Clinical Characteristics of Prostate Cancer in an Analysis of Linkage to Four Putative Susceptibility Loci


ABSTRACT

Purpose: Hereditary prostate cancer is an etiologically heterogeneous disease with six susceptibility loci mapped to date. We aimed to describe a collection of high-risk prostate cancer families and assess linkage to multiple markers at four loci: HPC1 (1q24–25), PCaP (1q42.2–43), HPCX (Xq27–28), and CAPB (1p36).

Experimental Design: Medical record data on 505 affected men in 149 multiply-affected prostate cancer families were reviewed, and correlations of clinical traits within each family were calculated. Logarithm of odds (LOD) score and nonparametric (NPL) linkage analyses were performed; white families were stratified by age of diagnosis, grade and stage of disease, and evidence of linkage to the other loci to increase genetic homogeneity.

Results: Age at diagnosis was the most correlated clinical trait within families. A maximum NPL score of 2.61 (P = 0.007) appeared to confirm HPC1 linkage for families that had a prevalence of high-grade or advanced-stage prostate cancer and which were not likely to be linked to PCaP, HPCX, or CAPB. Because the NPL scores improved when families more likely to be linked to the other loci were excluded, HPC1 may act independently of the other loci. The relationship of HPC1 and aggressive disease was strongest in families with median age at diagnosis ≥65 years (NPL, 3.48; P = 0.0008).

Conclusions: The current results suggest that HPC1 linkage may be most common among families with more severe prostate cancer. Stratification by clinical characteristics may be a useful tool in prostate cancer linkage analyses and may increase our understanding of hereditary prostate cancer.

INTRODUCTION

Prostate cancer is a significant cause of morbidity and mortality in the United States; there were an estimated 198,100 new prostate cancer cases and 31,500 deaths attributable to the disease in the year 2001 (1). In addition to age and race, family history is a strong risk factor, indicating that genetics plays an important role in prostate cancer (2–4). Approximately 9% of cases are expected to occur in families with several affected family members (5). It is likely that hereditary prostate cancer is a genetically heterogeneous disease with several genes conferring susceptibility. Although numerous case-control studies have evaluated risk associated with common polymorphisms of several genes (6–15), none have been shown to be causal in high-risk families.

The existence of rare, highly-penetrant dominant susceptibility genes for prostate cancer has been supported by segregation analyses of high-risk prostate cancer families (5, 16–19). Genome-wide linkage analyses have implicated several regions that may be involved in inherited prostate cancer (Ref. 14, 20–26; reviewed in Refs. 27, 28). The first putative locus identified was HPC1 in the 1q24–25 chromosomal region (20). Confirmation linkage studies of HPC1 have had mixed results (21, 29–38), and a combined dataset of 772 families estimated that prostate cancer was linked to HPC1 in 6% of the families studied (39). Evidence for additional loci has been observed in other datasets: PCaP at 1q42.2–43, particularly among younger-onset families (21); HPCX at Xq27–28 (40); CAPB at 1p36 among prostate cancer families with a history of brain cancer (41); HPC20 at 20q13 (24); and HPC2/ELAC2 at 17p, which was cloned recently (14). Confirmation studies at these loci have produced disparate results; a small proportion of families may be linked to each of these loci (15, 34, 35, 37, 38, 42–49).

The lack of consistent confirmation of linkage for the putative loci HPC1, PCaP, HPCX, CAPB, and HPC20 may be because individual research groups are analyzing unique datasets of families. To the extent that clinical characteristics vary between datasets and reflect the influence of different genetic factors, such variation may confound linkage studies. Describing the clinical features of prostate cancer in families studied may assist in elucidating reasons for discrepant confirmation results. The lack of consistent confirmation may also be attributable to an excess of linked families in the initial datasets. If the frequency of linkage in high-risk families in general is lower, confirmation datasets may lack sufficient power to detect these loci. The power of linkage analysis can be increased not only by increasing sample size but also by the creation of genetically homogeneous subsets of families (50–52). Grouping families with similar clinical characteristics has proven useful in eluci-
dating linkage to susceptibility genes for several diseases (53–56). Age at diagnosis, number of affected men, pattern of disease transmission, and the presence of other cancers have been used to stratify prostate cancer families (21, 39, 41); however, consideration of additional clinical characteristics, such as tumor grade and cancer stage, may further improve power to confirm linkage findings. The goal of the current study was to describe the clinical characteristics of 149 high-risk prostate cancer families and to incorporate these data into examination of linkage to the first four identified putative loci, HPC1, PCaP, HPCX, and CAPB.

SUBJECTS AND METHODS

Study Subjects

Families with multiple cases of prostate cancer were recruited from 1995 to 2000 to participate in the Prostate Cancer Genetic Research Study (PROGRESS). Family inclusion criteria were: (a) three or more first-degree relatives with prostate cancer; (b) prostate cancer in three successive generations; or (c) prostate cancer in two living first-degree relatives diagnosed before age 65 years. All procedures were approved by the Fred Hutchinson Cancer Research Center institutional review board, and written informed consent was obtained from each family member. Details of this ascertainment and data collection are available elsewhere (29, 42).

Clinical Data

Prostate cancer diagnoses were confirmed by medical records and/or death certificates. When medical records could not be obtained, age at diagnosis was determined from written self-report. Additional clinical data were abstracted from medical records including results of digital rectal exam, prediagnosis PSA³ level, date of diagnosis, Gleason score on biopsy, Gleason score on prostatectomy, tumor grade, clinical stage, pathological stage, and primary treatment. Unaffected men 45 years of age or older were coded as having unknown affection status if they indicated that they had not had a PSA test within the last 5 years, if they did not know if they had had a PSA test, or if they had an elevated or abnormal PSA and did not have physician-diagnosed benign prostatic hyperplasia.

Tumor Grade. Two prostate cancer grading systems are currently in use, the American Joint Committee on Cancer’s grade I through IV system (57) and the Gleason grading system (Gleason score range, 2–10; Refs. 58–60). Because the larger tissue specimens from prostatectomy allow for fewer sampling errors than biopsy specimens, we used tumor grade from prostatectomy pathological reports, if available, and from biopsy pathological reports otherwise. Grade was classified as low-grade (well-differentiated, grade I, Gleason 2–4), moderate-grade (moderately differentiated, grade II, Gleason 5–6), or high-grade (poorly differentiated, grade III-IV, Gleason 7–10; Refs. 61, 62).

Tumor Stage. Clinical stage was recorded in terms of both the Tumor-Node-Metastasis and Jewett staging system. The Jewett staging system classifies prostate cancers as follows: stages A and B describe locally confined prostate cancers; stage C describes regionally spread cancer extending through the prostatic capsule or involving the seminal vesicles; and stage D represents prostate cancer that has metastasized to lymph nodes or distant sites (63). Stages C and D were considered to be advanced stages in this analysis. The Tumor-Node-Metastasis staging system is based on three significant events in the natural history of a cancer: local tumor growth (T), spread to regional lymph nodes (N), and distant metastases (M; Ref. 57). The current analysis is based on the pathological stage when available; otherwise the clinical stage was used.

Aggressive Disease. A binary variable indicating aggressive disease was created for each man with medical record data. If either high-grade disease (poorly differentiated, grade III-IV, Gleason 7–10) or advanced-stage disease (stage C or D) was noted, an affected man was considered to have had aggressive disease.

Genotyping

Individuals were genotyped using microsatellite markers in the HPC1 (1q24–25), PCaP (1q42.2–43), HPCX (Xq27–28), and CAPB (1p36) regions, including markers with peak LOD scores in the original linkage reports (20, 21, 40, 41): for HPC1, D1S1589, D1S2883, D1S2818, D1S2127, D1S318, and D1S1660; for PCaP, D1S235, D1S2785, D1S347, and D1S1609; for HPCX, DXS984, DXS8106, DXS8086, DXS1200, DXS297, DXS1193, DXS8069, and DXS8103; for CAPB, D1S1597 and D1S407. Intermarker distances were taken from the Marshfield Medical Research Foundation’s sex-averaged genetic map, and allele frequencies were determined using all individuals in the dataset (64, 65). Additional information about genotyping of DNA samples is summarized elsewhere (29, 42).

Statistical Analysis

Familial Correlations. Clinical features of affected men were described, including kappa statistics to assess the agreement between biopsy grade and prostatectomy grade. To assess familial clustering of each clinical characteristic, intraclass correlation coefficients among pairs of brothers were calculated with the program FCOR2, a component of S.A.G.E. 4.0 (66), and all pedigrees were weighted equally. The resulting statistic $r^2$ represents the similarity of a trait for all brother pairs in the dataset. Correlations were calculated for the continuous traits of age at diagnosis and Gleason score; for the dichotomous traits (yes, no) of high-grade, advanced-staged, and aggressive disease; and for the categorical trait of grouped PSA level (0–4, 4–9.99, 10–19.99, and 20+ ng/ml). Correlations of dichotomous traits were also calculated considering only pairs of men affected before certain ages (<60 years, ≥65 years, and >65 years).

Linkage Analysis. The LOD score method of linkage analysis was used to test the hypothesis of linkage between genetic markers at known locations and a putative prostate marker. The abbreviations used are: PSA, prostate-specific antigen; LOD, logarithm of odds; HLOD, heterogeneity LOD score; NPL, nonparametric linkage; ASP, affected sib-pair.

³Internet address: http://www.marshmed.org/genetics.
cancer susceptibility gene and to provide a maximum likelihood estimate of the recombination fraction (θ) between each marker and the putative gene. The model used for parametric LOD score analysis assumed age-dependent penetrance and autosomal or X-linked dominant inheritance of a disease allele with frequency equal to 0.003 (23). To eliminate false negatives attributable to differences in model specifications, exact models used in other studies that found significant linkage evidence at each locus were also used (20, 21, 40, 67). Parametric analyses were also performed assuming locus heterogeneity; the resulting HLOD is the maximum LOD score obtained over varying values of θ and the proportion of families linked (α). Two-point analyses (testing linkage of each marker independently) used the programs FASTLINK (68, 69) and HOMOG (70). Multipoint analyses (testing linkage of multiple markers simultaneously) used GENEHUNTER version 2.0 and GENEHUNTER-PLUS version 1.2 (X-linked) for parametric analysis, as well as NPL analysis, which does not require model specification (71–73). The resulting NPLALL score and corresponding P measure haplotype-sharing among affected individuals in each family (74).

**Family Stratification.** White families were stratified by median age at diagnosis (<60 years, 60–64 years, 65–59 years, and 70+ years), number of high-grade cases (0, 1, and 2), number of advanced-stage cases (0, 1, and 2), and the percentage of cases with aggressive disease (<33%, 33–66%, and >66%). Analyses at HPC1 were stratified by the most recent year of diagnosis per family to attempt replication of a recent report that evidence for HPC1 linkage was stronger among families with men diagnosed before 1990 than with families with men diagnosed in later years (75).

If a family’s prostate cancer is attributable to linkage at one of the four loci analyzed here, analysis at any of the other three loci would be confounded by the inclusion of this family. We sought to minimize such locus heterogeneity by repeating analysis at each locus when families with evidence for potential linkage to any of the other three loci were removed. A family was considered to have evidence for other potential linkage if a 2-point LOD score ≥0.1 was observed at any θ value at marker D1S518 in the HPC1 region, D1S2785 in the PCaP region, DXS984 in the HPCX region, or D1S407 in the CAPB region. This cutoff was chosen because approximately half of the dataset was removed for each analysis. This exceeded the expected proportion of families that were linked to other loci and therefore considered conservative. Because of the exploratory nature of this analysis and the high correlation between the multiple tests, Ps were not adjusted for the multiple strata considered, but they should be interpreted with caution considering the possible inflated type I error rate.

**RESULTS**

One hundred and forty-nine high-risk prostate cancer families (2410 individuals) including 662 affected men were ascertained nationally. There was an average of 4.2 affected men per family (range, 3–14), and the median age at diagnosis of sampled affected men per family was 67.0 years (range, 51.5–78.0). Six families were non-white. One thousand two-hundred and thirty-three people, including 514 affected men, were genotyped at HPC1, PCaP, HPCX, and CAPB markers.

**Clinical Characteristics**

Medical records confirming adenocarcinoma of the prostate were available for 505 affected men, with an average of 3.4 affected men/family having medical record data (range, 1–9). Table 1 describes the sources of disease confirmation and clinical data of these men. The majority (85%) of affected men were diagnosed after 1987, when PSA screening had come into common use as diagnostic tool; 364 affected men (72%) had an elevated PSA at the time of diagnosis. Only 56 men (11%), however, clearly had microscopic, unpalpable, unvisualizable, PSA-detected cancer (stage T1N0M0).

Tumor grade data were available for 471 men (93%; Table 1). Pathological analysis of the prostate after surgery can result in a different grade classification between the surgical specimen and the biopsy specimen in as much as 25–40% of cases (76–78). One hundred and fifty-seven men had both biopsy and prostatectomy Gleason scores available (186 men had biopsy Gleason scores only and 49 men had surgical Gleason scores only). Of the 157 men with both Gleason scores available, 52 men (33%) were “upgraded” when the entire prostate was examined, and 10 men (6%) were “downgraded”; a kappa statistic of 0.33 (P < 0.0001) indicates significant agreement between biopsy and prostatectomy grade.

Clinical stage was available for 471 men (93%; Table 1). Twenty-five men with unknown clinical stage, but treated with prostatectomy, were assumed to have had localized disease. In other reports, 50–59% of cases are upstaged at prostatectomy when pathological records indicate the cancer has spread more than was suspected at initial clinical examination (79, 80). Of 236 men in the current analysis with known clinical and pathological stage, 74 men (31%) were “upstaged” from having localized disease at prostatectomy, including 63 men (27%) found to have regional spread and 11 men (5%) found to have lymph node involvement or other metastases.

Among 452 men with known grade and stage, these two characteristics were not independent of each other (χ² = 26.2; P < 0.0001), a finding consistent with population-based data (81). Only 6% of men with localized disease had poorly differentiated tumors, whereas 20% of men with regional or distantly metastasized disease did. Considering grade and stage simultaneously, 206 men (41%) had aggressive disease (high-grade or advanced-stage disease).

Clinical data are summarized by family in Table 2. The vast majority of families are expected to include cases that were detected by PSA screening; all but three families included men diagnosed the 1990s. No significant association between median year of diagnosis and grade group was seen here. Forty-two of the 149 families (83 of the white families) were missing medical record data on at least one affected man. Because missing data could result in misclassification of families into clinical strata, we considered whether the amount of missing clinical data varied by family characteristics. Although more younger-onset
families were missing medical records than older-onset families ($\chi^2 = 9.29; P = 0.019$), the amount of missing medical records did not appear to be related to grade groups, stage groups, or aggressive-disease groups.

To test whether any clinical features cluster in families and thus are hypothesized to have the largest genetic component, correlations of clinical traits were calculated for pairs of affected brothers. Age at diagnosis showed the strongest correlation (646 pairs of brothers with age at diagnosis data, $r^2 = 0.43; SE, 0.05$). Using a categorical PSA variable, a correlation of 0.12 (265 pairs; SE, 0.09) was observed, suggesting some clustering of PSA level within families. Gleason score had a nonsignificant positive correlation (285 pairs; $r^2 = 0.08; SE, 0.10$). Correlations for grade, stage, and aggressive disease were positive but low; the strongest result was for grade (406 pairs; $r^2 = 0.08; SE, 0.05$). Because age at diagnosis was correlated in families, we also calculated correlations for grade, stage, and aggressive disease restricted to men diagnosed at certain ages. Tumor grade of men diagnosed at $\leq65$ years was more highly correlated (95 pairs; $r^2 = 0.28; SE, 0.26$) than grade among men diagnosed >65 years (148 pairs; $r^2 = -0.09; SE, 0.13$).

### Linkage Analyses

Consistent with our previously published findings using overlapping family sets (36, 41, 42, 45), there was no significant evidence for linkage to markers in any of the \text{HPC1}, \text{PCaP}, \text{HPCX}, and \text{CAPB} regions in unstratified analysis (detailed data not shown; see above references). The most suggestive result was for markers in the \text{CAPB} region with a maximum multipoint HLOD of 0.21 ($\alpha = 0.18$) and NPL of 1.50 ($P = 0.07$) at marker \text{D1S407}. At all loci, data were consistent with a linked subset of families with positive 2-point LOD scores at some $\theta$s >0 and some positive 2-point HLODs.

In an effort to improve locus homogeneity, analysis at each

### Table 1

Clinical characteristics of 505 men with prostate cancer in 149 families

<table>
<thead>
<tr>
<th>Clinical characteristic</th>
<th>Mean, median (range)</th>
</tr>
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<tbody>
<tr>
<td>Age at diagnosis ($n = 529)$, yr$^a$</td>
<td>65.8, 66.0 (40–87)</td>
</tr>
<tr>
<td>PSA level ($n = 374$)</td>
<td>25.4, 9.8 (0.9–1200.0)</td>
</tr>
<tr>
<td>Gleason score from biopsy ($n = 345$)$^b$</td>
<td>5.5, 6 (2–10)</td>
</tr>
<tr>
<td>Gleason score from prostatectomy ($n = 206$)$^b$</td>
<td>6.1, 6 (3–9)</td>
</tr>
</tbody>
</table>

$^a$ Age at diagnosis includes additional data from 24 men without medical records but with written self-report of age at diagnosis.

$^b$ A Gleason score is a measure from 2 to 10 of the amount of loss of differentiation in a tumor.

$^c$ Includes data on 16 men with an elevated PSA indicated in medical records but with exact PSA level unknown.

$^d$ Summary stage represents pathological stage for 237 for whom it was available; otherwise, clinical stage was used. Twenty-five men with unknown clinical and pathological stages who had surgery were assumed to have clinical stage A or B.
locus was repeated excluding families with 2-point LOD scores $\geq 0.1$ at the other three loci. This strategy should improve evidence for true linkage if the locus under consideration does not interact with the loci removed. Again, the most suggestive result was for markers at \textit{CAPB}, where we observed a maximum multipoint HLOD of 0.86 ($\alpha = 0.54$), and multipoint NPLs of 2.04 ($P = 0.02$) and 1.98 ($P = 0.03$). At \textit{HPC1}, \textit{PCaP}, and \textit{HPCX}, data were consistent with a linked subset.

**Stratified Analysis.** White families were stratified by median age at diagnosis (<60 years, 60–64 years, 65–69 years, and 70+ years), number of high-grade cases (0, 1, and 2), number of advanced-stage cases (0, 1, and 2), and percentage of aggressive (high-grade or advanced-disease) cases (<33, 33–66, and $\geq 66$%). Table 3 presents results for \textit{HPC1}, \textit{PCaP}, \textit{HPCX}, and \textit{CAPB} markers that produced an NPL $P \leq 0.05$ when families were analyzed in one of these strata either with or without families with other potential linkage. In general, the most suggestive results in the stratified analyses were seen at the \textit{HPC1} locus. Evidence for \textit{HPC1} linkage improved with increasing median age at diagnosis, increasing number of high-grade cases, increasing number of advanced-stage cases, and increasing percentage of aggressive cases. At \textit{PCaP} and \textit{HPCX}, only stratification by age of diagnosis revealed differences in linkage results between strata, and no clear trends were observed. Several strata revealed NPL scores with $P \leq 0.05$ at \textit{CAPB}, although results were not stronger than unstratified results, and no trends were observed.

**Analysis of \textit{HPC1}**. Although other studies of prostate cancer families revealed the most evidence for \textit{HPC1} linkage among younger-onset families (39), older-onset families showed stronger suggestion of \textit{HPC1} linkage in the current data. Forty-two white families with median age at diagnosis $\geq 70$ years had a peak 2-point LOD score of 1.52 ($\theta = 0$) at marker \textit{DIS15}, and NPL scores ranged from 0.70 to 1.59 with corresponding $Ps$ ranging from 0.23 to 0.06. Restricting this analysis to families with five or more affected men revealed consistent results with older-onset families (median ages, 65–59 and 70+ years) having positive NPL scores at all \textit{HPC1} markers. In contrast, younger-onset families with five or more affected men had NPL scores $< 0$ at most markers. When families with evidence for possible linkage at other loci were excluded, suggestive linkage results remained in the 70+ year median age group and were also seen among families with median age 65–69 years. A maximum multipoint HLOD of 0.39 ($\alpha = 0.76$) was seen among the group of 38 remaining families with median age $\geq 70$ years, and a maximum NPL of 1.56 ($P = 0.06$) was seen among 23 remaining families with median age of diagnosis 65–69 years.

In all family groups stratified by grade, some \textit{HPC1} markers produced positive LOD scores at high values of $\theta$ in all strata, consistent with the unstratified results. However, when the analysis was restricted to families with minimal evidence for linkage to the \textit{PCaP}, \textit{HPCX}, or \textit{CAPB} loci, the weak evidence for \textit{HPC1} linkage was strengthened among 21 families with two or more cases of high-grade disease (Table 3). Positive 2-point and multipoint HLODs and NPL scores were observed at all \textit{HPC1} markers including a maximum NPL score of 2.03 ($P = 0.03$) at marker \textit{DIS2818} (Table 3). When white families with two or more high-grade cases were further stratified by median age at diagnosis (<65 years and 65+ years), multipoint HLODs and NPL scores were negative at all \textit{HPC1} markers for the younger-onset group (data not shown) and positive at all markers for the older-onset group (Table 4). The weak evidence for linkage was also strengthened in the older-onset group after removing families with evidence of other potential linkage (Table 4).

Analysis of white families stratified by stage produced a peak NPL score of 1.35 ($P = 0.09$) among families with two or more advanced-stage cases (stage C or D; Table 3). When families with possible other linkages were excluded, increasing support for linkage with more advanced-stage cases was observed. Families with no advanced-stage cases produced negative NPL scores at all \textit{HPC1} markers, families with one advanced-stage cancer produced positive NPL scores ranging from 0.57 to 1.08 ($Ps$ of 0.13–0.27), and families with at least two advanced-cases produced NPL scores ranging from 1.06 to 2.44 ($Ps$ of 0.01–0.14). A peak NPL score of 2.44 ($P = 0.01$) was seen in the families with two or more cases of high-grade disease (Table 3). Median age at diagnosis again impacted evidence for linkage in the families with two or more advanced-stage cases. The older-onset families had positive NPL scores (Table 4), and younger-onset families produced consistently negative LOD and NPL scores. Removing possible locus heterogeneity improved evidence for linkage to \textit{HPC1} among families with median age $\geq 65$ years and two or more advanced-stage cases (peak NPL, 3.00; $P = 0.004$; Table 4).

Stratification by percentage of cases with aggressive disease also showed increasing LOD and NPL scores at \textit{HPC1} with increasing disease severity. Positive NPL scores were seen only
for the 32 families with >66% of cases with aggressive disease (Table 3). Removal of families potentially linked to PCaP, HPCX, or CAPB revealed positive LOD, HLOD, and NPL scores at all HPC1 markers among 21 remaining families with >66% aggressive disease cases. NPL scores of 2.55 (P = 0.008) were seen at consecutive markers D1S2883, D1S2818, and D1S2127, and a peak NPL of 2.61 (P = 0.007) was observed between markers D1S2883 and D1S2818. Analysis stratified by median age at diagnosis revealed that older-onset families with >66% aggressive cases may be more enriched for HPC1-linked families than younger-onset families (<65 years). Multipoint LOD scores and NPL scores were negative among the younger-onset group but peaked to an NPL of 2.53 (P = 0.009) in the older-onset group (Table 4). When families with evidence of linkage at other putative loci were removed, HLOD and NPL scores at HPC1 increased in the older-onset families with >66% aggressive disease cases (peak NPL, 3.48; P = 0.0008, between D1S2818 and D1S2127) but not in the younger-onset families.

**DISCUSSION**

The goal of the current analysis was to clinically characterize this collection of high-risk prostate cancer families and to explore evidence for linkage to four previously reported loci in a priori-defined subgroups of families based on clinical characteristics. Original estimates of the proportion of families linked to HPC1, PCaP, HPCX, or CAPB ranged from 15 to 50% (20, 21, 40, 41). Not surprisingly, confirmation studies have indicated that the proportion of families linked to these loci may be less than the original estimates (35, 45, 46, 82). Families studied by various research groups may differ with respect to clinical characteristics, and these clinical characteristics may be related to the likelihood of linkage to each locus.

Several studies have suggested that men with inherited prostate cancer may differ clinically from sporadic cases; most consistently, the potentially inherited cases tend to be diagnosed at younger ages than sporadic cases (83–89). Two survival analyses suggest that familial prostate cancer may have a more aggressive course because affected men with a family history of prostate cancer have lower 5-year biochemical relapse-free survival rates (P < 0.001; Refs. 90, 91). Other reports have shown no significant difference in any clinical variable other than age at diagnosis (86–88); however, two studies of prostatectomy specimens suggested that the hereditary or familial tumors were of lower grade than the sporadic tumors (84, 85), and an Australian population-based study suggested that regionally spread disease was more common among sporadic cases than among those with family history (83). Lower grade or stage at diagnosis in hereditary cases may reflect differences in screening behaviors compared with the sporadic group. In the current study, the clinical characteristic that showed the strongest correlation among affected brothers was age at diagnosis. Familial correlation of tumor grade was also observed, although to a lesser extent, and the correlations of grade were strongest among young men. These familial correlations suggest that age at diagnosis and grade may be controlled in part by genetic factors.

This analysis was not the first to clinically describe high-risk prostate cancer families studied in linkage analysis. A comparison of the current families with a published description of 74 North American families (92) suggests clinical similarity between the two datasets in terms of age at diagnosis, year of...
diagnosis, and median PSA. Excluding missing data, the two collections had similar grade and stage distributions. This suggests that differences in linkage results between these collections may not be solely attributable to clinical differences. However, the possibility that the missing data may not be random with respect to clinical features in each dataset leaves open the possibility of significant clinical differences between datasets.

Analyses of two other collections of high-risk families have been reported that considered clinical characteristics and specific putative prostate cancer loci. Grönberg et al. (92) classified 74 prostate cancer families as “potentially-linked” and “potentially-unlinked” to HPC1 based on haplotype-sharing of affected men. The authors concluded that affected men in families “potentially-linked” to HPC1 were younger at diagnosis and more likely to have high-grade tumors and advanced-stage disease than men in “potentially-unlinked” families (92). However, interpretation of these results is difficult because: (a) a trend toward higher-grade tumors in “potentially-linked” families was not seen (“potentially-linked” also had more low-grade tumors; Ref. 93); and (b) a collection of ASPs was assessed for linkage to prostate cancer grade as represented by Gleason score, and some suggestion of linkage between Gleason score and markers near the CAPB region was seen (P < 0.01; Ref. 25). Recently, Goddard et al. (26) performed a model-free likelihood analysis of linkage in this collection of ASPs allowing for the inclusion of covariates such as Gleason score. They observed a significant effect of Gleason score on linkage to the HPC1 region (P = 0.00012) and concluded that ASPs with high Gleason scores had the strongest evidence of HPC1 linkage; no age effect was reported (26). A Gleason score effect on linkage to PCaP was seen as well; ASPs with male-to-male transmission and low Gleason scores had most evidence of linkage to this loci (26).

The current study was the first to stratify prostate cancer families a priori into clinical groups and then assess linkage. At HPC1, white families having more severe disease (high-grade and/or advanced-stage) displayed the strongest evidence of linkage, consistent with the findings of Grönberg et al. (92). However, the current finding is particularly strong among older-onset families, which contrasts with the findings reported by Grönberg et al. and others (33, 38, 39, 67, 92, 94). We have reported previously that analysis of the same set of families was consistent with a small linked subset of families, and that positive NPL scores were seen in older-onset families and negative NPL scores were seen among younger-onset families (36). It appears that stratification by stage and grade in the current analysis has further elucidated the linked subset with an observed strengthening of evidence for linkage among the more severe, older-onset families. The observation that HPC1 linkage increases in this group after exclusion of families with possible linkage at PCaP, HPCX, or CAPB suggests that HPC1 acts independently of these loci.

Clinical stratification did not give any informative results for PCaP, HPCX, or CAPB. In the case of PCaP and HPCX, this is likely attributable to a lack of families linked to these loci in this dataset. Stratification for a family history of brain cancer may be more important than the characteristics considered here for detection of CAPB linkage (41). Several limitations are inherent in this type of family study. Medical records were available only for participating affected men; clinical data from deceased affected men were not available for inclusion in stage and grade distributions or in age of diagnosis calculations. Thus, a survival bias may be incurred by

### Table 4 Nonparametric multipoint linkage results at HPC1 for white prostate cancer families with median age at diagnosis ≥65 years stratified by grade, stage, or aggressive disease

<table>
<thead>
<tr>
<th>Family characteristic</th>
<th>No. of families</th>
<th>Marker</th>
<th>NPL</th>
<th>P</th>
<th>Excluding other linkage</th>
<th>NPL</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 or more high-grade cases</td>
<td>26</td>
<td>D1S1589</td>
<td>1.10</td>
<td>0.13</td>
<td>18</td>
<td>1.64</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D1S2883</td>
<td>1.55</td>
<td>0.06</td>
<td>2.08</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D1S2818</td>
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<td>0.02</td>
<td>2.60</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D1S2127</td>
<td>1.81</td>
<td>0.04</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td></td>
<td>D1S1660</td>
<td>0.30</td>
<td>0.36</td>
<td>1.05</td>
<td>0.14</td>
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</tr>
<tr>
<td>2 or more advanced-stage cases</td>
<td>14</td>
<td>D1S1589</td>
<td>0.61</td>
<td>0.25</td>
<td>9</td>
<td>1.31</td>
<td>0.10</td>
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<tr>
<td></td>
<td></td>
<td>D1S2883</td>
<td>0.99</td>
<td>0.15</td>
<td>1.81</td>
<td>0.04</td>
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<tr>
<td></td>
<td></td>
<td>D1S2127</td>
<td>1.82</td>
<td>0.04</td>
<td>2.33</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D1S18</td>
<td>2.45</td>
<td>0.01</td>
<td>3.00</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>D1S1660</td>
<td>1.55</td>
<td>0.06</td>
<td>2.37</td>
<td>0.01</td>
<td></td>
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<tr>
<td>&gt;66% of cases with aggressive disease</td>
<td>29</td>
<td>D1S1589</td>
<td>1.19</td>
<td>0.11</td>
<td>18</td>
<td>2.28</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>D1S2883</td>
<td>1.76</td>
<td>0.04</td>
<td>3.20</td>
<td>0.001</td>
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<td></td>
<td>D1S2818</td>
<td>2.38</td>
<td>0.01</td>
<td>3.40</td>
<td>0.0009</td>
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<td></td>
<td></td>
<td>D1S2127</td>
<td>2.53</td>
<td>0.009</td>
<td>3.47</td>
<td>0.0008</td>
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<td></td>
<td></td>
<td>D1S18</td>
<td>2.05</td>
<td>0.02</td>
<td>3.02</td>
<td>0.002</td>
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<tr>
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<td>D1S1660</td>
<td>1.55</td>
<td>0.06</td>
<td>2.66</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

* Median age is based on 529 sampled, affected men. Grade data are from prostatectomy specimens, if available. Stage represents pathological stage if available; otherwise, clinical stage was used. Advanced-stage is defined as stage C or D. Aggressive disease is defined as high-grade (Gleason 7–10) or stage C or D disease.

* Excludes families with LOD scores ≥0.1 at D1S2785, DXS984, or D1S407.

* Four families were common to all three groups.
the use of prevalent cases in these families. Clinical factors
associated with poor survival include young age at diagnosis,
high-grade disease, and advanced-stage disease (81, 95). Addi-
tionally, men diagnosed prior to PSA screening (prior to the late
1980s) have lower 5-year survival rates than men diagnosed
today (81, 95), perhaps because of lead-time bias or because
PSA-detected disease tends to be a lesser stage.

The type I error rate may be inflated here, because multiple
tests were performed. Ps were not adjusted to account for
multiple comparisons, however, because it is unclear how best
to adjust Ps from multiple strata that are not independent.
Furthermore, the prior probability of linkage to the regions
assessed is increased because this is a confirmation study. We
have previously examined linkage to the four regions analyzed
here in the entire unstratified dataset, and for most of the
regions, results were consistent with the presence of a small
linked subset (positive LOD scores at high values of θ). We
therefore chose characteristics based on a priori hypotheses that
differs significantly and unlinked families (we did not
expect linkage in each subgroup). Because of the controversy
over significance levels in linkage analysis (96–100), Witte et
al. (97) recommend providing results and Ps without correction
for adjustments, so that they could be interpreted by an informed
reader. We have considered consistency between adjacent mark-
ers, the presence of trends with increasing number or percentage
of severe cases, consistency of parametric and nonparametric
results and of 2-point and multipoint results, persistence of
results whether families with potential other linkage are in-
cluded, and the similar results for grade and stage strata in
interpreting the current results.

In summary, we have described clinical characteristics of a
collection of high-risk families and assessed evidence of linkage
to four putative prostate cancer loci (HPC1, PCaP, HPCX, and
CAPB) in a priori-defined clinical subgroups. No clinical char-
acteristics identified groups of families with possible linkage to
PCaP, HPCX, or CAPB. However, our results suggest that
families with men diagnosed at older ages with high-grade and/or
advanced-stage disease may be enriched for linkage to the
HPC1 locus. Exclusion of families possibly linked to other
putative loci improved evidence for HPC1 linkage in subgroups,
suggesting that HPC1 acts independently of these other loci.
These suggestions made by the current findings should be exam-
inied in other prostate cancer family collections so that the
possible role of these loci in prostate cancer susceptibility can be
further clarified.

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participated in this research. We thank Michael Brannan and Laurie
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Clinical Characteristics of Prostate Cancer in an Analysis of Linkage to Four Putative Susceptibility Loci
