Am. J. Hum. Genet. 65:134–140, 1999

In Swedish Families with Hereditary Prostate Cancer, Linkage to the *HPC1* Locus on Chromosome 1q24-25 Is Restricted to Families with Early-Onset Prostate Cancer

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Summary

Prostate cancer clusters in some families, and an estimated 5%–10% of all cases are estimated to result from inheritance of prostate cancer–susceptibility genes. We previously reported evidence of linkage to the 1q24-25 region (*HPC1*) in 91 North American and Swedish families each with multiple cases of prostate cancer (Smith et al. 1996). In the present report we analyze 40 (12 original and 28 newly identified) Swedish families with hereditary prostate cancer (HPC) that, on the basis of 40 markers spanning a 25-cM interval within 1q24-25, have evidence of linkage. In the complete set of families, a maximum two-point LOD score of 1.10 was observed at D1S413 (at a recombination fraction [θ] of .1), with a maximum NPL (nonparametric linkage) *Z* score of 1.64 at D1S202 ($P = .05$). The evidence of linkage to this region originated almost exclusively from the subset of 12 early-onset (age <65 years) families, which yielded a maximum LOD score of 2.38 at D1S413 ($\theta = 0$) and an NPL *Z* score of 1.95 at D1S422 ($P = .03$). Estimates from heterogeneity tests suggest that, within Sweden, as many as 50% of early-onset families had evidence of linkage to the *HPC1* region. These results are consistent with the hypothesis of linkage to *HPC1* in a subset of families with prostate cancer, particularly those with an early age at diagnosis.

Received November 20, 1998; accepted for publication April 23, 1999; electronically published May 21, 1999.

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0002-9297/99/6501-0018$02.00

Introduction

Several epidemiological studies have demonstrated that prostate cancer aggregates in some families. Estimates of the relative risk for first-degree relatives of men with prostate cancer has been in the range 1.5–8.7 (Steinberg et al. 1990; Spitz et al. 1991; Grönberg et al. 1996). In general, the relative risk increases with an increase in the number of affected individuals in the families and with a decrease in the age at diagnosis of prostate cancer in relatives (Steinberg et al. 1990). Segregation analyses in families with prostate cancer suggest that hereditary prostate cancer (HPC) is inherited in an autosomal dominant mode (Carter et al. 1992*a*; Grönberg et al. 1997*a*). Other epidemiological studies have suggested that a recessive or X-linked inheritance also is present in a subset of families with HPC (Monroe et al. 1995; Narod et al. 1995), since the relative risk for an individual with an affected brother are higher than that for an individual with an affected father. This observation has very recently been confirmed through linkage analysis (Xu et al. 1998)

In 1996 the first prostate cancer–susceptibility locus (*HPC1*) was mapped, to chromosome 1q24-25 (Smith et al. 1996). The initial linkage was reported in 91 families with HPC (i.e., families with at least three members effected with prostate cancer) that were from North America or Sweden. The maximal multipoint LOD score was 5.43 under heterogeneity. In a follow-up study (Grönberg 1997*b*), the majority of the evidence for linkage was derived from families with a greater number (i.e., at least four) of affected individuals who had early-onset (mean age <65 years) prostate cancer. So far, five independent data sets have been reported that have attempted to confirm linkage to chromosome 1q24-25. Two of the studies have been able to confirm linkage to *HPC1;* the first study was an analysis of 20 families with HPC and reported a maximum NPL (nonparametric linkage) *Z* score of 1.72 ($P = .0451$) at marker D1S466

(Cooney et al. 1997). The second study was of 56 families and reported an NPL Z score of 1.91 ($P = .036$) (Hsieh et al. 1997). Three other data sets have not been able to confirm the linkage to *HPC1* (McIndoe et al. 1997; Berthon et al. 1998; Eeles et al 1998).

There are several potential problems in the mapping of genes causing HPC. Prostate cancer is diagnosed at a late age (in Sweden, mean age at diagnosis is 74 years)—and HPC is reported as having been diagnosed only 7–8 years earlier. Prostate cancer is also a common disease in elderly men, with a lifetime risk, in the Swedish population, of 15% at age 85 years. These facts make the problem of phenocopies potentially large, since it is difficult to distinguish, solely on the basis of age, between sporadic and hereditary cases. Genetic heterogeneity is an obvious problem, since linkage to *HPC1* has been reported in only ~30% of families with HPC (Smith et al. 1996).

In the present study, we have studied 40 families with HPC that have been collected throughout Sweden, in an attempt to confirm linkage to *HPC1* on 1q24-25, with special attention to age at diagnosis in the families.

Families and Methods

Families

Families with HPC have been examined by our institution since 1995 and have been identified mainly on the basis of referrals from urologists throughout Sweden. We have received ~300 referrals during the past 3 years. From these referrals we have selected for our study 40 families with HPC, from which we have collected at least three DNA samples (blood or tissue) from each of the men with prostate cancer (table 1). Twelve of these families were included in our earlier report (Smith et al. 1996). However, in two families the affected status has been changed since the first report (two men have subsequently been diagnosed as having prostate cancer), and, in all families, more markers have been typed to establish haplotypes. Blood has been collected for DNA extraction, both from affected men and their spouses and from the children, so that inferred genotypes can be determined. From the files of different pathology departments in Sweden, we have extensively collected paraffin-embedded tissue samples from men with prostate cancer. A single experienced pathologist reviewed all tissue samples, and microdissection was performed to enrich tumor tissue versus normal tissue. Only normal tissue was used for genotyping. All diagnoses of prostate cancer in the families were confirmed both by reference to the Swedish National Cancer Registry and direct examination of medical records.

Genotyping

Details of DNA extraction and genotyping have been described in an earlier report (Smith et al. 1996). Genotyping was performed by means of an ABI Prism 377 DNA sequencer, and data analysis was performed with ABI Prism GeneScan Analysis™ 2.1 and ABI Prism Genotyper™ 2.0, to allocate the alleles. The genotyping was performed both at Umeå University and at the National Human Genome Research Institute, with the same markers and PCR protocol.

Genotyping of 28 families (families 2–40) was performed with 40 multiplex markers that were from the D1S2799–D1S1660 interval and that spanned the 25-cM region of interest. A list of markers is available from the authors on request. In 12 families (families 41–55), only five markers were genotyped: D12S452, D1S218, D1S158, D1S422, and D1S413.

Statistical Analysis

Both parametric and NPL approaches were used in this study. Both a parametric two-point analysis and multipoint analysis were used, as described in detail in previous reports (Smith et al. 1996; Grönberg et al. 1997*b*), and a dominant disease allele with a frequency of .003 was assumed. Affected men were assumed to be carriers of a rare dominant gene, with a fixed 15% phenocopy rate. All unaffected men <75 years of age and all women were assumed to be of unknown phenotype. In men >75 years of age, the lifetime penetrance of gene carriers was estimated to be 63%, and the lifetime risk of prostate cancer for noncarriers was 16% in this age class. The computer software packages FASTLINK and ANALYZE were used for two-point analysis. In both

Table 1

Characteristics of 40 Swedish Families with HPC

	Families in Which Mean Age at Onset Is		
	<65 years	>65 years	Overall
No. of families	12	28	40
Mean age at onset (years)	61.0	71.1	68.0
No. affected/family (range)	3.83 (3–6)	4.78 (3–10)	4.5 (3–10)
No. affected and typed/family (range)	3.5 (2–6)	3.75 (3–9)	3.68 (2–9)

the multipoint parametric analysis and the NPL analysis and when creating haplotypes, we used all the information from the 40 typed markers (in families 2–40), together with the software package GENEHUNTER. The admixture test as implemented in HOMOG (Ott 1985) was used to test for genetic heterogeneity and to estimate the proportion of linked families (α). Blood samples from 50 unaffected Swedish men were used as controls, for calculation of the allele frequencies in the Swedish population, for the 40 markers in the analysis.

Results

The results of the parametric and the nonparametric analysis of 8 selected markers ranging from D1S452 to D1S413 are summarized in Table 2. For all 40 families, a maximum multipoint NPL Z score of 1.64 ($P = .05$) at D1S202 and a maximum two-point LOD score (at a recombination fraction [θ] of .1) of 1.10 at D1S413 were observed. The maximum NPL Z score for the 28 families not reported previously was 1.45 ($P = .07$) at marker D1S2877 (~5.0 cM telomeric of D1S158).

When the data were stratified on the basis of mean age at diagnosis of prostate cancer in the families (i.e., <65 years [$n = 12$] vs. >65 years [$n = 28$]), the evidence of linkage to the 1q24-25 region was seen mostly in families with an early age at onset. In the 12 families with an early age at onset, the NPL Z score was significant for several markers in the region, with a maximum multipoint NPL Z score of 1.95 ($P = .03$) at D1S422, and the multipoint parametric analysis yielded a peak LOD score of 1.09 ($P = .05$) close to marker D1S413. Most of the LOD scores in the parametric analysis were positive for the markers in this region in this subset of families, with a maximum two-point LOD score of 2.38 at D1S413 ($\theta = 0$). When the three families (families 2, 17, and 27) previously reported (Smith et al. 1996) were excluded from the analysis, the maximum NPL Z score for the remaining nine families was 1.17 at D1S422 ($P = .12$). By contrast, there was virtually no evidence for linkage in families in which the average age at onset was >65 years. The scores are negative in these 28 families—a maximum two-point LOD score of only 0.35 at D1S2883 and an NPL Z score of 0.85 ($P = .19$) at D1S202 were observed. Figure 1 shows the multipoint NPL Z scores for all 40 typed markers, for both the early-onset families and the late-onset families. When the proportion of families with linkage was estimated

Table 2

Results of Parametric Two-Point Linkage and of Multipoint Analysis and Nonparametric Multipoint Linkage, for Eight Markers in 1q24-25 in 40 Swedish HPC Families, by Mean Age at Diagnosis of Prostate Cancer

	Two-Point LOD Score at θ =			Parametric Multipoint	Nonparametric Analysis	
Group and Marker (Position [cM])	.0	.1	.3	LOD Score	NPL Z	P
All families:						
D1S452 (.0)	−13.12	−1.47	.21	.00	−.21	.57
D1S218 (2.3)	−13.19	−2.78	−.16	.00	−.44	.66
D1S2883 (7.5)	−5.86	−.11	.55	.12	.84	.20
D1S212 (7.6)	−8.94	−.55	.46	.02	.53	.29
D1S158 (14.9)	−21.70	−3.21	−.14	.11	.95	.17
D1S202 (17.6)	−5.89	−.23	.20	.31	1.64	.05
D1S422 (21.1)	−11.26	−.64	.54	.23	1.39	.09
D1S413 (26.5)	−2.88	1.10	.63	.28	.75	.22
Families in which age at onset is <65 years ($n = 12$):						
D1S452 (.0)	−3.73	−.49	.05	.00	−.01	.48
D1S218 (2.3)	−2.18	−.43	.07	.09	.29	.37
D1S2883 (7.5)	.21	.58	.2	.88	1.49	.08
D1S212 (7.6)	1.17	.96	.31	.88	1.48	.08
D1S158 (14.9)	−2.89	−.11	.24	1.05	1.73	.05
D1S202 (17.6)	−.05	.19	.06	1.04	1.71	.05
D1S422 (21.1)	−.54	1.03	.39	1.05	1.95	.03
D1S413 (26.5)	2.38	1.70	.48	1.09	1.79	.04
Families in which age at onset is >65 years ($n = 28$):						
D1S452 (.0)	−9.44	−1.5	−.05	.00	−.24	.58
D1S218 (2.3)	−10.51	−1.83	−.06	.00	−.71	.76
D1S2883 (7.5)	−6.07	−.69	.35	.00	.04	.46
D1S212 (7.6)	−10.11	−1.51	.15	.00	.32	.61
D1S158 (14.9)	−18.71	−3.1	−.36	.00	.01	.47
D1S202 (17.6)	−5.84	−.42	.14	.00	.84	.19
D1S422 (21.1)	−10.82	−1.67	.15	.00	.40	.33
D1S413 (26.5)	−5.08	−.62	.15	.00	−.26	.59

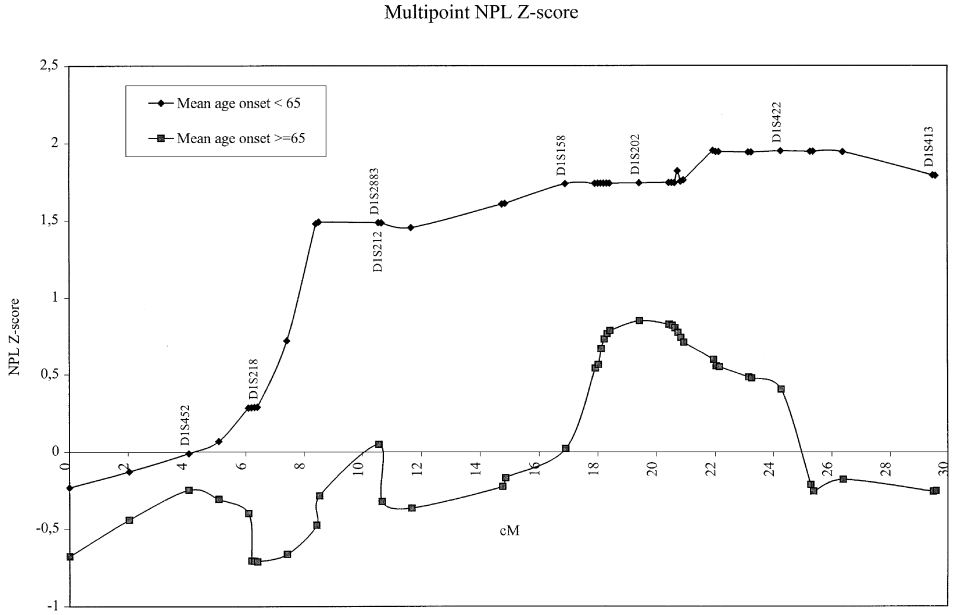

Figure 1 Multipoint NPL *Z* scores for 40 markers in the 1q 24-25 region in 40 Swedish families with HPC, divided by mean age at onset of diagnosis of prostate cancer in the family.

under the assumption of heterogeneity, α was 50% in the early-onset families and was 0% in the late-onset families.

In 8/12 (67%) of the early-onset families, all affected individuals within a single family share the same haplotype in the entire 1q24-25 region; however, we observed no founder haplotype in these families. By contrast, only 3/28 (11%) of the affected men in the late-onset families share the same haplotype within the *HPC1* region. In figure 2, the largest family with HPC in our study (family 032) is shown with haplotypes for seven markers spanning the *HPC1* region. This family has prostate cancer in two generations and three different cousinships. The mean age at onset in this family is 71 years. Eight of the 10 men affected with prostate cancer in this family share the same haplotype in region 1q24-25. However, there are two men with prostate cancer in two different cousinships not sharing this haplotype, thereby making both the overall multipoint LOD score and the NPL *Z* score negative for this family. Since the lifetime risk of prostate cancer in Sweden is 13% at age 85 years, it is possible that 1 or 2 of the 10 affected men have sporadic prostate cancer and that the family has linkage to the *HPC1* region.

Discussion

In the 40 Swedish families with HPC, the multipoint NPL *Z* score is 1.64 ($P = .05$). Of these 40 families, 12 were included in the first report of linkage to the *HPC1* locus (Smith et al. 1996), and the maximum NPL *Z* score of the remaining 28 families is 1.45 ($P = .07$). In a follow-up study of the 79 North American families included in the first study (Grönberg et al. 1997*b*), the evidence of linkage to the *HPC1* locus was mainly confined to early-onset families. The NPL *Z* score was 5.57 ($P = .000009$) for early-onset families and was only 0.70 ($P = .23$) in late-onset families. In the present report, there are 12 early-onset families (9 of which had not been reported previously) in which the NPL *Z* score is significant. The maximum NPL *Z* score for these families is 1.95 ($P = .033$) at D1S422 (table 2 and fig. 1), with an estimated 50% of the families linked when heterogeneity is assumed. We have genotyped only 12 families with early-onset HPC, so the LOD scores and NPL *Z* scores are not to be expected to be higher, since the average score per family is almost equivalent to that in the North American families in the first linkage report (Smith et al. 1996). In most families, 40 markers spanning a 25-cM region have been typed, to ensure that maximum information is obtained from each family and to provide a stable linkage result. A fact that further supports evidence for linkage in these early-onset families is that, in 8/12 (67%) of them, all affected men share the same haplotype in this region. It is not surprising that linkage to *HPC1* is strongest in early-onset families, since similar findings have been presented in the initial reports of linkage to the breast cancer–susceptibility genes *BRCA1* and BRCA2. In the Breast Cancer Linkage Consortium's report of 214 fam-

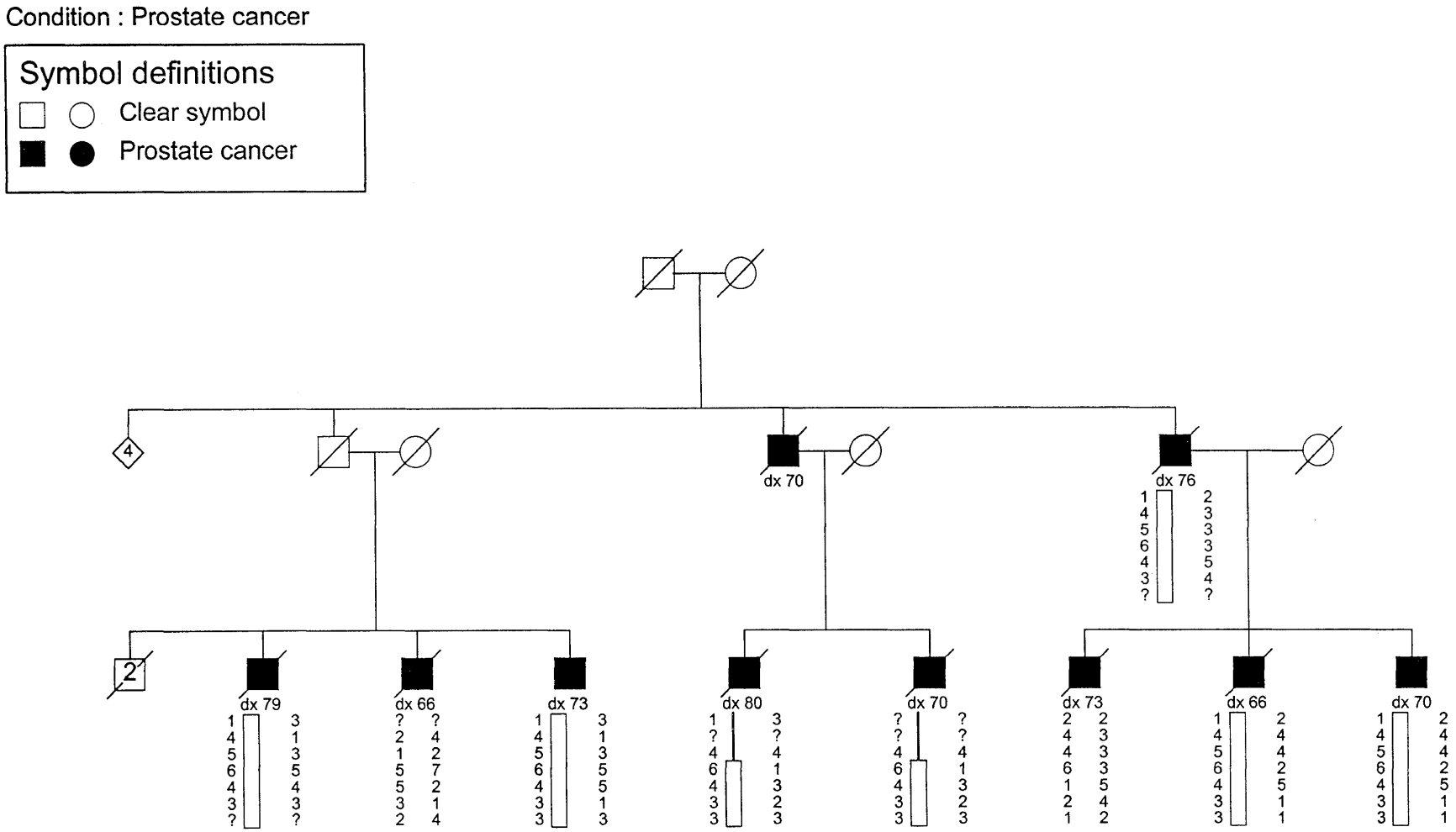

Figure 2 Family 032, with HPC and haplotypes following markers D1S452, D1S2883, D1S212, D1S158, D1S202, D1S422, and D1S413 in the 1q24-25 region

ilies with hereditary breast cancer, the strongest evidence of linkage to *BRCA1* was in families with breast/ovarian and in families with early-onset (at age <45 years) breast cancer (Easton et al. 1993). Almost no evidence of linkage was seen in families in which the average age at onset was >45 years, an age that is 20 years younger than the average age at onset of sporadic breast cancer. When the *BRCA1* and *BRCA2* genes were later identified, mutations were identified mostly in families with a very early age at onset—for example, 44.5 years in 34 Swedish families with *BRCA1* mutations (Johannsson et al. 1996). The same results have been seen in hereditary nonpolyposis colon cancer, in which the majority of families with *MLH1* and *MSH2* mutations are in early-onset (mean age <50 years) families that meet the Amsterdam criteria (Wijnen et al. 1997).

There are conflicting data with regard to linkage to the *HPC1* locus. Two reports have been able to confirm the linkage (Cooney et al. 1997; Hsieh et al. 1997), but three others have been unable to do so (McIndoe et al. 1997; Berthon et al. 1998; Eeles et al. 1998). There are several possible explanations for these discrepant results. Since the evidence for linkage, in both the Swedish families that we have studied and the families studied at Johns Hopkins University, is provided mainly by families with early-onset (age <65 years) prostate cancer, it is crucial to investigate HPC families meeting these criteria, to be able to confirm any linkage. It is difficult to estimate, on the basis of the published reports, what proportion of families fulfill these criteria. The number of genotyped affected persons in each family is also of importance, since strong evidence of linkage is difficult to obtain if only two or three affected individuals are genotyped in each family. In the families that we studied, we genotyped, on average, 3.83 affected men, since we could obtain and use archival tissue samples from deceased men with prostate cancer. Age at diagnosis of prostate cancer is highly dependent on the diagnostic traditions among physicians in a particular area. The introduction of PSA (prostate-specific antigen) screening in the general population—and, in particular, in high-risk families—will lower the age at diagnosis by ⩾5 years, on average (Carter et al. 1992*b*) On the other hand, in areas in which men do not seek medical attention until they experience pain due to bone metastasizes or other symptoms, the diagnosis is probably 10 years later, compared with cases detected by PSA screening in asymptomatic men (Carter et al. 1992*b*). This is illustrated by family 032 (fig. 2), which originated from a rural area in northern Sweden and in which several men with prostate cancer were diagnosed at late stages, thus substantially affecting the mean age at diagnosis of prostate cancer. A great majority of the families in the present and in the initial linkage report (Smith et al 1996) were identified before the introduction of PSA screening. This might not be the case in some studies in which no linkage to the 1q 24-25 region was found (McIndoe et al 1997; Berthon et al. 1998).

It is also likely that the introduction of PSA screening increases the number of phenocopies in the families with HPC, since the recommendation today is that annual screening in these high-risk families should begin at age 40 years. In later-onset families, the likelihood of phenocopies is even higher, which makes it even more difficult to find linkage in those families. Locus heterogeneity in families with HPC is obvious, since, even in highly selected families, the *HPC1* locus cannot be responsible for the disease in >50% of the families with HPC. A recent report (Berthon et al. 1998) has suggested this; it shows that both a multipoint LOD score, under the assumption of heterogeneity, of 2.2 and a two-point LOD of 2.7 at 1q42-43, suggesting an additional locus more distal on the 1q arm.

In conclusion, we have found significant evidence of linkage to the *HPC1* locus on 1q24-25 in Swedish families with HPC that have an early age at onset (i.e., <65 years). However, the mapping of prostate cancer–susceptibility genes is going to be difficult, owing to the following factors: locus heterogeneity, a high phenocopy rate, the fact that no specific phenotypic features of HPC have yet been identified, and the difficulty of finding large informative families with many affected individuals. These factors might explain the conflicting results of linkage to the *HPC1* locus that have been published by different groups thus far.

Acknowledgments

We would like to thank all the family members who participated in this study. In addition, the assistance of physicians across Sweden who referred patients to this study is gratefully appreciated. This work was supported by grants from the Swedish Cancer Society (Cancerfonden), from the Lion's Cancer Foundation, from the Department of Oncology, from Umeå University, from the Swedish Medical Association, and from the National Human Genome Research Institute (National Institute of Health, Bethesda).

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