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Genetic Epidemiology of Prostate Cancer

by

Fredrik Wiklund

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To Lena and Isabella

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Abstract

Prostate cancer is a major health burden throughout the world being the most common cancer among men in most developed countries, yet the etiology of prostate cancer is poorly understood. Evidence has accumulated supporting the existence of a hereditary form of prostate cancer. In linkage analyses several chromosomal regions have been suggested and fine mapping of promising loci have identified three candidate genes. However, conflicting reports of the effects of these genes, and failure to replicate suggested regions indicates a much more complex genetic basis of prostate cancer than first anticipated. Improved understanding of the genetic mechanisms underlying the development and progression of prostate cancer would be a major advance for improved prevention, detection and treatment strategies. This thesis evaluates different aspects of the genetic epidemiology of prostate cancer.

A genomic scan in Swedish hereditary prostate cancer (HPC) families localized two chromosomal regions with suggestive evidence for linkage. The strongest support was found on chromosome 19p with an allele sharing LOD score of 2.91 (genome-wide P value = 0.032). The second region showing suggestive evidence of linkage was observed in the centromeric region of chromosome 5. Linkage analyses of densely spaced markers on chromosome 8p22-23 confirmed (P = 0.03) previously reported linkage to this region. A systematic evaluation of the possible impact that the RNASEL gene have on prostate cancer was performed. No support for a role of the rare truncating mutation E265X was found, and analyses of common sequence variants provided limited evidence for association with prostate cancer risk. Considering the large and well characterized study population and the high quality in genotyping (0.3% error rate) these results provide strong evidence against a role of RNASEL in prostate cancer etiology in Sweden. In a comprehensive evaluation of occurrence of other malignancies in HPC families, previously reported association between gastric and prostate carcinoma was confirmed. The increased risk was of the same magnitude in early and late onset HPC families and confined to only male relatives. A genome-wide linkage analysis, stratified by occurrence of gastric carcinoma, identified a novel susceptibility locus on chromosome Xp21.

In summary, chromosome 5q and 19p represents the regions most likely to harbor susceptibility genes predisposing to prostate cancer in the Swedish population. A common genetic basis for both gastric and prostate cancer has been confirmed and a novel susceptibility locus on chromosome Xp21 has been identified. Our results provide further support for the assumption that genetics play a critical role in prostate cancer susceptibility. A growing body of evidence advocates the existence of multiple genes with small to moderate effect in the development of this disease. Future genetic epidemiological efforts utilizing efficient strategies to decrease the complexity of prostate cancer genetics will likely succeed in the unraveling of the genetic influences on this common disease.

Key words: Prostate cancer, epidemiology, genetics, linkage analysis, genome-wide scan

Original papers

I. Wiklund F, Gillanders EM, Albertus JA, Bergh A, Damber JE, Emanuelsson M, Freas-Lutz DL, Gildea DE, Göransson I, Jones MS, Jonsson BA, Lindmark F, Markey CJ, Riedesel EL, Stenman E, Trent JM, Grönberg H**.** Genome-wide scan of Swedish families with hereditary prostate cancer: suggestive evidence of linkage at 5q11.2 and 19p13.3. Prostate. 2003 Dec 1;57(4):290-7.

II. Wiklund F, Jonsson BA, Göransson I, Bergh A, Grönberg H. Linkage analysis of prostate cancer susceptibility: confirmation of linkage at 8p22-23. Hum Genet. 2003 Apr;112(4):414-8.

III. Wiklund F, Jonsson BA, Brookes AJ, Strömqvist L, Adolfsson J, Emanuelsson M, Adami HO, Bälter K, Grönberg H. Genetic analysis of the RNASEL gene in hereditary, familial and sporadic prostate cancer. Submitted.

IV. Wiklund F, Emanuelsson M, Stenman E, Gillanders EM, Trent JM, Grönberg H. Comprehensive evaluation of malignancies associated with hereditary prostate cancer: confirmation of a common genetic basis for prostate and gastric cancer and suggestive linkage to Xp21. Submitted.

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Abrevations

AR	Androgen receptor gene
BCLC	Breast Cancer Linkage Consortium
BRCA1	Breast cancer gene 1
BRCA2	Breast cancer gene 2
CAPS	Cancer Prostate in Sweden
CI	Confidence interval
cM	CentiMorgan
CNS	Central nervous system
DASH	Dynamic allele-specific hybridization
DHT	Dihydrotestosterone
DZ	Dizygotic
FDR	First-degree relative
FPC	Familial prostate cancer
FRR	Familial relative risk
HLOD	Heterogeneity LOD
HPC	Hereditary prostate cancer
htSNP	Haplotype tagging SNP
HWE	Hardy Weinberg equilibrium
IBD	Identical by descent
ICPCG	International Consortium for Prostate Cancer Genetics
IGF-I	Insulin growth factor-I
LD	Linkage disequilibrium
MSR1	Macrophage scavenger receptor 1
MTM	Male-to-male
MZ	Monozygotic
NPL	Non-parametric linkage
OR	Odds ratio
PSA	Prostate-specific antigen
RNASEL	Ribonuclease L gene
RR	Relative risk
SIR	Standardized incidence ratio
SNP	Single nucleotide polymorphism
SPC	Sporadic prostate cancer
SRD5A2	5α-reductase type II gene

Introduction

Prostate cancer is a major health burden throughout the world being the most common cancer among men in most developed countries. In year 2000, over 500,000 new cases of prostate cancer and 240,000 deaths resulting from this disease was estimated worldwide[1]. Despite the common occurrence, little is known about the etiology of prostate cancer. Older age, African ancestry and a positive family history for prostate cancer have been recognized as important risk factors, yet the unraveling of the complex genetic and environmental influences on this disease is only at an early stage.

Over the past two decades, evidence has accumulated supporting the existence of a hereditary form of prostate cancer. However, despite the strong evidence for genetic susceptibility to this disease no predisposing genes have yet been identified and a much more complex genetic basis than first anticipated has been recognized. Improved understanding of the genetic mechanisms underlying the development and progression of prostate cancer would be a major advance for improved prevention, detection and treatment strategies. This thesis evaluates different aspects of the genetic epidemiology of prostate cancer.

Incidence

A substantial variation in prostate cancer incidence is observed between different countries. The highest incidence rates are observed in North America, Scandinavia and Australia (48-137 cases per 100,000 person-years during 1992-1998), European countries have intermediate rates (24-31 cases per 100,000 person-years), and Asian countries the lowest rates (2-10 cases per 100,000 person-years) [1]. There are also substantial ethnic differences in incidence of prostate cancer within countries, as an example, in the US, the African American population shows a two-fold increased risk compared to the white population. Interpretation of this variation in prostate cancer incidence must be done with caution since many factors are likely to contribute. Differences in access to medical care, screening methods, diet, and environmental risk factors are all plausible explanations for the observed variation of prostate cancer occurrence between countries and populations. However, there is consistent evidence from epidemiological studies across different ethnical populations that a positive family history for prostate cancer increase the risk for a man to get prostate cancer. In Sweden, over 7,800 new diagnoses of prostate cancer was recorded in year 2002 and the life-time probability (up to age 85 years) of developing prostate cancer was 20% (1 in 5). A significant increase in incidence has been observed in Sweden during the last decades. In 1970, 3000 new diagnosis was recorded and the average annual increase of the age-adjusted incidence was 3.2% during 1993-2002[2]. A considerable part of this increase is probably explained by a more frequent use of diagnostic tools, including prostate-specific antigen (PSA) test, since the increase has mainly been confined to non-fatal low-grade tumors [3]. In Sweden, no organized PSA screening exists and approximately 18% of all prostate cancer cases are PSA detected[4]. In contrast, in the US the advent of widespread PSA testing has led to a shift in disease stage towards early-stage and less aggressive cancers[5].

Risk factors

The observation that prostate cancer risk increases for migrant populations which move from low-incidence to high-incidence regions [6-9] suggests that environmental and lifestyle (e.g. diet) factors play an important role in prostate cancer development.

Diet

Total fat intake has been associated with increased risk for prostate cancer in both case-control and cohort studies (see Kolonel et al. [10] for a review). Although many studies have failed to show this association, results from ecological studies support an association between fat intake and prostate cancer risk[11, 12]. In positive studies, the strongest association has been related to high intake of red meat. High consumption of red meat has also been associated with metastatic prostate cancer in one study [13]. The pronounced difference in occurrence of prostate cancer in Japan compared to Europe and USA has implicated an association between consumption of soybean products and prostate cancer risk. Soya contains isoflavones that have been shown to inhibit development and metastasis of human prostate cancer [14, 15], and it has been shown that the plasma concentration of isoflavones is considerably higher in Japanese men who eat traditional Japanese food compared to Finnish men eating a traditional Western diet [16].

Consumption of tomatoes and tomato products has been related with a lower risk of prostate cancer [17]. Lycopene, a carotenoid compound in raw and processed tomato products, has been suggested to be responsible for the lower risk. Selenium is an essential trace element, found mainly in fish, meat and grains, which mediates the cellular antioxidant defense system [18]. High intake of selenium has been associated with reduced prostate cancer risk [19, 20]. In a double-blind cancer prevention trial, a total of 974 men with a history of either a basal cell or squamous cell carcinoma were randomized to either a daily supplement of 200 mg of selenium or a placebo [19]. Selenium showed no protective effects against the primary endpoint of squamous and basal cell carcinomas of the skin; however, a significant reduction in the secondary endpoint of prostate cancer incidence (relative risk [RR] = 0.37, P = 0.002) was observed.

Hormonal and other factors

Normal growth and differentiation of the prostate is under androgen control. Androgen ablation, either surgically or by medical castration, is an effective strategy in the treatment of advanced prostate cancer. Men with congenital abnormalities in androgen metabolism and those who underwent castration before puberty do not develop prostate cancer [21]. However, epidemiological studies of plasma testosterone concentrations have not been convincingly associated with prostate cancer risk [22].

The insulin growth factor-I (IGF-I) is known to regulate the proliferation and differentiation of tumor cells and to prevent them from undergoing apoptosis. Several epidemiological studies support the association between plasma levels of IGF-I and prostate cancer risk [23-25]. In a recent meta-regression analysis [26] of six case-control studies, high concentrations of IGF-I were associated with an increased risk of prostate cancer (odds ratio [OR] comparing 75th with 25th percentile 1.5, 95% confidence interval [CI] 1.3-2.1). The IGF-system may explain the association between the Western lifestyle and prostate cancer risk: high intake of fat and caloric diets stimulates growth hormone and insulin production that in turn increase IGF-I production. Men with diabetes mellitus

have been associated with reduced risk of prostate cancer. In a large population-based cohort study in Sweden [27] a 9% lower risk of prostate cancer in men hospitalized for diabetes mellitus was found. Obesity is a strong risk factor for diabetes [28] and a key characteristic of obesity is reduced insulin sensitivity of several tissues and a rise in plasma insulin levels. Elevated insulin level reduces plasma concentration of IGF-I and circulating testosterone [29, 30], which may explain the inverse association between diabetes and prostate cancer risk.

A large number of epidemiological studies have evaluated the association of prostate cancer with behavioral risk factors, such as use of tobacco and alcohol, physical activity, and sexual activity. In a review by Grönberg [31] the general conclusion is that these factors do not affect the risk of prostate cancer.

Evidence of inherited susceptibility to prostate cancer

In 1956, the first description of familial aggregation of prostate cancer was reported[32]. Since then, a large number of epidemiological studies have confirmed that a positive family history is one of the strongest risk factor for this disease. The aggregation of prostate cancer within families may be due to a common exposure of environmental risk factors; however, as will be shown, strong evidence supports the existence of inherited susceptibility to prostate cancer.

Case-control and cohort studies

Numerous case-control studies have been reported demonstrating an increased risk for prostate cancer among men with a positive family history for prostate cancer. In a recent meta-analysis of 16 case-control studies and eight cohort studies reporting the relationship between risk of prostate cancer and family history, defined as having a father, brother, any first- or second-degree relative or other relative affected with prostate cancer, estimated a pooled RR of 1.9 (95% CI 1.6-2.3) for any affected family member [33]. The pooled estimates of RRs were 2.1 (95% CI 1.8-2.5) if a father was affected and 2.9 (95% CI 2.2-3.7) if a brother was affected. The RR associated with an affected brother was found to be significantly higher than the RR associated with an affected father ($P < 0.03$), which has been suggested to indicate X-linked or recessive genetic components [34, 35]. However, possible bias due to increased intensity of medical examinations among brothers to affected cases compared to sons of affected cases could explain this difference.

A trend of increasing risk has consistently been observed with increasing number of affected family members and with decreased age at diagnosis of affected family members. Taken together, these results provide solid evidence for an association between prostate cancer risk and a positive family history of prostate cancer, supporting the existence of a genetic component in prostate cancer etiology.

Twin studies

Monozygotic (MZ) twins are genetically identical, except for postzygotic somatic genetic changes, while dizygotic (DZ) twins share half their genes on average. Genetic characters should therefore show a higher concordance in MZ twins compared to DZ twins; hence twin studies constitutes a powerful epidemiological design for elucidating both the existence and the relative importance of genetic factors in disease etiology.

Lichtenstein et al. [36] studied the contribution of inherited genes to the development of malignant diseases in a large twin study comprising 44,788 pairs of twins listed in the Swedish, Danish, and Finnish twin registries. For prostate cancer, utilizing 7,231 male MZ twin pairs and 13,769 male DZ twin pairs, a pronounced difference in probandwise concordant rate was observed (21% for MZ twins and 6% for DZ twins). Furthermore, the proportion of the risk of prostate cancer that can be explained by heritable factors was estimated to 42%, which was considerably higher compared to colorectal- and breast cancer (35% and 27%, respectively).

The interpretation of heritability is not straightforward. Risch [37] has proposed that the familial risk ratio, defined as the risk of disease for a given type of relative divided by the population prevalence, is a meaningful measure for genetic effect. Furthermore, the ratio, $R_{MD} = (\lambda_M - 1)/(\lambda_D - 1)$ where λ_M and λ_D denotes the family risk ratio for MZ and DZ twins, respectively, can provide valuable insights to the underlying genetic mode of inheritance. For a disease caused by a rare dominant allele, with the possibility of different rare alleles in different families, this ratio is approximately two. For a recessive gene, this ratio is approximately four if the gene is rare, and approaches two for a more common allele, while for multiple interacting genes, this ratio can attain considerably higher values. Applied to the Scandinavian twin study, an estimated R_{MD} of 3.9 for prostate cancer was obtained. Risch interpretation from this was that inherited prostate cancer may be explained by recessive and/or multiple interacting genes, not by single rare autosomal dominant genes. Schaid [38] recently reanalyzed the results from another large twin study, comprising 13,487 World War II veteran twin pairs from the USA [39], using the method proposed by Risch. Schaid obtained an estimated R_{MD} of 5.4 for prostate cancer among the veteran twins. Schaid also provided an estimated pooled R_{MD} from both of these twin studies, using the total number of twin pairs from each study as weights, of 4.5.

Two important conclusions are provided by these results. First, there is strong evidence that a heritable form of prostate cancer exists. Secondly, the genetic inheritance of prostate cancer cannot be explained by rare autosomal dominant genes alone, rather is a more complex pattern consisting of both rare autosomal dominant, recessive, X-linked, and multiple interacting genes expected.

Family-based segregation analyses

Further support for a genetic component in prostate cancer comes from family-based segregation analyses. These provide a quantitative method to explore whether the observed aggregation of disease in a set of families can be explained by a hypothesized genetic model. These models are based on the mode of inheritance, the population frequency of the putative susceptibility allele, and the penetrance (possibly age-dependent) of the underlying genotype.

In the first published segregation analyses of prostate cancer the best fitting model was found to be a rare (population frequency 0.36%) autosomal dominant susceptibility allele with a high penetrance (88% up to age 85 years) for carriers [40]. This analysis was based on 691 nuclear US families ascertained via probands eligible for a radical prostatectomy, and hence affected with localized diseases of good prognosis. In two subsequent segregation analyses, also based on US probands who were eligible for radical prostatectomy, a rare autosomal dominant model was again found to provide the best fit with similar estimates of allele frequency and penetrance for carriers [41, 42]. Valeri et al. [43], using hospital-based probands from France, also found that an autosomal

dominant model provided the best fit. Furthermore, their analysis allowed for residual correlations among family members that were not explained by the genetic model, and they found that there was unexplained dependence between brothers under the simple autosomal dominant model. Unexplained departures from a simple dominant model was also seen in the results from Schaid et al. [41], indicating that there may be additional genetic or environmental factors not explained by the simple autosomal dominant model.

Grönberg et al. [44] conducted a segregation analysis based on probands selected from the Swedish Cancer Registry. Since PSA screening is uncommon in Sweden, especially before 1994, most of the cases were diagnosed because of clinical symptoms. Therefore, a large proportion of the cases were expected to have aggressive disease. This study also favored an autosomal dominant model, though with a more common susceptibility allele (1.7%) and a lower penetrance (63%).

A common feature of the above discussed studies is that the analyzed pedigrees all consisted of first-degree relatives (FDR) to the probands. Since information about prostate cancer risk mainly comes from fathers and brothers, since sons often are too young to be at risk for prostate cancer, these studies provide limited ability to discriminate between dominant or recessive and autosomal or X-linked mode of inheritance. This limitation is partly reduced in a study by Cui et al. [45], in which maternal and paternal uncles, in addition to fathers and brothers, of the probands were identified. This study was conducted in an Australian population and in accordance with the study by Grönberg et al.[44], probands were selected from a population-based cancer registry. In their analysis, genetic models that allowed for either one or two loci were evaluated, and the best fit was provided by a model including a rare autosomal dominant allele, contributing to risk at younger ages, and a more common allele that is either X-linked or autosomal recessive, contributing to risk at older ages. Interestingly, the allele frequency (1.7%) and penetrance (67%) of the autosomal dominant allele are very similar to those estimated by Grönberg et al. [44].

Finally, both the analysis by Gong et al. [46] and Conlon et al. [47] supports a genetic model with autosomal dominant inheritance. However, both of these analyses support genetic models including multiple susceptibility loci. Gong et al. [46], using families of Caucasian, African American, and Asian-American ethnicity from the USA and Canada, found that a low-penetrance multi-factorial model fitted the data equally well as an autosomal dominant high-penetrance model. The analysis by Conlon et al. [47] used a different approach compared to other published segregation analysis of prostate cancer. They used highly selected multiplex pedigrees that were ascertained for a linkage study and applied a statistical method allowing for unlimited number of susceptibility loci. A model including 2-3 susceptibility loci, with the two largest effects being autosomal dominant, provided the best fit. Although the pedigrees used in this analysis is expected to provide greater ability to discriminate between dominant or recessive inheritance, it is not surprising that a dominant model provided the best fit, due to the fact that autosomal dominant transmission was favored in the ascertainment of pedigrees.

In summary, segregation analyses of prostate cancer give strongest supports for a dominant inheritance of susceptibility genes; however, also autosomal recessive or X-linked mode of inheritance has been implicated. Moreover, it seems not plausible that a single locus can explain all observed aggregation of prostate cancer within families.

Definition of hereditary prostate cancer

Based on family structure Carter et al. [48] proposed the following definition for hereditary prostate cancer (HPC):

1. A cluster of three or more relatives affected with prostate cancer in any nuclear family

or

2. The occurrence of prostate cancer in three successive generations in either of the proband's maternal or paternal lineages

or

3. A cluster of two relatives, both with a prostate cancer diagnosis before age 55 years

The clinical and pathological characteristics of hereditary prostate cancer are so far poorly understood. Utilizing the Carter criteria, Bratt et al. [49] assessed clinical characteristics in 201 patients with HPC and 402 sporadic prostate cancer cases who were matched for age and calendar year of diagnosis, and the hospital where the diagnosis were made. HPC was found to have an earlier onset than sporadic prostate cancer (6-7 years) but no other important difference in clinical characteristics was found, which is in agreement with previous reports [48, 50].

Malignancies associated with HPC

Most hereditary cancer syndromes are associated with more than one tumor site, such as hereditary breast and ovarian carcinoma caused by mutations in the breast cancer 1 (BRCA1) and breast cancer 2 (BRCA2) genes and the hereditary nonpolyposis colon carcinoma syndrome associated with colorectal, endometrial, gastric, as well as ovarian carcinoma. Several studies have evaluated occurrence of other malignancies in relatives of prostate cancer patients; however, results from different studies have been inconclusive.

To date, only two studies have been conducted in relatives of HPC cases. Isaacs et al. [51] reported an elevated risk of tumors in the central nervous system (CNS) (RR = 3.0, 95% CI 1.1-8.4) in 75 HPC families ascertained in the US. No other sites showed significant association with HPC in these families. Isaacs and coworkers also evaluated incidence of malignancies in relatives to 690 prostate cancer patients unselected for a positive family history, without finding any significant associations. The second study utilizing families affected with HPC was conducted by Grönberg et al. [52] in a Swedish population. A total of 1,364 FDRs of prostate cancer patients from 62 HPC families were evaluated and several tumor sites were found to be significantly associated with HPC. In male FDRs, a significantly increased risk of gastric carcinoma was found (standardized incidence risk [SIR] = 3.1, 95% CI 1.5-5.1). In female FDRs, incidence of breast cancer (SIR = 2.1, 95% CI 1.0-4.0) and kidney cancer (SIR 3.1, 95% CI 1.1-4.1) was significantly elevated. However, no significant association between brain/CNS tumors and HPC was found. In contrast, in a large nation-wide register cohort study in Sweden [53], comprising 10,684 children of all prostate cancer cases diagnosed between 1959 and 1963 in Sweden, no significantly elevated risks were found for any cancer site. Only in a subset analysis, restricted to children of fathers with a prostate cancer diagnosis before age 70 years, significantly increased risk of colon/rectum cancer was observed (SIR = 1.9, 95% CI 1.0-

3.1). A major strength in the study design used by Grönberg and coworkers is the utilization of a population-based cancer registry, providing unbiased information of cancer incidence in relatives. Furthermore, for each cancer site expected number of cases can be determined based on site-specific incidence rates derived from the entire population. In the study by Isaacs and coworkers, cancer incidence in FDRs of prostate cancer patients was compared with the cancer incidence in FDRs of control individuals, consisting of the patients' spouses or female companions. Occurrence of malignancies in relatives was assessed from interviews of patients and controls, which is clearly subject to recall bias. These differences in study design may explain the different results from these studies.

Some large registry-based studies evaluating malignancies associated with prostate cancer have been reported. Utilizing the Utah Population Database resource, Goldgar et al. [54] systematically assessed familial clustering at 28 distinct cancer sites. Sites with significantly elevated familial relative risk (FRR) in FDRs of 6,350 prostate cancer cases included colon (FRR = 1.3, 95% CI 1.1-1.4), rectal (FRR = 1.3, 95% CI 1.1-1.5), non-Hodgkin's lymfoma (FRR = 1.2, 95% CI 1.1-1.5), and brain/CNS (FRR = 1.3, 95% CI 1.0-1.5). Relatives of early onset (<60 years) prostate cancer cases were at increased risk of rectal cancer (FRR = 2.0, 95% CI 1.2-3.1). Proband cancer sites significantly associated with prostate cancer in FDRs included colon, rectal, breast, non-Hodgkins lymfoma, brain/CNS, and thyroid, all with FRRs ranging between 1.2 to 1.4. Matikainen et al. [55] investigated the incidence of malignancies in two cohorts of FDRs of early- (<60 years) and late (≥60 years) onset prostate cancer patients in Finland. All patients diagnosed with prostate cancer in Finland during 1988-1993 were selected and linking the population-based parish records with the Finnish Cancer Registry identified FDRs. In the early onset cohort (3,631 FDRs) significantly elevated risk of gastric cancer was seen (SIR = 1.9, 95% CI 1.3-2.7), observed only among male relatives. In the late onset cohort no increased risk of gastric cancer or any other site was observed. In a recent study of relatives of 371 prostate cancer cases in Iceland, Eldon et al. [56] reported significantly elevated risk of kidney cancer among 1,780 female FDRs (SIR = 2.5, 95% 1.1-5.7) and in 5,534 female second-degree relatives (SIR = 2.7, 95% 1.0-6.8). No other sites were significantly associated with prostate cancer.

Several studies have supported an association of breast cancer with prostate cancer in families, showing increased risk for breast cancer in relatives of prostate cancer patients [52, 57-59], and increased risk for prostate cancer in relatives to breast cancer patients [59-61]. Increased risk of prostate cancer has been reported in male carriers of the breast cancer predisposing genes BRCA1 and BRCA2. The Breast Cancer Linkage Consortium (BCLC) reported a RR of 4.7 (95% CI 3.5-6.2) for prostate cancer in 173 breast-ovarian cancer families with BRCA2 mutations [170], and Edwards et al. [62] found that BRCA2 mutations are responsible for a significant fraction of early-onset prostate cancer (23-fold risk by age 56 years). Although elevated risk of prostate cancer has been reported in BRCA1 mutation carriers [63] the role of this gene seems to be more limited for prostate cancer. The result from the BCLC showed a RR of 1.1 (95% CI 0.7-1.5) for BRCA1 carriers (with a RR of 1.8 before age 65 years) [64]. These results provide strong support for the BRCA2 gene as a high-risk susceptibility gene for prostate cancer, likely to explain a considerable part of the association between breast and prostate cancer. However, elevated risk for prostate cancer in relatives of early onset breast cancer cases without a BRCA mutation has also been reported [65].

Gene mapping

Linkage analysis is a statistical technique used to identify genes involved in disease etiology. The basic idea is to look for co-segregation between the disease and genetic markers (chromosomal variants with known locations) in families affected with the disease. This approach provides an efficient method to screen the entire genome for regions possibly harboring disease-causing genes.

Human genetics and recombination

In humans the DNA material is divided into 23 segments called chromosomes. Each child being born receives one copy of each chromosome from its father and one from its mother for a total of 46 chromosomes, one pair of sex chromosomes (XX or XY) and 22 pairs of autosomes (any chromosome other than X or Y). During meiosis (cell division leading to the formation of gametes [egg or sperm cells]) crossing over between homologous pairs of chromatids (one strand of a chromosome) may occur which result in what is referred to as a recombination (Figure 1). The recombination fraction θ is the probability that two loci become recombinant during meiosis. Alleles of loci on different chromosomes segregate independently of each other ($\theta = 0.5$) as do alleles of loci on the opposite end of the same chromosome. However, when two loci are located close together on the same chromosome their alleles no longer segregate independently ($\theta < 0.5$) and such loci are said to be linked. The genetic distance (in the units of Morgans [M]) between two loci is defined as the average number of crossovers per meiosis occurring between the loci. Importantly, genetic distance is correlated with physical distance, although this relation depends on the chromosomal region considered.

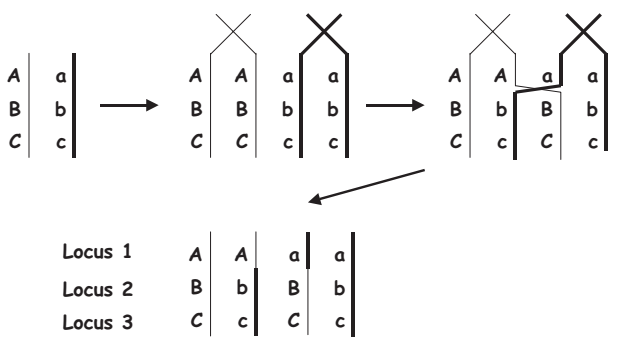

Figure 1. Illustration of a cross over during meiosis. Three loci (chromosomal positions) are indicated, each with two alleles represented by capital and small case letters. A recombination occurred between loci 1 and 2 and generated two recombinant sister chromatids.

Linkage analysis

The goal of linkage analysis is to determine the chromosomal location of e.g. a disease gene relative to a map of genetic markers with known locations. The approach is based on evaluating co-segregation between genetic markers and the disease phenotype in families including members affected with the disease. In Figure 2, a sample pedigree with two unaffected and two affected (with some disease) offspring is illustrated. Furthermore, based on each individuals disease status (phenotype) alleles at an hypothetical dis-

ease locus has been indicated, with "D" denoting the disease allele and "+" the normal allele. In this example we further assume that it is sufficient to carry one copy of the disease allele for the phenotype to manifest (dominant genetic effect). By genotyping a marker with known chromosomal position (alleles illustrated by different numbers in Figure 2) we can observe how many recombinant meiosis that have occurred and estimate θ, the recombination fraction, and perform statistical tests for linkage ($\theta = 0.5$ vs. $\theta < 0.5$). In the example in Figure 2, all recombinant and non-recombinant meiosis can be counted unambiguously and the likelihood function for θ is given by the binomial probability function

$$L(\theta) = \binom{n}{r} \theta^r (1-\theta)^{n-r},$$

where n and r denotes number of meiosis and number of recombinant meiosis, respectively. Given the likelihood function, test for linkage can be performed utilizing a likelihood ratio test statistic $2\ln L(\theta)/L(0.5)$, however for historical reason it is common practice to use the logarithm (base 10) of the likelihood ratio. This statistic

$$Z(\theta) = \log_{10} \frac{L(\theta)}{L(\theta = 0.5)}$$

is known as the LOD score (for log-odds) and the null hypothesis of no linkage ($\theta = 0.5$) can be rejected for large LOD scores. Positive LOD scores indicate evidence in favor of linkage and the conventional critical value used in linkage analysis for calling a test significant is $Z \geq 3$, which corresponds to a P value of 0.0001 under the assumption of an asymptotic chi-square distribution with one degree of freedom (one-sided test). The reason for requiring such a low significance level in linkage analysis is that of multiple testing.

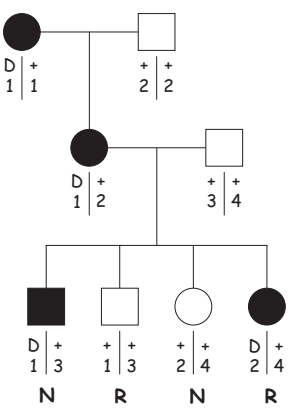

Figure 2. A pedigree with four affected individuals (black symbols) illustrating recombination. The disease allele is denoted "D", the normal allele "+", and alleles at the marker locus are illustrated by different numbers. Two offspring are recombinant (R) and two are non-recombinant (N) for the disease and marker locus.

In the sample pedigree in Figure 2 all meiosis can be scored unambiguously as recombinant or non-recombinant with regard to a disease locus and a marker locus. However, this is rarely possible in human genetic linkage analysis due to several complicating factors, such as unknown phase, phenocopies (non-disease gene carriers affected with disease), incomplete penetrance (disease gene carriers not manifesting the disease), unknown mode of inheritance, missing marker or phenotype data, marker and/or disease locus homozygosity, and diagnostic uncertainty. In these situations the likelihood of the pedigree can be quite extensive and practically impossible to compute by hand. Therefore, a number of computer software has been developed specifically for computation of pedigree likelihoods (a comprehensive set of programs are available for download from http://linkage.rockefeller.edu/soft/).

Parametric likelihood-based linkage analysis requires specification of the disease allele frequency, mode of inheritance, as well as the penetrance of the disease allele. However, for complex diseases this is not trivial since these characteristics of the underlying genetic model rarely are well known. An alternative to parametric analysis is non-parametric linkage (NPL) analysis, which do not require an explicitly specified genetic model. NPL methods typically evaluate amount of allele sharing identical-by-descent (IBD, a pair of related individuals shares an allele IBD if that allele has a common ancestral source) among affected individuals within a pedigree. In order to test if a specific locus is linked to a disease locus the amount of allele sharing IBD among affected individuals is compared to the expected amount of IBD sharing under the null hypothesis of no linkage. In 1996 Kruglyak et al. [140] proposed a procedure based on a scoring function defined with respect to the amount of IBD sharing among affected. Kong and Cox [137] further extended this approach by applying a one-parameter allele-sharing model, which under general missing-data patterns allows exact calculation of likelihood ratios and LOD scores.

Linkage analyses of prostate cancer

During the last decade numerous chromosomal regions have been reported to harbor prostate cancer susceptibility genes using genetic linkage studies. However, attempts to replicate reported regions in independent populations have yielded conflicting results. This is, however, not surprising considering all of the difficulties involved in mapping susceptibility genes predisposed to genetic complex diseases. For prostate cancer, there is accumulating evidence for a considerable degree of genetic heterogeneity, with multiple disease causing genes and multiple mode of inheritance (dominant, recessive, and X-linked). Furthermore, even within families affected with HPC, phenocopies (non-mutant carriers with disease) are likely to be common due to the high prevalence of prostate cancer. In addition, due to the late age of disease onset sampling of DNA is difficult for more than one generation. Other complications are differences in how families have been ascertained and changing diagnostic criteria, mainly through the introduction of PSA screening.

Table 1 summarizes prostate cancer susceptibility loci that have been identified through linkage analyses from different groups during the last decade. In 1996, the first prostate cancer susceptibility locus, known as HPC1, was mapped to the long arm of chromosome 1 [66]. Using 91 multiple case families from North America and Sweden

significant evidence of linkage of a prostate cancer susceptibility gene to markers on chromosome 1q23-25 was reported, with a maximum heterogeneity LOD (HLOD) score of 5.4, and an estimated 34% of families linked to this region. In stratified analyses, in the same set of families, evidence of linkage was most pronounced in families with an early age of diagnosis as well as in large families with many affected members[67].

Despite the initial strong evidence of linkage to HPC1, subsequent studies have proven inconclusive; some confirmed linkage [68-71], whilst others did not [72-75]. In an effort to thoroughly evaluate the linkage to HPC1, the International Consortium for Prostate Cancer Genetics (ICPCG) performed a pooled analysis of 772 families from nine international groups [76]. This revealed confirmatory evidence of linkage with an HLOD of 1.4 in the complete set of families. Stronger evidence of linkage was found in families with early-onset disease, with several members affected, and in families with male-to-male (MTM) disease transmission. For the 48 families that met all three criteria a maximum HLOD of 2.3 (P = 0.001) was observed. This combined analysis estimated that a segregating prostate cancer susceptibility gene in the HPC1 region could explain 6% of the families.

Table 1. Hereditary prostate cancer loci identified through linkage analyses.

Locus	Study (year)	Population	No. families	LOD score
1q24-25 (HPC1)	Smith (1996) [66]	US, Sweden	91	5.4
1q42.2-43 (PCAP)	Berthon (1998) [72]	France, Germany	47	2.2
Xq27-28 (HPCX)	Xu (1998) [77]	US, Sweden, Finland	360	3.9
1p36 (CAPB)	Gibbs (2000) [79]	US, Canada	12	3.2
16q23	Suarez (2000) [90]	US	230	2.2
20q13 (HPC20)	Berry (2000) [85]	US	162	3.1
17p11 (HPC2/ELAC2)	Tavtigian (2001) [91]	US	33	4.5
19p13	Hsieh (2001) [89]	US	98	1.8
8p22-23	Xu (2001) [86]	US	159	1.7
8p23	Matsui (2004) [156]	Japan	53	1.1

After the initial report of the HPC1 locus several regions have been implicated as harboring prostate cancer susceptibility genes. Berthon et al. [72] reported linkage to 1q42.2-43 (PCAP) in 47 families ascertained in France and Germany with an HLOD of 2.2 in the complete set of families. Stronger evidence was observed among early-onset families (HLOD =3.3) and they estimated 50% of the families to be linked to PCAP. In 360 families, ascertained in North America, Sweden, and Finland, Xu et al. [77] found evidence of linkage to markers on the X chromosome with a maximum HLOD of 3.9. The 129 families without evidence of MTM transmission provided stronger evidence of linkage (HLOD = 2.5) compared to families with MTM transmission (HLOD = 1.5), consistent with X-linked inheritance. Importantly, each independent family collection provided positive evidence for linkage to this region, although the linkage signal was weak in the 41 Swedish families. This region, denoted HPCX, was estimated to account for 15% of the US and 41% of the Finnish families. Further analyses revealed that Finnish families linked to HPCX are characterized by late age of diagnosis, in addition to absence of MTM transmission [78].

Based on observed co-aggregation of prostate cancer with brain tumors Gibbs et al. [79] analyzed 12 families with a history of both prostate and primary brain tumors. On chromosome 1p36 (CAPB) a maximum two-point LOD score of 3.2 was found and the

six early onset families provided a maximum two-point LOD score of 3.6. However, in a recent updated analysis by the same group, including 21 HPC families with occurrence of primary brain tumors, very limited evidence (LOD = 0.5) for linkage was observed at the CAPB locus [80]. Instead a LOD score of 2.3 was observed on chromosome 6, a region also implicated in the complete set of families. Attempts to replicate linkage to CAPB have been inconclusive with one confirmatory study [81] and three studies with limited [82] or no evidence for linkage [83, 84].

Berry et al. [85] reported suggestive linkage for chromosome 20q13 (HPC20). In 162 families, using a recessive model, a two-point LOD score of 3.1 was found. Families without evidence of MTM transmission, with late age of disease onset, and less than five affected men provided the strongest evidence for linkage. A linkage analysis by Xu et al. [86] indicated chromosome 8p22-23 as harboring susceptibility genes (allele sharing LOD = 1.8). Strongest evidence for linkage was found in families with a late age of disease onset (allele sharing LOD = 2.6). Gibbs et al. [87] also reported positive linkage to this region with strongest support in late onset families (allele sharing LOD = 0.89). The same region was also implicated in a recent genome-wide analysis in a Japanese population [156]. Chromosome 8p has been implicated in prostate carcinogenesis by the observation of frequent deletions of this region in prostate cancer cells [88]. Further linkage peaks have been reported on chromosome 16q23, 17p11 (HPC2) and 19p13 [89-91].

In addition to the above reported regions, Witte et al. [92] found evidence for linkage on chromosome 5q, 7q, and 19q using the Gleason score as a quantitative trait in linkage analysis. Refined analyses [93, 94] and two independent studies [95, 96] confirmed linkage to chromosome 7q and 19q and also revealed additional loci with suggestive linkage. The Gleason score is a grading system of prostate cancer that is based on the architectural pattern of tumor specimens. The score ranges from 2 to 10 and high scores (8-10) are associated with a poor prognosis, while patients with low scores (2-5) rarely die from the disease.

The chromosomal regions diplayed in Table 1 has mainly been implicated from linkage findings in the total of each set of families. In addition to these regions, several more loci have been suggested from subset- and updated analyses, and it is clear from the current experience that prostate cancer is a genetically complex disease not explained by a few segregating genes.

Prostate cancer susceptibility genes

To date, based on identifed susceptibility loci through linkage analyses, three genes have been identified and proposed as candidate genes for prostate cancer susceptibility.

RNASEL

The ribonuclease L gene (RNASEL) maps to HPC1 and is involved in the interferon-regulated 2-5A system that mediates cell proliferation and apoptosis [97, 98] and has been suggested as a tumor supressor gene [99]. Six years after the initial linkage report of HPC1, Carpten et al. [100] identified RNASEL as a candidate gene for the HPC1 locus through a positional cloning and candidate gene approach. A truncating mutation (E265X) and an initiation codon mutation (M1I) was reported to co-segregate within two HPC families linked to HPC1. Furthermore, loss of the wild-type allele and reduced RNASEL activity was observed in microdissected tumors carrying a germline mutation.

Attempts to confirm these findings have provided mixed results. Wang et al. [101] found no truncating mutations in RNASEL among 326 prostate cancer cases from 163 HPC families. In 116 Finnish HPC families, E265X was detected in five (4.3%) prostate cancer cases; yet, no segregation with prostate cancer was observed in any family [102]. Chen et al. [103] detected one E265X carrier among 95 affected men from 75 multiplex prostate cancer families. In addition, three different missense mutations (G59S, I97L, and V247M) were found in three unrelated families. In a study of Ashkenazi Jews (AJ), a novel frame shift mutation (471delAAAG) was detected in six (6.9%, 95% CI 2.6-14.4%) patients with prostate cancer [104]. Lower frequencies of this mutation were found in eldery AJ without prostate cancer (2.4%) and in young AJ females (4.0%). However, in a subsequent study of 111 AJ probands affected with prostate cancer only one carrier of the 471delAAAG mutation was detected [105].

Table 2. Reported studies of the association between RNASEL missense mutations R462Q and D541E with prostate cancer risk.

			OR (95% CI)			
	No. of		R462Q		D541E	
Study	cases	controls	Heteroz[1]	Homoz[2]	Heteroz	Homoz
Casey [106]	423	454	1.5 (1.1-2.0)	2.1 (1.2-3.8)	—	—
Wang [101]	438	510	0.8 (0.6-1.1)	0.5 (0.3-0.9)	NS[3]	NS
Rökman [102]	66	176	0.7 (0.4-1.3)	1.6 (0.7-3.5)	1.3 (0.5-3.1)	1.7 (0.7-4.2)
Nakazato [108]	101	105	1.3 (0.7-2.2)	0.1 (0.0-0.1)	0.9 (0.5-1.5)	6.9 (4.0-12.1)

[1] OR for heterozygous carriers vs. non-carriers
[2] OR for homozygous carriers vs. non-carriers
[3] Not significant

In addition to rare mutations, two missense mutations (R462Q and D541E) in RNASEL have been associated with prostate cancer risk (Table 2). Casey et al. [106] estimated that heterozygous carriers of this variant have 50% greater risk of prostate cancer than non-carriers, and homozygous carriers have more than double the risk. They also reported a reduction in enzymatic activity of the variant to one-third of normal activity. Further functional evidence for this variants role in prostate cancer development comes from a recent study observing that the R462Q variant reduced the ability of RNase L to cause apoptosis in response to activation by 2-5A [107]. Wang et al. [101] also reported a significant association between this variant and prostate cancer risk among 473 affected men from 181 HPC families; however, they found an opposite trend with ORs of 0.8 for heterozygotes and 0.5 for homozygotes. Nakazato et al. [108] also found a significantly decreased risk associated with this variant in a Japanese population. In addition, the D541E missense variant was associated with an increased risk for prostate cancer.

MSR1

Deletions on chromosome 8p in prostate cancer cells and family-based linkage studies have implicated this region in the development of prostate cancer. The macrophage scavenger receptor 1 gene (MSR1) map to 8p22 and encodes proteins that function with host responses to infection, which may be of importance in susceptibility to prostate

cancer [109, 110]. In a mutation screening study in families affected with HPC, Xu et al. [111] identified six rare missense and one nonsense mutation in MSR1. A family-based linkage and association test indicated that these mutations co-segregate with prostate cancer (P = 0.0007). In addition, among men of European descent, MSR1 mutations were significantly more frequent among individuals affected with non-HPC (4.4%) compared with unaffected men (0.8%, P = 0.009). This association between MSR1 mutations and prostate cancer was also seen among African American men.

HPC2/ELAC2

A genome-wide scan in 33 high risk prostate cancer pedigrees from Utah provided evidence for linkage to a locus on chromosome 17p [91]. Within this region, positional cloning and mutation screening identified a gene, ELAC2, harboring mutations that segregated with prostate cancer in two pedigrees. In addition, two common missense variants, Ser217Leu and Ala541Thr, were found to be associated with prostate cancer risk.

Rebbeck et al. [112] reported significantly elevated prostate cancer risk in men carrying both Leu217 and Thr541 variants. Although these findings were supported by Suarez et al. [113], several additional studies provided no support for ELAC2 as a prostate cancer susceptibility gene [114-117].

Other candidate genes

An alternative to linkage analysis for disease gene detection is to select candidate genes involved in biological pathways expected to be essential in the etiology of prostate cancer. Genes functional in the metabolism of androgens, DNA repair, carcinogen metabolism, and inflammation processes have been evaluated in a large number of studies. In a recent MEDLINE search of articles published in English using the keywords "prostate," "cancer," and "polymorphisms" over 450 studies in which more than 70 different genes had been evaluated for association with prostate cancer risk were identified (Sara Lindström, personal communication). Two of the most commonly studied genes were the androgen receptor (AR) gene and the 5α-reductase type II (SRD5A2) gene, both involved in the metabolism of testosterone and other androgens.

The AR gene, located on chromosome Xq11-12, contains a variable number of trinucleotide repeats (CAG or GGC) in exon 1. Increased number of repeats is associated with decreased transactivation and binding affinity for androgens that may confer a protective effect in terms of prostate cancer risk [118]. The association of CAG and GGC repeat length and prostate cancer risk has been evaluated in several studies but the results are inconclusive (see Simard et al. [119] for a thorough review).

SRD5A2 maps to chromosome 2p22-23 and is expressed in the prostate and in genital skin where testosterone is converted irreversibly into the more active androgen dihydrotestosterone (DHT). A polymorphism involving the number of TA dinucleotide repeats in the untranslated region, and two common missense mutations (V89L and A49T) have been identified within the SRD5A2 gene. Longer TA alleles have been proposed to enhance the enzyme activity leading to increased DHT levels and increased risk of prostate cancer [118, 120, 121], and both missense mutations have been found to modify prostate cancer risk[122-124]. However, the evidence for prostate cancer risk associated with SRD5A2 variants is controversial. In a recent meta-analysis of 9 studies Ntais et al. [125] concluded that the V89L allele was not associated with prostate cancer risk (OR = 1.0,

95% CI 0.9-1.1) and that the A49T (OR = 1.6, 95% CI 0.9-2.6) and TA repeat allele may only have a modest effect (OR = 0.9, 95% CI 0.3-1.1).

The candidate gene approach has been utilized for numerous other genes with respect to prostate cancer susceptibility; see Coughlin and Hall [126] and Simard et al. [119] for comprehensive reviews. Although many of these genes have strong biological support, none has conclusively been shown to be associated with prostate cancer risk. However, most of these studies have been of limited size (a few hundred cases and controls) and much larger cohorts will likely be necessary in order to clarify the role of low-penetrant genes in prostate cancer etiology [127]. Similar to studies aiming to map susceptibility loci through linkage analysis, association studies of candidate genes have certain limitations. The impact of population stratification, due to which differences in allele frequency between cases and controls are caused by systematic differences in ancestry, rather than association of genes with disease, is of concern in case-control association studies. Freedman et al. [128] recently suggested that modest amount of stratification can exist even in well designed studies. Another complication is the fact that the polymorphism being tested is not itself the causal variant but is rather in linkage disequilibrium (LD) with the causal variant. This may explain some of the variable results of association studies found in different populations.

Aims

The specific aims of this thesis were:

- To conduct a genome-wide search for prostate cancer susceptibility loci in Swedish families affected with HPC.
- To evaluate linkage to chromosome 8p22-23 in Swedish HPC families.
- To assess the possible role of genetic sequence variants in the RNASEL gene on prostate cancer risk in a Swedish population.
- To systematically investigate whether other malignancies aggregate in families with HPC and to apply observed associations in stratified genome-wide linkage analyses.

Material and methods

HPC families ascertained for linkage analysis (Paper I, II, and IV)

Since 1995, our research group has collected families with HPC. Identification of families has been based mainly on referrals by collaborating urologists and oncologists throughout Sweden. All diagnoses of prostate cancer in the families were confirmed both by reference to the Swedish Cancer Registry and direct examination of medical records. Almost two-thirds of the cases were diagnosed before 1990, when PSA measurement was introduced as a diagnostic tool in Sweden. The majority of the cases (79%) had clinical symptoms at diagnosis and 60% of the cases were diagnosed with a locally advanced or metastatic tumor. Blood has been collected for DNA extraction, both from affected men and their spouses and from the children, so inferred genotypes can be determined.

For the genome-wide scan (Paper I), we selected 50 families, having a minimum of three men affected with prostate cancer, in which we have at least two affected men with known or inferred genotype (Table 3). In total, 200 individuals were genotyped of which 109 were affected with prostate cancer. From typed children and spouses inferred genotypes could be determined for a further 20 cases of prostate cancer. Reviewing medical records assessed tumor aggressiveness. Patients fulfilling at least one of the following criteria were categorized as having an aggressive tumor: a poorly differentiated tumor (GIII) or Gleason score ≥7, distant metastases, confirmed progress, and/or death due to prostate cancer. We were able to assess tumor aggressiveness for 191 (82%) of the 234 reported cases of prostate cancer in the families. For the stratified genome-wide screen (Paper IV), the same set of families was used. In the linkage analysis of markers at chromosome 8p22-23 (Paper II) an additional seven families were included for study.

Table 3. Characteristics of 50 Swedish HPC families included in the genome-wide scan.

	Families with mean age at diagnosis of		
	<66 years (N = 12)	≥66 years (N = 38)	All (N = 50)
Mean age at onset (years)	60.7	73.4	69.1
Mean no. of affected/family (range)	3.8 (3-6)	4.9 (3-14)	4.7 (3-14)
Mean no. of affected and typed/family (range)	2.7 (2-3)	2.6 (2-5)	2.6 (2-5)
Mean proportion of aggressive tumors/family	44%	60%	56%

Patients with prostate cancer and controls (Paper III)

In analysis of association between RNASEL sequence variants and prostate cancer risk (Paper III), men enrolled to the population based case-control study CAPS (Cancer Prostate in Sweden) was utilized. The CAPS study comprise all prostate cancer patients, aged ≤79 years, diagnosed between July 1, 2001 and September 30, 2002 from the central and northern part of Sweden, and all patients, aged ≤65 years, diagnosed during the same calendar period from the southeastern part of Sweden and the area of Stockholm. In total, 1,961 prostate cancer patients were invited for participation and 1,444 (74%) agreed to participate. All these cases donated a blood sample and answered a questionnaire including questions regarding family history for prostate cancer. For all cases with at least one reported family member with prostate cancer, based on the initial questionnaire, a more detailed family history was obtained through a second questionnaire followed by a telephone interview. Of the 1,444 cases included in the study, 143 were classified as familial prostate cancer (FPC, defined as two cases of prostate cancer in a nuclear family) cases and 51 as HPC cases. Cases were classified as either localized (tumor stage I or II, and PSA<100) or advanced (tumor stage III or IV, or PSA≥100).

From the Swedish Population Registry control subjects were randomly selected, frequency matched according to geographical origin (northern part of Sweden vs. southeastern part of Sweden and the area of Stockholm) and to the expected age distribution of the cases (within 5-year age groups). The controls were recruited during the same calendar period as the cases. Of 1,697 controls invited, 866 (51%) agreed to participate by completing a blood draw and answering a questionnaire (same as for cases). Subsequently, eight controls were excluded since linkage to the Swedish Cancer Registry revealed that they were diagnosed with a cancer of the prostate prior to inclusion. At the time of this study, DNA samples were available for 801 controls and 1,427 cases. In addition, all affected men with available DNA samples from HPC families were included into the study cohort, resulting in a total study population of 1,636 prostate cancer patients and 801 unaffected controls (Table 4).

Table 4. Pertinent characteristics of the study population (Paper III).

Patient group	No.	Mean age[1] (range)	Proportion of advanced tumors[2]
Controls	801	67.8 (45.5-80.0)	
Sporadic prostate cancer	1,247	66.7 (47.3-80.4)	512/1,182 (43.3%)
Familial prostate cancer	156	65.8 (47.0-80.0)	51/124 (41.1%)
Hereditary prostate cancer	233	65.3 (43.0-81.0)	15/51 (29.4%)

[1] Age defined as age at diagnosis for cases and age at blood sampling for controls

[2] Information of tumor characteristics only available for cases ascertained through the CAPS study

Cohort of relatives of patients affected with HPC (Paper IV)

Three different data sources were utilized in order to collect families affected with HPC. The first set of families was selected from the ongoing ascertainment of families suitable for linkage analysis described earlier. We selected all 86 HPC families that met the hereditary prostate criteria as defined by Carter et al.[48]. In the identification of FDRs' of affected family members only relatives of men with a prostate cancer diagnosis after January 1, 1958, when the Cancer Registry started, were considered. The identification of FDRs were initially based on questionnaires, sent out to each living affected man, and were subsequently verified by local parish offices throughout Sweden. The 86 HPC families included 364 members with a prostate cancer diagnosis after 1958 and 1,310 FDRs of these prostate cancer cases. Of the 1,310 FDRs, 69 had deceased or emigrated from Sweden before 1958. For the remaining 1,241 FDRs, we were able to retrieve a complete personal identification code for 1,144 (92%). Of the 86 HPC families, 62 were included in a previous study of associated malignancies from our group [52].

The second set of HPC families was collected from the CAPS study. In total, 48 families with at least three FDRs with a confirmed diagnosis of prostate cancer were identified, comprising 141 family members with prostate cancer and 481 FDRs of these men. Tracing of FDRs of affected men was conducted as described above and we were able to retrieve a complete personal identification code for 433 (90%) of the FDRs.

The third set of families affected with HPC was identified via a nation-wide register cohort study in Sweden[129]. In that study, all men with histologically or cytologically verified prostate cancer reported to the Swedish Cancer Registry between 1959 and 1963 were selected. Through local parish offices, a total of 11,950 children were identified and personal identification codes could be obtained for 10,893 (91%) of these children. After exclusion of children deceased or emigrated from Sweden before 1958, 10,608 children (5,554 sons and 5,054 daughters) remained for study. Referral of the cohort of children to the Cancer Registry for the period 1958-2001 revealed 75 families affected with HPC. Of the 75 HPC families, 12 were already identified via the ongoing ascertainment of HPC families or via the conducted CAPS study. The remaining 63 HPC families comprised 204 cases of prostate cancer and 177 FDRs to these cases.

Our total study cohort for analysis of malignancies associated with HPC comprised 1,764 FDRs of HPC patients (Table 5).

Table 5. Number of Swedish hereditary prostate cancer families, number of first-degree relatives of prostate cancer patients within families and cumulative person-years during the follow-up period 1958-2001.

Source	Number of families	Number (person-years) of first-degree relatives					
		Female		Male		Total	
Referred	86	591	(23,408)	553	(21,414)	1,144	(44,823)
CAPS	48	240	(9,352)	203	(7,903)	443	(17,254)
Cohort	55	106	(3,928)	71	(2,371)	177	(6,300)
Total	189	937	(36,688)	827	(31,689)	1,764	(68,377)

Genotyping

All four papers utilized genetic data from genotyped DNA samples. Genomic DNA was prepared from blood samples using standard techniques. In the family-based studies, PEDCHECK was used to test for Mendelian errors. Different methods for genotyping were applied for each study, which is described below.

Genome-wide markers (Paper I and IV)
DNA samples were genotyped using 405 short tandem repeat markers with an average inter-marker spacing of 8.6 cM and average heterozygosity of 80%. PCR reactions, using fluorescently labeled primers, were set up using a TECAN Genesis 200 robot. All PCR reactions were done in a 15 ml volume containing 20 ng of genomic DNA, 0.33 mM each primer, 0.25 mM each dNTP, 2.5 mM MgCl2, 10 mM Tris-HCl, 50 mM KCl, and 0.5 U Taq polymerase. PCR amplification was performed using the GeneAmp 9600 or 9700 thermocyclers (Applied Biosystems, Foster City, CA). PCR cycling conditions were as follows: 95°C for 12 minutes; followed by 10 cycles of 94°C for 15 seconds, 55°C for 15 seconds, 72°C for 30 seconds, and 20 cycles of 89°C for 15 seconds, 55°C for 15 seconds, 72°C for 30 seconds, with a final extension of 72°C for 10 minutes. Depending on the PCR yield, 5-15 μl of product was combined with those of up to 20 other markers of appropriate size and fluorescent label. PCR products were separated using the ABI 3100 DNA sequencer, allowing multiple fluorescently labeled markers to be run in a single lane. The ROX 400 size standard was run as an internal size-standard.

Allele sizing was calculated using the local southern algorithm available in the GENESCAN software program (Applied Biosystems, Foster City, CA). Allele calling and binning was done using the GENOTYPER software (Applied Biosystems, Foster City, CA). All genotyping was done including a CEPH control individual (1347-02) for quality control purposes. In order to evaluate genotyping error rate, 1% of study samples were included as blind duplicates, resulting in an estimated error rate of 0.09%.

Markers at 8p22-23 (Paper II)
Thirteen microsatellite markers, selected from the Marshfield comprehensive human genetic maps (Broman et al. 1998), spanning ~33 cM at 8p22-23 were genotyped. PCR reactions and analysis were done as in the genome wide scan with minor modifications of the PCR-protocols. Reactions were performed in a 15 ml volume containing 60 ng of genomic DNA, 0.33 mM each primer, 0.25 mM each dNTP, 2.25 mM MgCl2, 1 x buffer II, and 0.6 U AmpliTaq Gold polymerase. PCR cycling conditions were as follows: 95°C for 12 minutes; followed by 10 cycles of 94°C for 15 seconds, 55°C for 15 seconds, and 23 cycles of 89°C for 15 seconds, 55°C for 15 seconds, with a final extension of 72°C for 10 minutes.

Sequence variants of the RNASEL gene (Paper III)
To comprehensively evaluate association between prostate cancer risk and common RNASEL sequence variants, the following approach was used to select sequence variants for analysis. First, we defined the target region for selection of single nucleotide polymorphisms (SNP) as 3 kb of the promoter, all exons, introns, and 3'UTR. We then searched the public database dbSNP within this target region, resulting in the identification of 34 SNPs. A subset of nine SNPs was then selected, using the criteria of a re-

ported minor allele frequency of at least 5%, a density of one SNP per three kb, and as low as possible proportion of repetitive sequences. Preferentially validated SNPs were selected and additional attention was paid to SNPs in the promoter region. Besides the two reported missense variants, R462Q and D541E, which were decided to be included in the analyses in advance, this subset consisted of four SNPs in the promoter region, one SNP in intron four, one SNP in intron five, and one SNP in 3'UTR. These nine SNPs were genotyped in a randomly selected subset of 96 control subjects from the CAPS study. This resulted in the elimination of four SNPs due to failed assay (one SNP), PCR failure (two SNPs), and monomorphic result (one SNP). The remaining five SNPs were all in Hardy Weinberg equilibrium (HWE). Haplotypes were estimated using a method proposed by Stephens et al. [130], as implemented in the software PHASE. Utilizing a Markov Chain Monte Carlo approach this method incorporates a statistical model for the distribution of unresolved haplotypes based on coalescent theory. Haplotype tagging SNPs (htSNP) were determined by using the htSNP2 software. Since removing any one of the five SNPs resulted in a subset of htSNPs that retained less than 95% of the diversity observed among the 96 control individuals, all five SNPs were genotyped in the total study population. In addition to the five SNPs, the truncating stop mutation E265X was also genotyped in all individuals.

Each DNA plate contained two CEPH controls, a water blank and blinded internal replicates. Two methods were used for genotyping. The stop mutation (E265X) and the two missense mutations (R462Q and D541E) were genotyped using a 5' nuclease assay with TaqMan MGB probes. Primers and probes were designed using the Assay-by Design service, Applied Biosystems (Applied Biosystems Inc., Foster City, CA). All reactions were performed in a 10 μl volume consisting of 10 ng genomic DNA, 900 nM of each primer, 200 nM of each probe, and 5 μl of TaqMan universal master-mix. PCR cycling conditions were as follows: 50°C for 2 minutes, 95°C for 10 minutes, followed by 40 cycles of 92°C for 15 seconds and 60°C for one minute. The samples were analyzed on an ABI PRISM 7900 HT sequence detection system.

A dynamic allele-specific hybridization (DASH) technique was adapted to genotype the remaining three SNPs. In order to thoroughly evaluate the genotype results, the missense variant R462Q was also genotyped with the DASH technique. DASH was performed as previously described [131-133]. For this, two PCR primers and one DASH probe per target SNP were designed by means of custom DFold software [134] provided by DynaMetrix Ltd (UK). These oligonucleotides were provided and HPLC purified, by Biomers GmbH (Germany). The DASH PCRs entailed amplifying short genomic fragments spanning the variant of interest, with one of the primers carrying a 5'-biotin label. Amplifications were performed in 5 μL volume, containing 1-2 ng genomic DNA, 0.38 μM biotinylated primer, 0.75 μM non-biotinylated primer, 0.03 units AmpliTaq Gold (PE Biosystems, CA), 10% dimethylsulphoxide, 1 x AmpliTaq Gold Buffer including 1.5 mM of MgCl2 (PE Biosystems, CA) and 0.2 mM each dNTP. Thermal cycling was conducted on an MBS 384 device (Thermo-Hybaid, UK) as follows: 1 x (10 minutes at 94°C), 35 x (15 seconds at 94°C, 30 seconds at annealing temperature). To verify successful amplification, 0.5 μl of several randomly chosen samples were examined on a 3.0 >% low-melt agarose gel.

DASH analysis of the PCR product was conducted on membrane macro-arrays, using the DASH-2 protocol [132]. Briefly, this entailed transferring samples to the membrane array by centrifugation [135] or robotic gridding. Resulting individual arrays with up to

9,600 distinct features were rinsed in 0.1 M NaOH to denature the PCR products. They were then exposed to 2 ml HE buffer (0.1 M HEPES, 10 mM EDTA, pH 7.9) containing 4 nmol of the appropriate ROX end-labeled probe. After heating to 85°C and cooling to room temperature, the membrane was briefly rinsed in HE buffer. The array was then soaked in 40 ml HE-buffer containing SYBR GreenI dye at 1:20,000 dilution for up to 3 hours. Using a DASH-2 device (DynaMetrix Ltd, UK), the membrane was taken through a DASH heating ramp (heating at 3°C/minute from room temperature to 85°C) whilst fluorescence from the ROX acceptor dye on the probe was monitored. Data was collected at intervals of 0.5°C. We used fluorescence changes with temperature (DNA melting profiles) to distinguish different alleles. This was done by means of the DASH-2 device software, which uses negative derivatives of fluorescence against temperature to reveal peaks of denaturation rate (target-probe melting temperatures; Tm) and thereby automatically assign DNA samples into genotype groups.

Statistical analysis

Linkage analysis (Paper I, II and IV)

Because the majority of families contained ungenotyped founders, and, since there is an increased tendency to get false positive evidence of linkage in situation where an allele, which occurs commonly in the population, is called rare in the analysis, marker allele frequencies were estimated empirically from all genotyped individuals.

Multipoint linkage analyses, using the Haldane map function, were performed utilizing both parametric and non-parametric methods. For the parametric analysis, we assumed a disease-susceptibility allele frequency of 0.003 and an autosomal dominant model. The model used for this analysis is the same used by Smith et al. [66] in the first reported linkage finding for hereditary prostate cancer and has since then been used in several prostate cancer linkage studies. In summary, affected men were assumed to be disease-gene carriers, with a fixed phenocopy rate of 15%, whereas all unaffected men under 75 years of age and all women were assumed to be of unknown phenotype. In men aged over 75 years, the lifetime penetrance of gene carriers was estimated to 63%, and the lifetime risk of prostate cancer for a non-carrier was 16% in this age class. Linkage in the presence of heterogeneity was assessed by use of Smith's admixture test for heterogeneity [136].

In non-parametric multipoint linkage analysis, the estimated marker IBD sharing of alleles for the various affected relative-pairs was compared to the values expected under the null hypothesis of no linkage. Computation of Kong and Cox allele sharing LOD score [137] was based on the linear model option, the NPL-all scoring function, and with equal weight assigned to all families.

Parametric two-point LOD scores (Paper II) were computed by the FASTLINK software [138]. Multipoint parametric analyses were performed using the ALLEGRO [139] (Paper I) and GENEHUNTER-PLUS [140] (Paper II) software. Non-parametric multipoint analyses were performed using the MERLIN [141] (Paper I and IV) and GENEHUNTER-PLUS software [140, 142] (Paper II).

Test for HWE (Paper III)
We tested HWE for each sequence variant and pair-wise LD between all sequence variants using a replication method [143]. For each test, 10,000 permutations were performed and the Fisher probability test statistic of each replicate was calculated from the new corresponding multilocus table. Empirical P values for each test were estimated as the proportion of replicate data sets found to be as probable as or less probable than the observed data, as implemented in the software package Genetic Data Analysis (GDA).

Association analysis (Paper III)
Unconditional logistic regression was used to evaluate the association of each of the sequence variants with prostate cancer risk using the statistical software STATA [144]. To adjust for the matching we included indicator variables, representing each combination of age category (5-year age groups) and geographical region (northern part of Sweden vs. southeastern part of Sweden and the area of Stockholm), in the regression model. A likelihood ratio test of a covariate equal to number of rare alleles (0, 1 or 2), corresponding to multiplicative allelic effects on the genotype relative risks, was used to test for association between genotypes and disease. To account for genotype correlation among individuals from the same family (in comparing FPC or HPC cases with controls), a modified sandwich variance estimator in clustered logistic regression analysis was used [145]. Since the likelihood used for estimation is not a true likelihood, a Wald test was applied to test for association between genotypes and prostate cancer risk.

Haplotype analysis (Paper III)
Association between haplotypes and prostate cancer risk was evaluated using a score test proposed by Schaid et al. [146], as implemented in the software HAPLO.SCORE for the R programming language. Based on a generalized linear model, this method allows adjustment for possible confounding variables and provides both global and haplotype-specific tests. In these analyses, age and geographical region was adjusted for as described earlier and haplotypes with frequencies < 0.005 were pooled into a common group. Empirical P values, based on 10,000 simulations, were computed for the global score test and each of the haplotype-specific score tests.

Malignancies associated with HPC (Paper IV)
The study cohort was referred to the Swedish Cancer Registry for the period 1958-2001 to obtain information about incident malignant tumors. Linkage to the Swedish Cause-of-Death Registry and the Swedish Population Registry were performed to obtain times of emigration and death and thus time at risk.

Cancer incidence in FDRs of HPC cases was analyzed by calculating SIRs, the ratio of observed to the expected number of cancer cases. For each cancer site, only first primary malignancies were considered in the analyses. Only sites that had at least 10 observed cases in the cohort before age 85 were analyzed. Follow-up for malignancies among FDRs started on the date of their birth or on January 1, 1958, whichever was later, and ended at death, emigration or on December 31, 2001, whichever was first.

The number of observed cases of cancer and person-years at risk were stratified according to gender, calendar year, and 5-year age groups. For each cancer site, the expected number of cases was calculated by multiplying the observed person-years by

gender-, age-, and calendar-specific cancer incidence rates derived from the entire Swedish population. To adjust for possible over dispersion, due to dependency within families caused by hereditary and/or environmental factors, a sampling-based standard error was determined for the SIR[147]. Note that the dependency within families does not bias the estimate of the SIR. CI was estimated under the assumption of a normal distribution.

Results and comments

Genome-wide scan (Paper I)

Markers spanning all 22 autosomes and the X chromosome were genotyped in 50 families affected with HPC, and the data analyzed utilizing multipoint linkage analyses. Both a parametric rare dominant model and non-parametric allele sharing methods were utilized and provided similar results.

In the complete set of families two chromosomal regions provided suggestive evidence for linkage (Figure 3). The strongest support was found on chromosome 19p13.3 at marker D19S209, ~11 cM from 19pter with an allele sharing LOD score of 2.91 (P=0.0001). The evidence for linkage was seen at flanking markers centromeric to D19S209, with allele sharing LOD scores greater than 0.5 extending a region of ~43 cM. Interestingly, Hsieh et al. [89] also found the strongest evidence for linkage (LOD = 1.79) in the same region (~5 cM centromeric to D19S209) in 98 HPC families ascertained in North America and Canada. Our finding represent the first replication of this region, and, in fact, constitutes the first replication of a suggested linkage region with a LOD score greater than 2.

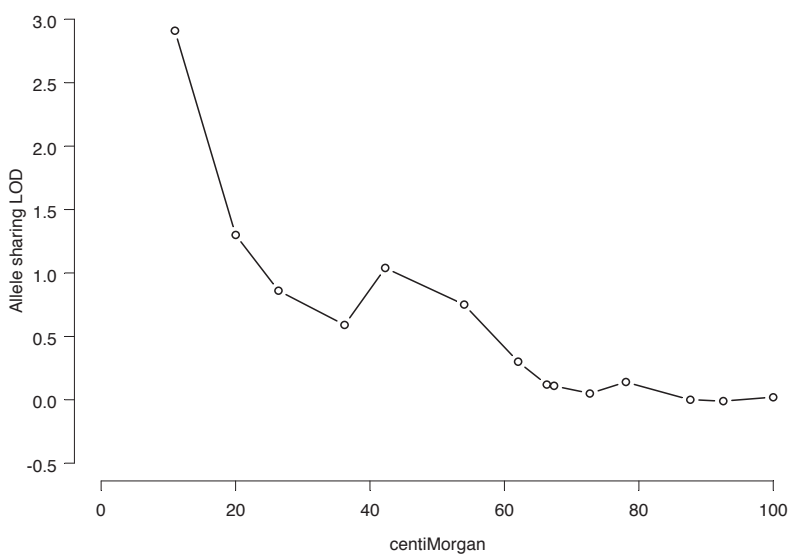

Figure 3. Result of multipoint non-parametric linkage analysis by using 14 markers across chromosome 19 in 50 Swedish HPC families.

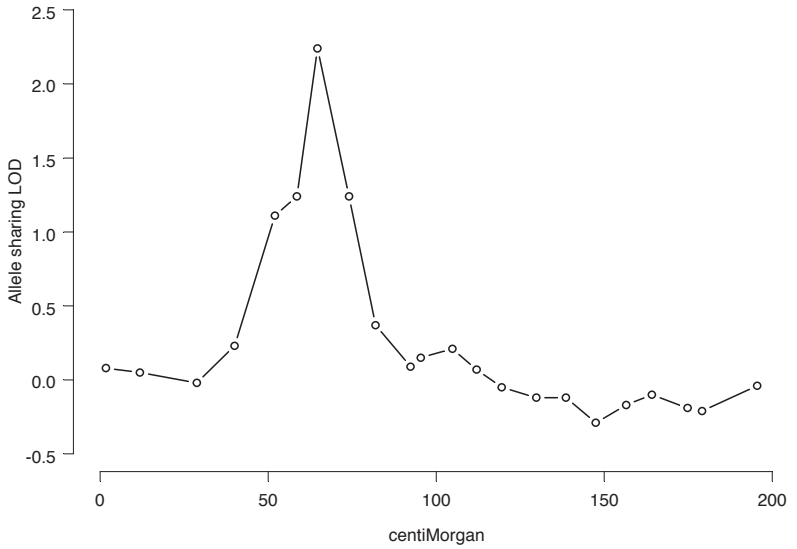

Figure 4. Result of multipoint non-parametric linkage analysis by using 22 markers across chromosome 5 in 50 Swedish HPC families.

The second region showing suggestive evidence of linkage was observed on chromosome 5 (Figure 4). In the centromeric region 5p13.2-5q13.3, flanked by markers D5S426 and D5S424, allele sharing LOD scores greater than 1 were observed with a peak score of 2.24 (P=0.0007) at marker D5S407 on 5q11.2. Remarkably, the genome scan by Hsieh et al. [89] also identified this region, with a peak allele sharing LOD of 0.85 at marker D5S2858, ~5 cM from our linkage peak. Moderately elevated LOD scores were reported in this region in the scan by Smith et al. [66]. By including Gleason score as a covariate in a model free likelihood analysis, Goddard et al. [69] reported significant (P=0.0006) evidence for linkage on 5p13.1, approximately 9 cM from our peak location.

No other regions with LOD scores in excess of 2 were observed in the complete set of families, neither in analyses stratified by family mean age at diagnosis, number of affected individuals, male-to-male transmission, or tumor aggressiveness. Stratified analyses revealed that all support for 19p and 5q was attributed to families with less than 5 affected members, possibly indicating a higher proportion of phenocopies in larger families. At 19p13.3 equal evidence of linkage was found in early- and late onset families, while all evidence of linkage at 5q11.2 was contributed to late onset families. Moderate evidence of linkage to HPC1 has been reported in a subset of the families described here [70]. The support for linkage originated almost exclusively from early-onset (<65 years) families, with a maximum LOD score of 2.38 at D1S413. However, in the slightly ex-

panded collection of the present families no support for linkage to HPC1 was observed, even in the subset of early-onset families. This lack of support is probably attributable to two significant differences between the present genome scan and the earlier linkage analysis. First, a much denser set of markers was used in the earlier study with a resolution of approximately 3.5 cM. Secondly, tissue samples from affected family members were used for genotyping in the former analysis, resulting in an average of 3.7 affected and typed individuals per family. In the present analysis only blood samples have been used for genotyping, resulting in an average of 2.6 affected and typed individuals per family. These differences contribute to a decrease in power for the genome scan compared to the former linkage analysis.

A region on chromosome 19q12 has been reported [92] and confirmed [95] as a susceptibility locus that regulates tumor aggressiveness of prostate cancer. We found no support for this in our families; neither families with high proportion of aggressive tumors, nor families with homogeneity of tumor aggressiveness provided strengthened evidence of linkage in this region.

A simulation study was performed to evaluate the genome-wide statistical significance of the linkage finding at 19p and 5q. We randomly simulated 1,000 replicates of the genome-wide scan data using the simulate option in the computer program MERLIN. In this procedure, marker data are simulated under the null hypothesis of no linkage or association to observed phenotype conditional on family structure and actual marker spacing and allele frequencies, as well as missing data patterns. In 32 out of 1,000 replicates a LOD score of 2.91 or above anywhere in the genome was observed, suggesting a genome-wide P value of 0.032 for the linkage at 19p. The chance to observe a LOD in excess of 2.24 anywhere in the genome was estimated to 17%. Moreover, the chance to obtain two chromosomal regions with LOD over 2 in a single replicate was 3%. Therefore, our finding of two regions with LOD over 2 provided significant evidence that at least one of these regions are likely to harbor prostate cancer susceptibility genes.

Recently, eight independent studies, including our study, reporting genome-wide searches for prostate cancer susceptibility loci, were published in the same issue of *The Prostate* [80, 148-154]. Together, these studies include 1,293 families comprising over 4,600 prostate cancer cases. Across all eight studies, eleven different chromosomal regions with suggestive evidence for linkage (LOD >2) were observed. Remarkably, among these eight studies no chromosomal region was reported with a LOD score of at least 2 by more than one group and previously reported regions received limited support; the only exception being the 19p locus suggested by Hsieh et al. which was significantly replicated (LOD = 2.91) in our study.

In addition to non-parametric allele sharing analyses, used in all eight studies, three studies also used a recessive model to assess evidence for linkage. In fact, the highest reported LOD score across all studies (HLOD = 4.77 at chromosome 20 [149]) was obtained under a recessive model. The corresponding allele sharing LOD score was 3.46. In our linkage scan, utilizing a rare dominant- or allele sharing model, no evidence for linkage was observed on chromosome 20. In order to check if we missed any linkage regions due to miss-specification of the genetic model, families were re-evaluated for linkage under a recessive model. In this model the disease allele frequency was assumed to be 7.7% with penetrances equal to those in the dominant model. This revealed no

further regions with LOD scores in excess of 1; on chromosome 20 the maximum HLOD obtained was 0.06.

Linkage analyses at 8p22-23 (Paper II)

Frequent loss of heterozygosity on 8p22-23 in prostate cancer cells [155], linkage studies in HPC families [66, 69, 87], and reported mutations in the MSR1 gene [111] have implicated this region in the development of prostate cancer. We performed linkage analyses in 57 Swedish families affected with HPC using 13 microsatellite markers on the short arm of chromosome 8 in an attempt to confirm linkage at this region

In the complete set of families evidence of linkage was found at 8p22-23, with a peak HLOD of 1.08 (P = 0.03) approximately 1 cM centromeric to the MSR1 gene. At marker D8S1135, the closest marker to MSR1, a HLOD of 1.07 (P = 0.03) was observed. Strongest evidence of linkage was seen in families with early age of disease onset and in families with small number of affected. Non-parametric analyses provided similar results. Further linkage support for this region comes from a recent genome-wide search in 53 Japanese sib-pairs [156]. In that study the best evidence for linkage was detected at 8p23 with a maximum allele sharing LOD score of 1.10.

In addition to rare germline mutations in the MSR1 gene, Xu et al. [157] reported five common variants of MSR1 significantly associated with prostate cancer risk. Haplotype analyses provided consistent findings and the disease-associated haplotype did not harbor any of the known rare mutations, suggesting that the association of common variants and prostate cancer risk are independent of the effect of the rare mutations. Additional support for an association of MSR1 variants with prostate cancer was provided by Miller et al. [158] in evaluating common sequence variants and rare MSR1 mutations in an African-American population. Lindmark et al. [159] recently identified several sequence variants, both novel and previously reported, when screening the MSR1 gene for mutations in 83 probands from Swedish HPC families. The previously reported [111] nonsense mutation R293X was found in two of the 83 families, and two potential splice site mutations were identified in one family each. Due to limited access of DNA samples it was not possible to determine whether the nonsense mutation R293X segregated with disease. Additional evaluation of these rare mutations, and the five common variants reported by Xu et al. [157], in 215 unselected prostate cancer cases and 425 age-matched controls revealed no significant differences in genotype frequency between cases and controls. Two others studies also found no association of MSR1 variants with risk of prostate cancer [160, 161].

Our findings support the assumption that a prostate cancer-susceptibility gene is located on chromosome 8p22-23; however, whether MSR1 represents this gene remains to be dissolved.

Genetic analysis of RNASEL (Paper III)

Linkage analyses of prostate cancer families provide convincing evidence that the HPC1 locus is likely to harbor a prostate cancer susceptibility gene and RNASEL has been suggested as a plausible candidate. To test the hypothesis that RNASEL sequence variants are associated with prostate cancer risk, we genotyped five common SNPs, including the

missense variants R462Q and D541E, and the rare stop mutation E265X in 801 unaffected men and 1,636 prostate cancer patients.

In total, 14,466 genotypes were assayed of which 13,988 (96.7%) provided a successful result. Quality control of genotyping results, based on included CEPH controls (59 samples), repeated study samples (from 115 individuals), and independent genotyping of the R462Q variant in two different laboratories (utilizing two different genotyping methods), provided an estimated error rate of 0.3%. Repeated samples revealed 19 discordant genotyping results out of 2,901 samples verified. Repeated samples with discordant genotyping result were blanked in the analysis. All CEPH samples gave consistent genotypes. No significant deviation of the genotype frequencies from those expected under HWE was observed for any of the six sequence variants, neither among the 801 control individuals nor among the 1,504 unrelated prostate cancer cases (all P values > 0.19, range = 0.19-1.00). We found strong evidence for pair-wise LD between these variants (all P values < 0.0001) and pair-wise D' estimates between adjacent SNPs were high (mean = 0.94, range = 0.76-1.00).

There was no significant difference in proportion of E265X mutation carriers between controls and sporadic prostate cancer (SPC) cases, or between controls and FPC or HPC cases. The prevalence of E265X carriers among unaffected controls and sporadic prostate cancer patients was almost identical (1.9 and 1.8% in controls and sporadic cases, respectively), and even lower among FPC and HPC patients (1.4%). No evidence for segregation of E265X with disease was observed within any HPC family.

In the initial report of RNASEL as a candidate susceptibility gene for prostate cancer [100], an index case from each of 26 families, of which eight were linked to the HPC1 region, at high risk for prostate cancer were screened for mutations in the RNASEL gene. In this selected set of families, the stop mutation E265X, detected in one (3.8%) of the index cases, co-segregated with disease status within that family. Rökman et al. [102] reported the detection of E265X in five (4.3%) index patients from 116 Finnish families with HPC. In one of these five families, suggestive evidence of segregation between the mutation and prostate cancer was observed. In a study of 95 men with HPC, originating from 75 unrelated families, Chen et al. [103] found the E265X mutation in one family, with two of three affected brothers carrying the mutation. In 163 families, defined as having FPC, Wang et al. [101] detected no carriers of the E265X mutation among 326 prostate cancer cases. From these results it is clear that only a small fraction of prostate cancer families may be explained by segregation of the E265X variant.

Despite the functional and epidemiological findings supporting a role for R462Q in prostate cancer susceptibility, no significant associations between this variant and prostate cancer risk was observed in our study. Nor did we find any significant association with prostate cancer risk for any of the other four common sequence variants studied, except for the missense mutation D541E which under a recessive genetic model provided marginally significant evidence for association with SPC (P = 0.03), with an estimated OR of 0.77 (95% CI 0.59-1.00) for homozygous carriers compared to homozygous non-carriers. However, due to the large number of tests conducted in our study this could represent a false positive finding. Moreover, no differences were observed between patients with FPC or HPC and controls for this variant, irrespective of choice of genetic model (recessive, multiplicative, or dominant).

Global tests for association between haplotypes and prostate cancer risk were all non-significant. Individual haplotype analyses revealed two haplotypes significantly asso-

ciated with prostate cancer risk. The frequency of the AAGAA haplotype of SNPs -2575 A>G, R462Q G>A, D541E G>T, +10721 G>A, and +13861 A>C was significantly decreased in SPC cases compared to controls (2.9 vs. 1.5%, P = 0.010). This haplotype also occurred at lower frequency among FPC and HPC cases (1.6%) compared to controls, yet there was no significant difference (P = 0.181). The other haplotype significantly associated with prostate cancer risk was the GGTGC haplotype, which also occurred at higher frequencies among controls (3.1%) compared to SPC cases (2.5%, P = 0.415) and FPC/HPC cases (0.8%, P = 0.020). It is puzzling that both of these haplotypes indicated protective effects being completely discordant for each of the five SNPs, and, as discussed above, due to the many statistical tests performed the chance of false positive findings has to be considered.

We performed a systematic evaluation of the possible impact that the RNASEL gene have on prostate cancer. We failed to support a role of the rare truncating mutation E265X, and analyses of common sequence variants provided limited evidence for association with prostate cancer risk. Considering the large and well characterized study population and the high quality in genotyping (0.3% error rate) these results provide strong evidence against a role of RNASEL in prostate cancer etiology in Sweden.

Associated tumors (Paper IV)

Stratification of HPC families using biologically relevant covariates, such as co-aggregation of associated tumors, is expected to reduce locus heterogeneity and thereby improve the power to map susceptibility genes. For prostate cancer it is less clear how such stratification should be performed since reported co-occurrence of prostate cancer with other malignancies has not been consistent across different studies. We performed a comprehensive evaluation, using nation-wide cancer and population registries, of other malignancies associated with HPC. Furthermore, genome-wide linkage analyses, stratified on the basis of co-occurrence of associated malignancies, were performed.

In total, 1,764 FDRs of patients affected with HPC were available for study. These individuals contributed 68,377 person-years of follow-up during 1958-2001. The mean follow-up time was 25 years (range 5 to 41 years), the mean age at cohort entry was 22 years, and 37% of the follow-up time covered the age range of 50-84 years. In total, 279 tumors at 48 different sites were observed among the FDRs.

The main finding was a significantly 2-fold elevated risk of gastric carcinoma. The increased risk was of the same magnitude in early and late onset HPC families and confined to only male relatives (Table 6). Co-aggregation of prostate cancer with gastric cancer was first reported by Grönberg et al. [162], using a subset of the families analyzed in this study. Importantly, previously not reported families provided an estimated risk of the same magnitude, although not significant, as in previously reported families (Table 7). Furthermore, since the majority (82%) of the new families represents a population-based sample of Swedish HPC families it is unlikely that selection-bias, due to over-sampling of families with an excess of malignancies other than prostate cancer, has a major impact on this finding. Besides gastric cancer, breast cancer was the only site with significantly increased risk. The elevated risk was restricted to families with early age of onset of prostate carcinoma and young age in the relatives (Table 6).

Table 6. Risk of gastric and breast cancer in first-degree relatives of hereditary prostate cancer patients as a function of age at follow-up and family mean age at diagnosis of prostate cancer.

Family mean age[1]	Age at risk of first-degree relatives								
	0-65 years			66-84 years			0-84 years		
	Obs[2]	SIR[3]	95% CI[4]	Obs	SIR	95% CI	Obs	SIR	95% CI
Gastric cancer (males)									
≤ 65 years	1	2.00	0.00-5.97	3	2.32	0.00-4.78	4	2.23	0.16-4.29
> 65 years	7	3.87	1.18-6.56	9	1.78	0.65-2.91	16	2.33	1.22-3.44
All families	8	3.46	1.18-5.75	12	1.89	0.86-2.92	20	2.31	1.33-3.29
Breast cancer (females)									
≤ 60 years	7	5.33	0.12-10.5	1	1.21	0.00-3.72	8	3.74	0.22-7.26
> 60 years	32	1.49	0.96-2.01	5	0.28	0.05-0.52	37	0.94	0.63-1.25
All families	39	1.71	1.12-2.29	6	0.32	0.07-0.57	45	1.08	0.74-1.43

[1] Family mean age at diagnosis of prostate cancer
[2] Obs, observed number of cases
[3] SIR, standardized incidence ratio
[4] CI, confidence interval

Table 7. Risk of gastric cancer in first-degree male relatives of hereditary prostate cancer patients by appearance in previously reported study and type of data source used for ascertainment of families.

	No. of families	Obs[2]	SIR[3]	95% CI[4]
Previously reported families	62	12	2.43	1.10-3.76
New families	127	8	2.15	0.71-3.60
Population-based families[1]	104	6	2.51	0.52-4.49
All families	189	20	2.31	1.33-3.29

[1] Among the 127 new families 104 were ascertained through the CAPS study or the register-cohort study
[2] Obs, observed number of cases
[3] SIR, standardized incidence ratio
4 CI, confidence interval

Based on the confirmed association between gastric and prostate cancer, a genome-wide linkage analysis, restricted to families with occurrence of gastric cancer cases, was performed (none of the early onset HPC families including early onset breast cancer cases was available for linkage analysis). Of the 50 HPC families included in the genomic scan (paper I), nine families included at least one FDR of a prostate cancer case with a confirmed (through the Swedish Cancer Registry) gastric carcinoma. Non-parametric multipoint linkage analysis in these families identified only one chromosomal region with a LOD score over 1. At marker DXS1068, located on chromosome Xp21, a LOD score of 1.65 (P = 0.003) was observed. Notably, in the 41 families without confirmed cases of gastric carcinoma no evidence of linkage was found in this region. In addition, the nine gastric families provided no support for the two regions on chromosome 5 and 19 that were linked in the rest of the families, supporting the expected genetic homogeneity within these prostate-gastric families (Figure 5).

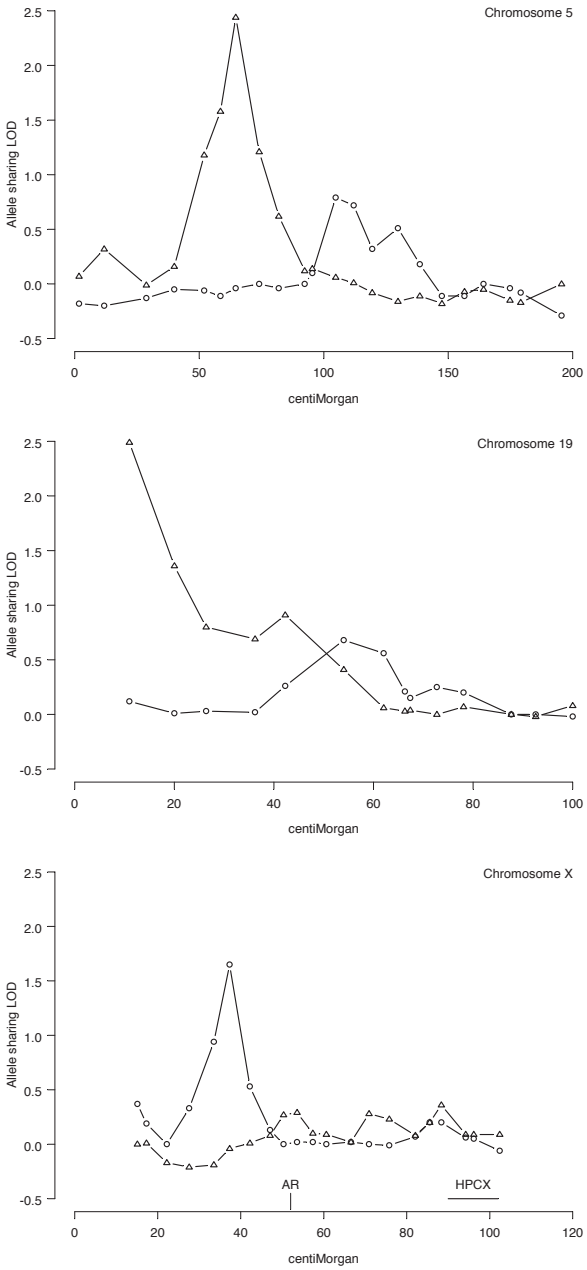

Figure 5. Result of multipoint non-parametric linkage analysis at chromosome 5, 19 and X in nine hereditary prostate cancer families with occurrence gastric cancer (O) and 41 hereditary prostate cancer families without gastric cancer cases (▵).

A simulation study was performed to evaluate the genome-wide statistical significance of the linkage finding at Xp21. We randomly simulated 1,000 replicates of the genome-wide scan data using the simulate option in the computer program MERLIN. In this procedure, marker data are simulated under the null hypothesis of no linkage or association to observed phenotype conditional on family structure and actual marker spacing and allele frequencies, as well as missing data patterns. In 464 out of 1,000 replicates a LOD score of 1.65 or above anywhere in the genome was observed, suggesting a genome-wide p-value of 0.46 for the observed linkage at Xp21. Although the observed linkage at Xp21 could represent a false positive finding it is intriguing. The absence of excess risk of gastric cancer among female relatives is compatible with an X-linked recessive mode of inheritance in these families. In three of the nine families with gastric cancer cases no evidence of male-to-male transmission was observed and two of these families provided positive scores at Xp21. Additional linkage studies utilizing stratification based on occurrence of gastric carcinoma are needed to clarify the significance of this finding.

Linkage to chromosome X has been reported previously. In a combined analysis of 360 families from US, Sweden, and Finland significant linkage to Xq27-28 (HPCX) was reported [77]. However, more than 60 cM separates HPCX from our observed linkage region suggesting different susceptibility genes for these loci. The AR gene has been hypothesized to play a key role in prostate cancer etiology through its involvement in the androgenic response. Variable number of trinucleotide repeat lengths (CAG and GGC) has been associated with prostate cancer risk in several studies, but not all (see Simard et al. [119] for a review). Interestingly, Chang et al. [167] reported positive but weak linkage in the AR region (HLOD = 0.49, P = 0.12); however, stronger evidence for linkage was found in the subset of families whose proband had short GGC repeats (HLOD=0.70, P=0.07) or by using a male-limited X-linked transmission/disequilibrium test (z'=2.65, P = 0.008). High level of AR expression in gastric cancer tumors has been associated with metastasis and worse prognosis [168], and malignant evolution in gastric tumors has been reported to be associated with extensive loss of heterozygosity of the X chromosome [169]. The AR gene is located on chromosome Xq11-12, approximately 10 cM from our linkage region, and represents an interesting candidate gene for the observed co-aggregation of gastric and prostate cancer.

In order to evaluate malignancies associated with HPC our study design has several advantages. First, analysis of malignancies associated with HPC is most efficiently performed in relatives of prostate cancer cases affected with HPC. Furthermore, the existence of nation-wide population- and cancer registers in Sweden provides valuable tools both for population-based sampling of high-risk families and for unbiased information of incidence of other malignancies within families. Our study also had certain limitations. First, not all families were ascertained in an unbiased manner that could introduce selection bias into the analyses. However, subset analysis of gastric cancer risk in population-based families provided no indication of such a bias. Secondly, we were not able to locate all FDRs of prostate cancer patients in our HPC families; yet, with a detection rate over 90% we are confident that this has no major impact on our conclusions. Thirdly, utilizing families heavily affected with disease could introduce a bias due to increased medical surveillance among these individuals. It is our experience, from review of collected medical records from most families, that no such screening for cancer except for prostate carcinoma has been conducted within these families. Lastly and most impor-

tantly, we had a very limited number of HPC families with appearance of gastric cancer cases available for linkage analysis. This implies that we were underpowered to identify linkage at a significant level and prompts for the need to combine data from existing collection of HPC families.

In summary, we have provided strong evidence supporting a common genetic basis for both prostate cancer and gastric cancer. Previously reported excess risk of gastric cancer in relatives of HPC patients was confirmed. The elevated risk is found only in male relatives and is of equal magnitude in both early and late onset prostate cancer families. Linkage analysis indicated a novel susceptibility locus at Xp21 predisposing to both prostate and gastric cancers. These findings encourage further evaluation of HPC families with occurrence of gastric cancer in order to advance our understanding of the complexities of prostate cancer genetics.

Discussion and perspectives

During the last decade tremendous efforts have been made to localize prostate cancer susceptibility genes, however — no major predisposing gene has yet been identified. This is despite the strong evidence supporting the existence of a hereditary form of prostate cancer. Increased risk for prostate cancer attributed to a positive family history has been consistently reported across different ethnical populations [38] and twin-studies clearly indicates that a substantial proportion of the familial aggregation is due to genetic factors [36, 39]. However, given the many reported prostate cancer susceptibility loci, and the difficulty of finding susceptibility genes within these regions, it seems likely that the major part of inherited forms of prostate cancer is due to multiple genes with small to moderate effect on disease risk.

Considering all the factors contributing to the complexity of prostate cancer linkage analysis, the success of future efforts will depend on our ability to reduce the genetic heterogeneity among studied families and thereby increase the linkage signal. Several approaches to increase homogeneity are available including:

1. Stratification on clinical characteristics
2. Stratification on family characteristics
3. Stratification based on occurrence of other malignancies
4. Population isolates

The major problem with the first approach is the complete lack of clinical characteristics separating hereditary forms of prostate cancer from sporadic forms. New knowledge providing criteria that with high specificity and sensitivity distinguish between sporadic and inherited forms (and even between different inherited forms) of prostate cancer would be an invaluable asset in this context.

The second approach has been applied in earlier reported linkage studies. Although these attempts have revealed some new susceptibility loci, they have not contributed substantially to an increased consistency between studies. However, these analyses have not been performed under equal conditions regarding marker maps, stratifying criteria and statistical analysis methods. The International Consortium for Prostate Cancer Genetics currently plans combined analysis of most available HPC families, with predefined conditions of study design and analysis method. The large number of families (~1,200) included, and the stringent criteria for subset definitions, will provide a considerable increase in statistical power to detect susceptibility regions in this combined analysis.

Co-occurrence of other malignancies within HPC families has not been well exploited in linkage analysis. Cancer sites that have been considered in conjunction with prostate cancer include breast, ovarian and brain. In this thesis we have confirmed previously reported association between gastric cancer and HPC and also identified a novel susceptibility locus on chromosome X. Utilizing HPC families with appearance of gastric cancer cases is a promising strategy to increase genetic homogeneity and deserves future evaluation in combined sets of families.

Finally, families ascertained from population isolates are expected to be genetically more homogeneous compared to families collected from more outbred populations. This approach has been applied in susceptibility gene mapping for several complex diseases with successful results [163-165]. Recently, Laitinen et al. [163] reported the identification of haplotypes on chromosome 7p conferring risk to asthma-related traits. This discovery was initially based on a genome-wide search [166] in 86 families originating from an isolated population in eastern central Finland (Kainuu province). Initially, two chromosomal regions with moderate evidence of linkage were found (LOD = 1.4 and 0.9 on chromosome 4 and 7, respectively). After genotyping of additional markers in the two regions the significance of linkage increased considerably (LOD = 3.3) in a 20 cM region of chromosome 7p. Further genotyping with increased density of markers, with intermediate association analyses, finally revealed the disease-associated haplotypes. Importantly, the relative risk for asthma conferred by these haplotypes were moderate (range 1.4 to 2.5) and excess of IBD sharing of these haplotypes suggested that the majority of the initial linkage signal was because of the observed risk haplotypes. In accordance with prostate cancer, asthma is a complex disease with a proven genetic etiology and several chromosomal regions have been reported potentially linked to an increased susceptibility for asthma-related traits. Therefore, this successful mapping effort provides future hope for mapping of prostate cancer predisposing genes through linkage studies.

In our genome-wide scan two chromosomal regions were implicated. These need to be further evaluated using densely spaced markers before their potential importance in prostate cancer genetics can be evaluated. In addition, we are currently investigating possible relatedness among ascertained HPC families based on genealogical information from church records. Currently, four families with HPC from the region of Skellefteå have been merged into one pedigree with common ancestors in the middle of the 17th century (Figure 6). We believe that such large pedigrees will be highly valuable for prostate cancer susceptibility gene identifications.

Furthermore, the fast development in genotyping technology will in the future allow high-density searches of association in large case-control populations. Candidate gene approaches, based on biological pathways relevant in prostate cancer etiology, have been applied in numerous studies with mixed results. However, in most of these efforts only variation in coding and promoter regions have been studied and the complete genetic variation attributable to all variants within the gene has not been evaluated. Successful assessment of candidate genes in association studies will essentially depend on complete information of all the genetic variation present, large sample sizes and high quality of genotyping.

In summary, although our understanding of the genetics underlying inherited forms of prostate cancer has grown during the past decades, it is clearly limited. However, future efforts utilizing efficient strategies to decrease the complexity of prostate cancer genetics will likely succeed in the unraveling of the genetic influences on this common disease.

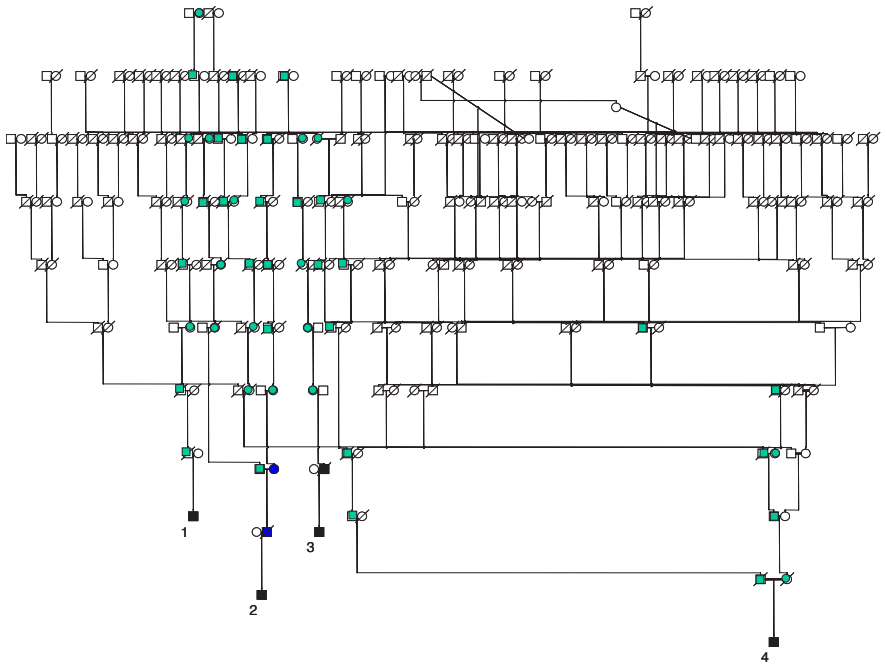

Figure 6. Four families with HPC from the region of Skellefteå. Based on genealogical information from church records these families have been merged into one pedigree with common ancestors in the middle of the 17th century.

Conclusions

- Genome-wide searches for prostate cancer susceptibility genes have identified several chromosomal regions in different populations, indicating the likely heterogeneous genetic basis of this disease and the existence of multiple genes with moderate effect. In Sweden, chromosome 5q and 19p represents the regions most likely to harbor susceptibility genes predisposing to prostate cancer.
- A linkage analysis of densely spaced microsatellite markers on chromosome 8p22-23 has confirmed previously reported linkage to this region.
- In a large population-based association analysis only limited support was obtained for the hypothesis that common sequence variants in the RNASEL gene are associated with prostate cancer risk. In addition, no support was found for a role of the rare truncating mutation E265X in familial aggregation of this disease. Considering the large and well characterized study population and the high quality in genotyping, these results provide strong evidence against a role of RNASEL in prostate cancer etiology in Sweden.
- A comprehensive evaluation of malignancies associated with HPC supported the existence of a common genetic basis for gastric and prostate cancer. A genomic scan of nine HPC families with confirmed cases of gastric carcinoma indicated a novel susceptibility locus on chromosome Xp21.

Populärvetenskaplig sammanfattning (summary in Swedish)

Prostatacancer utgör ett stort hälsoproblem som en av de vanligaste cancerformerna bland män i de flesta industrialiserade länderna. Det uppskattas att det under år 2000 upptäcktes över 500,000 nya fall av prostatacancer över hela världen, samt att 240,000 män avled i denna sjukdom detta år. Ett flertal epidemiologiska studier har entydigt visat att män med nära släktingar vilka drabbats av prostatacancer har en förhöjd risk att själva insjukna. Att en betydande del av denna riskökning är genetiskt betingad stöds av resultat från tvillingstudier och så kallade segregationsanalyser och det uppskattas idag att ca 5-10% av all prostatacancer orsakas av ärftliga faktorer.

De senaste två decennierna har betydande ansträngningar gjorts för att identifiera gener vilka orsakar prostatacancer. Med så kallade kopplingsanalyser har ett flertal områden på olika kromosomer identifierats som troliga att innehålla prostatacancergener. Det första området lokaliserades hösten 1996 till den långa armen av kromosom 1. Efter intensiva studier av detta område presenterades sex år senare en gen kallad RNASEL som en kandidatgen för detta område. Efter denna upptäckt har ytterligare två gener föreslagits som möjliga prostatacancergener, en lokaliserad på kromosom 8 samt en på kromosom 17. Försök att verifiera dessa fynd i oberoende populationer har dock varit motsägelsefulla och det existerar idag inga kända gener vars betydelse för prostatacancer är odiskutabel. Värdet av att identifiera dessa gener är stort, både vad gäller möjligheten att diagnostisera prostatacancer på ett tidigt och botbart stadium samt för att öka förståelsen av de biologiska orsakerna till denna sjukdoms uppkomst.

I denna avhandling har olika aspekter av de genetiska grunderna till uppkomsten av prostatacancer utvärderats. I svenska familjer med frekvent förekomst av prostatacancer har nedärvningsmönster studerats med hjälp av genetiska markörer utspridda över samtliga kromosomer. Två områden med trolig förekomst av prostatacancergener lokaliserades, ett område på kromosom 5 samt ett på kromosom 19. Dessa resultat stöds av liknande fynd från andra forskargrupper och mer detaljerade analyser av dessa två områden är planerade. Ett specifikt område på kromosom 8 har studerats. Resultatet verifierar tidigare gjorda fynd och uppmuntrar fortsatta studier av detta område. I en populationsbaserad studie av 1,636 män med prostatacancer samt 801 friska män genomfördes en systematisk utvärdering av RNASEL genen. Den generella slutsatsen från denna analys är att RNASEL har en mycket begränsad betydelse för uppkomsten av prostatacancer i Sverige. En utvärdering av cancerformer associerade med ärftlig prostatacancer konfirmerade tidigare rapporterad överrepresentation av magsäckscancer. En kopplingsanalys av familjer med förekomst av både prostata- och magsäckscancer identifierade ett ej tidigare rapporterat område på kromosom X.

En växande kunskap talar för att ett flertal gener lokaliserade på olika kromosomer ligger bakom den medfödda benägenheten för prostatacancer. I Sverige representerar kromosom 5 och 19 de mest troliga områdena för prostatacancergener. Kunskapen att prostata- och magsäckscancer har en gemensam genetisk grund är av stort värde i framtida studier av de genetiska orsakerna till prostatacancer.

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