Similar high increase and now decline have been observed in most Western industrialized countries [3].

Extensive efforts have been made to reveal the genetics behind PRCA susceptibility [4]. Early linkage studies and more recent genome-wide association analyses have revealed multiple susceptibility loci for PRCA [5-7]. However, both causal variants and genes of the associated loci in are still not known and the identified PRCA risk variants only account for a fraction of the overall genetic variance for PRCA risk [8-10].

DNA repair pathway genes have an undisputed role in cancer progression and inherited mutations in them have been strongly associated with different cancers. *BRCA1* and *BRCA2* are both tumor suppressor genes involved in DNA repair and mutations in these genes predominantly predispose carriers to breast and ovarian cancers [11]. Potential links to PRCA have been studied for both of these genes and mutations in *BRCA2* lead to an increased risk for the disease in multiple studies [12,13]. In an Icelandic study, mutations in *BRCA2* (999del5, Icelandic founder mutation) are associated with a poorly differentiated, advanced type of PRCA [14]. These results are also consistent with results from a recent study in the UK, where a significantly higher Gleason score was observed among *BRCA2* mutation carriers than with non-carriers. These findings suggest that *BRCA2* is a high-risk PRCA susceptibility gene and mutational analysis could be used as a prognostic marker for aggressive PRCA [15]. However, Finnish *BRCA1/2* founder mutations do not associate with PRCA predisposition among Finnish PRCA patients [16]. This does not exclude the possibility of these genes to be involved in PRCA susceptibility but no studies have been published where the whole genes would have been sequenced in large data sets. Nevertheless an increased risk for PRCA in Finnish breast cancer families carrying *BRCA2* mutations has been observed [17]. Moreover, mutations of *CHEK2*, a gene acting in the same DNA repair route as *BRCA1/2*, have been shown to have a significant role in PRCA susceptibility in Finland [18].

PALB2 is a BRCA2 binding protein and the BRCA2-PALB2 interaction is essential for BRCA2-mediated DNA repair [19]. Recently it was shown that proper PALB2 function is necessary for the homologous recombination repair via interaction with BRCA1, revealing that PALB2 is actually a linker between BRCA1 and BRCA2 [20]. In the Finnish study by Erkko and colleagues a novel *PALB2* founder mutation (c.1592delT) was identified among Finnish breast cancer families (OR 11.3, CI 1.8-57.8) increasing the risk to breast cancer 4-fold [21]. In the same study the c.1592delT mutation was observed also from a patient with familial PRCA (1/164) but none of 475 unselected cases were carriers of the mutation. No statistically significant association with PRCA and c.1592delT was detected and no other variants were screened for in PRCA patients. Here, to investigate the role of possible other *PALB2* variants, we screened all *PALB2* exons(exons 1-13) in a cohort of 938 Finnish PRCA patients including both familial and unselected cases.

Material and methods

Patients and controls

A population-based cohort was collected from 1999 to 2005 from patients diagnosed with PRCA in the Pirkanmaa Hospital District that serves a population of a half a million inhabitants. The mean age at diagnosis for the 760 unselected patients was 62 (range 43-77), The median primary PSA and Gleason score were 8.9 and 6.4, respectively. The controls (n = 760) consisted of DNA samples from anonymous male blood donors obtained from the Finnish Red Cross.

A detailed description of the collection of the PRCA families has been previously described elsewhere [22]. In this study, we analysed 178 families (youngest affected male from each family) with two or more affected first-degree relatives. The average number of affected family members was three. Familial cancer data was attained from the Finnish Cancer Registry and detailed clinical information, including Gleason score, WHO grade, PSA at the time of diagnosis, TNM stage and primary treatment from hospital records.

To examine the association between the variants and the disease aggressiveness a subgroup of 380 with clinically aggressive disease was analyzed. Consequently clinically aggressive disease patients were selected according to Gleason score over seven and an age at diagnosis less than 61 years.

Mutation analysis of PALB2

All familial samples had been previously screened for the 11 Finnish *BRCA1* and seven *BRCA2* founder mutations [16]. DNA samples from each family (n = 178), 285 unselected cases and 470 control samples were initially used for direct sequencing of the entire coding region and splice sites of *PALB2*. Unselected cases for screening the whole gene were early onset cases with Gleason score over seven. Additional analyses were carried out on four variants that showed a trend for association (*PALB2* c.1592delT, 1674A>G, 2993G>A and 3300T>G). Unselected cases for additional analysis included 95 early onsets, aggressive cases and 368 non-aggressive cases with average age at diagnosis 67 years (range 63-77). Control samples in both primary and additional analysis were anonymous male blood donors from the Finnish Red Cross.

Mutation detection was performed through resequencing using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit with the ABI 3130xl sequencer

(Applied Biosystems). Primers and the naming of sequence variants were produced to correspond to the GenBank reference sequences for *PALB2* (NM_024675.3). Primer sequences are available on request. Variants were identified using Sequencher software 4.7 (Gene Codes Corporation, Ann Arbor, Mi). Loss of heterozygosity (LOH) analysis was carried out on two available tumor samples from the family with the segregating *PALB2* 1592delT. Selected sections of formalin fixed paraffin embedded tumors were obtained according to pathologist declaration. After tumor deparaffinization, DNA was extracted using a standard proteinase K protocol. Sequence from the tumor samples was compared to the patients' DNA sample from their peripheral blood.

DNA damage response in PALB2 deficient cell line

Peripheral blood leucocytes from two cancerous individuals carrying *PALB2* 1592delT mutation were immortalized with Epstein-Barr virus (EBV) and sensitized to DNA crosslinking agent mitomycin C (MMC). Cells were treated with 0 nM, 1 nM, 5 nM, 10 nM and 50 nM MMC for 96 h and their viability was assayed by CellTiter-Blue® Cell Viability Assay (Promega Corporation, Madison, WI, USA).

Statistical analyses

To validate significance of variants, the odds ratios and 95% confidence intervals were calculated and association analyses were made using the Fisher's exact test. Analyses were done using SPSS 12.0 statistical software. Pairwise linkage disequilibrium between three most frequently detected variants (1674A>G, 2993G>A and 3300T>G) in *PALB2* were analysed with Haploview 4.1. To define the haplotypes of cases familial and unselected cases were combined.

Results and Discussion

Mutation analysis

Prompted by the previous observation that the Finnish truncating founder mutation, *PALB2* c.1592delT, was identified segregating in one family with four PRCA cases [21], we wanted to study the contribution of other possible *PALB2* mutations. Probands from 178 Finnish HPC (hereditary prostate cancer) families and 285 unselected cases were screened for the entire coding region of *PALB2*. Total of six variants were detected in the coding regions and the exon-intron boundaries of *PALB2* (Table 1). All the variants have been previously described and only the truncating mutation (c.1592delT) has been shown to have functional consequences on DNA damage response. Association of four detected variants (c.1592delT, 1674A>G, 2993G>A and 3300T>G) were assessed in more detail in a larger set of unselected cases and controls. The truncating c.1592delT mutation was detected in two sporadic cases in addition to the previously described family 310 and in one control sample [21]. Interestingly, family 310 has now been found to have also stomach and skin cancer, in addition to earlier described breast cancer, as indicated in Figure 1 and Table 2. Three of the detected variants, 1674A>G in exon 4, 2993G>A in exon 9 and 3300T>G in exon 12, co-existed in six patients out of 178 familial samples (OR 1.52 CI 95% 0.6-3.9), 20/748 unselected cases (OR 1.20 CI 95% 0.6-2.3) and in 17/760 of the controls. To assess question whether these three variants have a stronger joint effect that would strengthen the trend seen with singletons a haplotype analysis was performed. The strongest linkage was between 1674A>G and 2993G>A (D'= 0.975; r^2 = 0.14, LOD 34.7) when 1886 case chromosomes and 1714 control chromosomes were analysed. Although these two variants appeared to have a frequency in PRCA cases that is higher than the Finnish population control frequency, their combined haplotype gave no improved results, i.e. none of the haplotype combinations showed significant association with the disease. Variant 2993G>A is now reported for the first time in the Finnish population since it did not come up in the study of Finnish breast cancer patients [21]. As reported by Rahman and colleagues [23] the 2993G>A variant is possibly

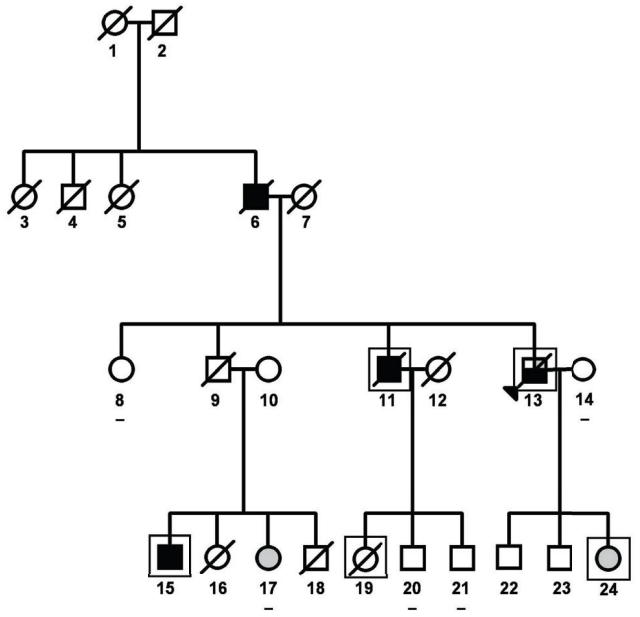

Figure 1
Family 310 segregating the *PALB* mutation. Minus-symbol signifies a person that has been screened for the *PALB2* 1592delT mutation, but found negative. Clinical information on this family is found in Table 2. Index person is marked with a black triangle, black square denotes persons with prostate cancer, grey circle indicates breast cancer cases and black square with white and grey corners signifies patient with prostate, stomach and skin cancers.

Table 1: Observed *PALB2* variants among the Finnish familial and unselected prostate cancer cases.

Exon/Intron	Nt change	Amino acid Change	Carrier Frequency (OR; 95% CI)		
			Familial	Unselected	Controls
PALB2					
Ex 4	1592delT	Leu531>Fs>Stop	1/178 (0.6%) (4.3; 0.3-68.8)	2/748 (0.3%) (2.0; 0.2-22.2)	1/760 (0.2%)
Ex 4	1674A>G	Gln559Agr	36/178 (20.2%) (1.2; 0.8-1.8)	153/748(20.5%) (1.2; 0.9-1.5)	134/760(18.0%)
Ex 8	2794G>A	Val932 Met	4/178 (2.2%) (1.0; 0.3-3.4)	7/285(2.5%) (1.2; 0.4-3.1)	10/470 (2.1%)
Ex 9	2993G>A	Gly998Glu	8/178 (4.5%) (1.6; 0.7-3.5)	35/748 (4.9%) (1.6; 0.9-2.8)	22/760 (2.9%)
Ex 12	3300T>G	Thr1100Thr	6/178 (3.4%) (0.8; 0.3-2.0)	30/748 (3.9%) (1.0: 0.6-1.7)	30/760 (3.9%)
5'UTR	G>A	-	8/178 (4.5%) (1.8; 0.7-4.4)	14/285 (4.9%) (2.0; 0.9-4.3)	12/470 (2.6%)

damaging to protein function, based on SIFT (sorting intolerant from tolerant) analysis.

We found that the 2993G>A variant exhibits a borderline significant odds ratios in a subgroup of 380 unselected cases with a Gleason score over seven and an age at diagnosis less than 61 years (20/380; OR 1.9; 95% CI 1.0-3.5). Edwards and colleagues [24] have presented similar age related observation with BRCA2 mutations in prostate cancer. They reported protein truncating mutations in BRCA2 to associate with PRCA diagnosed at or before age of 55. Our subgroup analysis also revealed that the variant 1674A>G has an OR of 1.4 (86/380; 95% CI 1.0-1.9) in a group of 368 patients with an average age at diagnosis of 67 years. However, the 1674A>G variant is commonly found in the Finnish population and well tolerated according to SIFT analysis, indicating no clear contribution to cancer formation. Similarly, the Val932 Met (2794G>A) change in exon 8 is well tolerated and has no effect on protein function [23].

In order to obtain information about loss of heterozygosity in the tumors from c.1592delT mutation positive patients, two available paraffin-embedded tumors were analysed. Repeated PCR-analysis did not reveal existence of LOH, which argues against the role as tumor suppressor gene. However the sample size is very small and therefore more tumor samples from *PALB2*-associated tumors needs to be characterized to be able to reliable assess PALB2 function in tumor formation. To date there is only one report indicating LOH in *PALB2* related tumors. Unfortunately also this study lacks a statistical power to make an appropriate conclusion about the role of PALB2 in tumorigenesis [25].

DNA damage response in PALB2 deficient cell line

Previously, MMC sensitivity test to c.1592delT mutation has been done in a reporter cell line [21]. To test whether the previously described *PALB2* c.1592delT mutation has functional consequences also in a heterozygous form, as present in all our patients, we predisposed two lymphoblast cell lines with c.1592delT and a wild type carrying control cell line to DNA cross linking agent MMC (Figure 2). c.1592delT mutation carriers were patients (15 and 24) from family 310 earlier described by Erkko et al. [21]. In both cell lines MMC-induced growth inhibition was observed suggesting PALB2 haploinsufficiency. It is also possible that dominant-negative effect is affecting in tumor formation in these patients.

Geographical and ethnical differences in the PRCA susceptibility alleles have been previously reported in the

Table 2: Clinical characteristics of family 310. The patient number refers to the number in the pedigree in Figure 1.

Patient	Cancer	Histology	Age at diagnosis	Reason for diagnosis	First treatment	Primary PSA value	WHO grading	Gleason grading	TNM grading	IHC
6	Prostate	Adenocarcinoma	83	NA	NA	NA	NA	NA	NA	
11	Prostate	Adenocarcinoma	68	Symptoms	Orchiectomy	NA	III	Na	T3NXMX	
13	Stomach	Adenocarcinoma	70	Symptoms	Gastrectomy	NA	NA	Na	T4N0 M0	
13	Skin	Carcinoma epidermoides	75	NA	Resection	NA	NA	Na	NA	
13	Prostate	Adenocarcinoma	76	Elevated PSA	Orchiectomy	47	III	2+3 = 5	T2NXMX	
15	Prostate	Adenocarcinoma	69	Elevated PSA	Brakytherapy	7.7	I	2+3 = 5	T1cNXMX	
17	Breast	Ductal carcinoma	52	Screening mammography	Mammary resection	NA	I	Na	T1N0 M0	ER+, PR+
24	Breast	Ductal carcinoma	49	Mammography	Mammary resection	NA	I	Na	T1N0 M0	NA

IHC = immunohistochemistry, NA = not available

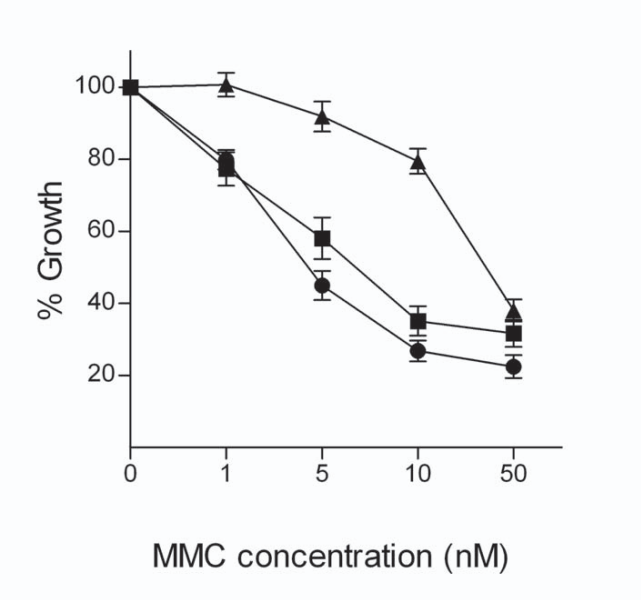

Figure 2
Mitomycin C growth inhibition in lymphoblastoid cell lines from the 310 family. Black circle and square depicts cell lines derived from affected individuals with *PALB2* 1592delT mutation. Black triangle signs for control sample from healthy individual.

CHEK2 gene that acts in the same pathway as *PALB2*, and therefore, variation in the *PALB2* mutation spectrum in different populations would not be surprising. In the present study and in the study by Erkko et al. [21], the number of mutations found in *PALB2* is relatively small among both Finnish breast and PRCA patients, with nine and six variants detected, respectively, compared to the British breast cancer family study by Rahman and colleagues [23] where a total of 50 variants and five frameshift mutations were reported. Recently, Tischkowitz and colleagues [26] reported a study of 95 US PRCA patients from Michigan, all diagnosed at < 55 years of age. Eleven variants in *PALB2* were found, but none of them were truncating and no association with the disease was observed. When comparing our patients to those very early-onset patients studied by Tischkowitz and colleagues [26] there is a clear difference in the mean age of onset between the two datasets. On the other hand, no variants were found in the 14 Ashkenazi-Jewish and 21 French-Canadian PRCA cases with family history of cancer [27]. In Finland, the smaller number of variation found likely reflects the known genetic homogeneity and founder effect of the population [28].

Our findings indicate that no other deleterious *PALB2* variants, except 1592delT mutation, contribute even marginally to PRCA risk in Finland. However, it remains possible that other genes from the BRCA1-PALB2-BRCA2 pathway have PRCA-predisposing alleles

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
SP gathered the clinical data and aggregated the family structures of PRCA families and drafted the manuscript. TW and SS genotyped the cases and the controls. TW did the statistical calculations, contributed to study design and coordination and revised the manuscript. MP performed the MMC sensitivity test. MM and TT are the clinical contributors. JS participated in study design, interpreted the results and critically revised the manuscript. All the authors have read and approved the final manuscript.

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References
1. Carter HB, Coffey DS: **The prostate: an increasing medical problem.** *Prostate* 1990, **16:**39-48.
2. **Finnish Cancer Registry** *Cancer incidence and mortality in Finland. Cancer Statistics* 2007 [http://www.cancerregistry.fi].
3. Kvale R, Auvinen A, Adami HO, Klint A, Hernes E, Moller B, Pukkala E, Storm HH, Tryggvadottir L, Tretli S, Wahlqvist R, Weiderpass E, Bray F: **Interpreting trends in prostate cancer incidence and mortality in the five Nordic countries.** *J Natl Cancer Inst* 2007, **99:**1881-1887.
4. Schaid DJ: **The complex genetic epidemiology of prostate cancer.** *Hum Mol Genet* 2004, **13(Spec No 1):**R103-21.
5. Tavtigian SV, Simard J, Teng DH, Abtin V, Baumgard M, Beck A, Camp NJ, Carillo AR, Chen Y, Dayananth P, Desrochers M, Dumont M, Farnham JM, Frank D, Frye C, Ghaffari S, Gupte JS, Hu R, Iliev D, Janecki T, Kort EN, Laity KE, Leavitt A, Leblanc G, McArthur-Morrison J, Pederson A, Penn B, Peterson KT, Reid JE, Richards S, Schroeder M, Smith R, Snyder SC, Swedlund B, Swensen J, Thomas A, Tranchant M, Woodland AM, Labrie F, Skolnick MH, Neuhausen S, Rommens J, Cannon-Albright LA: **A candidate prostate cancer susceptibility gene at chromosome 17p.** *Nat Genet* 2001, **27:**172-180.
6. Carpten J, Nupponen N, Isaacs S, Sood R, Robbins C, Xu J, Faruque M, Moses T, Ewing C, Gillanders E, Hu P, Bujnovszky P, Makalowska I, Baffoe-Bonnie A, Faith D, Smith J, Stephan D, Wiley K, Brownstein M, Gildea D, Kelly B, Jenkins R, Hostetter G, Matikainen M, Schleutker J, Klinger K, Connors T, Xiang Y, Wang Z, De Marzo A, Papadopoulos N, Kallioniemi OP, Burk R, Meyers D, Gronberg H, Meltzer P, Silverman R, Bailey-Wilson J, Walsh P, Isaacs W, Trent J: **Germline mutations in the ribonuclease L gene in families showing linkage with HPC1.** *Nat Genet* 2002, **30:**181-184.
7. Xu J, Zheng SL, Komiya A, Mychaleckyj JC, Isaacs SD, Hu JJ, Sterling D, Lange EM, Hawkins GA, Turner A, Ewing CM, Faith DA, Johnson JR, Suzuki H, Bujnovszky P, Wiley KE, DeMarzo AM, Bova GS, Chang B, Hall MC, McCullough DL, Partin AW, Kassabian VS, Carpten JD, Bailey-Wilson JE, Trent JM, Ohar J, Bleecker ER, Walsh PC, Isaacs WB, Meyers DA: **Germline mutations and sequence variants**

of the macrophage scavenger receptor 1 gene are associated with prostate cancer risk. *Nat Genet* 2002, **32:**321-325.
8. Rokman A, Ikonen T, Mononen N, Autio V, Matikainen MP, Koivisto PA, Tammela TL, Kallioniemi OP, Schleutker J: **ELAC2/HPC2 involvement in hereditary and sporadic prostate cancer.** *Cancer Res* 2001, **61:**6038-6041.
9. Rokman A, Ikonen T, Seppala EH, Nupponen N, Autio V, Mononen N, Bailey-Wilson J, Trent J, Carpten J, Matikainen MP, Koivisto PA, Tammela TL, Kallioniemi OP, Schleutker J: **Germline alterations of the RNASEL gene, a candidate HPC1 gene at 1q25, in patients and families with prostate cancer.** *Am J Hum Genet* 2002, **70:**1299-1304.
10. Seppala EH, Ikonen T, Autio V, Rokman A, Mononen N, Matikainen MP, Tammela TL, Schleutker J: **Germ-line alterations in MSR1 gene and prostate cancer risk.** *Clin Cancer Res* 2003, **9:**5252-5256.
11. King MC, Marks JH, Mandell JB, New York Breast Cancer Study Group: **Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2.** *Science* 2003, **302:**643-646.
12. Sigurdsson S, Thorlacius S, Tomasson J, Tryggvadottir L, Benediktsdottir K, Eyfjord JE, Jonsson E: **BRCA2 mutation in Icelandic prostate cancer patients.** *J Mol Med* 1997, **75:**758-761.
13. Friedenson B: **BRCA1 and BRCA2 pathways and the risk of cancers other than breast or ovarian.** *Med Gen Med* 2005, **7:**60.
14. Tryggvadottir L, Vidarsdottir L, Thorgeirsson T, Jonasson JG, Olafsdottir EJ, Olafsdottir GH, Rafnar T, Thorlacius S, Jonsson E, Eyfjord JE, Tulinius H: **Prostate cancer progression and survival in BRCA2 mutation carriers.** *J Natl Cancer Inst* 2007, **99:**929-935.
15. Mitra AV, Bancroft EK, Eeles RA, IMPACT Steering Committee Collaborators: **A review of targeted screening for prostate cancer: introducing the IMPACT study.** *BJU Int* 2007, **99:**1350-1355.
16. Ikonen T, Matikainen MP, Syrjakoski K, Mononen N, Koivisto PA, Rokman A, Seppala EH, Kallioniemi OP, Tammela TL, Schleutker J: **BRCA1 and BRCA2 mutations have no major role in predisposition to prostate cancer in Finland.** *J Med Genet* 2003, **40:**e98.
17. Eerola H, Pukkala E, Pyrhonen S, Blomqvist C, Sankila R, Nevanlinna H: **Risk of cancer in BRCA1 and BRCA2 mutation-positive and-negative breast cancer families (Finland).** *Cancer Causes Control* 2001, **12:**739-746.
18. Seppala EH, Ikonen T, Mononen N, Autio V, Rokman A, Matikainen MP, Tammela TL, Schleutker J: **CHEK2 variants associate with hereditary prostate cancer.** *Br J Cancer* 2003, **89:**1966-1970.
19. Xia B, Sheng Q, Nakanishi K, Ohashi A, Wu J, Christ N, Liu X, Jasin M, Couch FJ, Livingston DM: **Control of BRCA2 cellular and clinical functions by a nuclear partner, PALB2.** *Mol Cell* 2006, **22:**719-729.
20. Zhang F, Ma J, Wu J, Ye L, Cai H, Xia B, Yu X: **PALB2 links BRCA1 and BRCA2 in the DNA-damage response.** *Curr Biol* 2009, **19:**524-529.
21. Erkko H, Xia B, Nikkila J, Schleutker J, Syrjakoski K, Mannermaa A, Kallioniemi A, Pylkas K, Karppinen SM, Rapakko K, Miron A, Sheng Q, Li G, Mattila H, Bell DW, Haber DA, Grip M, Reiman M, Jukkola-Vuorinen A, Mustonen A, Kere J, Aaltonen LA, Kosma VM, Kataja V, Soini Y, Drapkin RI, Livingston DM, Winqvist R: **A recurrent mutation in PALB2 in Finnish cancer families.** *Nature* 2007, **446:**316-319.
22. Matikainen MP, Schleutker J, Morsky P, Kallioniemi OP, Tammela TL: **Detection of subclinical cancers by prostate-specific antigen screening in asymptomatic men from high-risk prostate cancer families.** *Clin Cancer Res* 1999, **5:**1275-1279.
23. Rahman N, Seal S, Thompson D, Kelly P, Renwick A, Elliott A, Reid S, Spanova K, Barfoot R, Chagtai T, Jayatilake H, McGuffog L, Hanks S, Evans DG, Eccles D, Breast Cancer Susceptibility Collaboration (UK), Easton DF, Stratton MR: **PALB2, which encodes a BRCA2-interacting protein, is a breast cancer susceptibility gene.** *Nat Genet* 2007, **39:**165-167.
24. Edwards SM, Kote-Jarai Z, Meitz J, Hamoudi R, Hope Q, Osin P, Jackson R, Southgate C, Singh R, Falconer A, Dearnaley DP, Ardern-Jones A, Murkin A, Dowe A, Kelly J, Williams S, Oram R, Stevens M, Teare DM, Ponder BA, Gayther SA, Easton DF, Eeles RA, Cancer Research UK/Bristish Prostate Group UK Familial Prostate Cancer Study Collaborators British Association of Urological Surgeons Section of Oncology: **Two percent of men with early-onset prostate cancer harbor germline mutations in the BRCA2 gene.** *Am J Hum Genet* 2003, **72:**1-12.
25. García MJ, Fernández V, Osorio A, Barroso A, Llort G, Lázaro C, Blanco I, Caldés T, de la Hoya M, Ramón Y, Cajal T, Alonso C, Tejada MI, San Román C, Robles-Díaz L, Urioste M, Benítez J: **Analysis of FANCB and FANCN/PALB2 fanconi anemia genes in BRCA1/2-negative Spanish breast cancer families.** *Breast Cancer Res Treat* 2009, **113:**545-51.
26. Tischkowitz M, Sabbaghian N, Ray AM, Lange EM, Foulkes WD, Cooney KA: **Analysis of the gene coding for the BRCA2-Interacting protein PALB2 in hereditary prostate cancer.** *Prostate* 2008, **68:**675-678.
27. Tischkowitz M, Xia B, Sabbaghian N, Reis-Filho JS, Hamel N, Li G, van Beers EH, Li L, Khalil T, Quenneville LA, Omeroglu A, Poll A, Lepage P, Wong N, Nederlof PM, Ashworth A, Tonin PN, Narod SA, Livingston DM, Foulkes WD: **Analysis of PALB2/FANCN-associated breast cancer families.** *PNAS* 2007, **104:**6788-6793.
28. de la Chapelle A: **Disease gene mapping in isolated human populations: the example of Finland.** *J Med Genet* 1993, **30:**857-865.