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Evidence for a Prostate Cancer–Susceptibility Locus on Chromosome 20

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Recent studies suggest that hereditary prostate cancer is a complex disease involving multiple susceptibility genes and variable phenotypic expression. While conducting a genomewide search on 162 North American families with ⩾3 members affected with prostate cancer (PRCA), we found evidence for linkage to chromosome 20q13 with two-point parametric LOD scores >1 at multiple sites, with the highest two-point LOD score of 2.69 for marker D20S196. The maximum multipoint NPL score for the entire data set was 3.02 ($P = .002$) at D20S887. On the basis of findings from previous reports, families were stratified by the presence ($n = 116$) or absence ($n = 46$) of male-to-male transmission, average age of diagnosis (<66 years, $n = 73$; ⩾66 years, $n = 89$), and number of affected individuals (<5, $n = 101$; ⩾5, $n = 61$) for further analysis. The strongest evidence of linkage was evident with the pedigrees having <5 family members affected with prostate cancer (multipoint NPL 3.22, $P = .00079$), a later average age of diagnosis (multipoint NPL 3.40, $P = .0006$), and no male-to-male transmission (multipoint NPL 3.94, $P = .00007$). The group of patients having all three of these characteristics ($n = 19$) had a multipoint NPL score of 3.69 ($P = .0001$). These results demonstrate evidence for a PRCA susceptibility locus in a subset of families that is distinct from the groups more likely to be linked to previously identified loci.

Introduction

Prostate cancer is one of the most common human cancers, occurring in as many as 15% of men in the United States (Kosary et al. 1995). While the majority of cases of prostate cancer are sporadic, it has long been recognized that familial clustering exists, with an increased relative risk occurring in relatives of affected men (Woolf 1960; Cannon et al. 1982; Meikle and Stanish 1982; Carter et al. 1990; Steinberg et al. 1990; Spitz et al. 1991; Goldgar et al. 1994; Whittemore et al. 1995). Segregation analysis of prostate cancer suggests the presence of at least one dominant susceptibility locus that may account for up to 10% of all prostate cancers (Carter et al. 1992; Schaid et al. 1998). Although genetic linkage analysis is a powerful technique for the identification of disease susceptibility loci, it is confounded by several factors in prostate cancer families. These include a late age at onset, a high phenocopy rate, and a lack of distinguishing features between the hereditary and sporadic forms of the disease.

In spite of such problems, two putative prostate cancer–susceptibility loci (*HPC1* [MIM 601518] and *PCAP* [MIM 602759]) (Smith et al. 1996; Berthon et al. 1998), plus a rare prostate cancer–brain cancer–susceptibility locus (*CAPB* [MIM 603688]) (Gibbs et al. 1999*b*), have been localized to regions on chromosome 1 through linkage studies of high risk prostate cancer families. In addition to these candidate regions on chromosome 1, evidence for linkage of a prostate cancer susceptibility locus to Xq27-28 (*HPCX* [MIM300147]) has also been reported in a combined study population (Xu et al. 1998). Although the initial report of linkage to the *HPC1* (1q24-25) region indicated that as many as 34% of familial PRCA (MIM 176807) may be linked to this region (Smith et al. 1996), a subsequent pooled analysis of 772 families showed the actual proportion to be much lower, probably ~6% (Xu et al. 2000). Studies have shown that families meeting the three criteria—male-to-male transmission, early average age at diagnosis (<65 years), and ⩾5 affected family members—are more likely to be linked to *HPC1* (Grönberg et al. 1997; Xu et al. 2000).

Since the initial report of *HPC1,* there have been several studies aimed at confirming linkage to this region. Four studies have shown weak evidence of linkage using nonparametric methods (Cooney et al. 1997; Hsieh et al. 1997; Neuhausen et al. 1999; Berry et al. 2000), whereas three other studies have shown no evidence of linkage to *HPC1* (McIndoe et al. 1997; Berthon et al. 1998; Eeles et al. 1998). For the *PCAP* (1q42.2-43) region, Berthon et al. (1998) estimated that as many as 40%–50% of their families might be linked. However, three subsequent studies of this region found no significant evidence for linkage with either parametric or nonparametric methods (Gibbs et al. 1999*a*; Whittemore et al. 1999; Berry et al. 2000). Therefore, although a mi-

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nority of families affected by prostate cancer may be linked to the *PCAP* region, the proportion is likely considerably less than 40%. The *CAPB* (1p36) region in families with both brain and prostate cancers was not confirmed in a followup study (Berry et al. 2000). To date, there has been one report attempting to confirm the linkage to the *HPCX* region (Lange et al. 1999), which, in the original report, was thought to account for an estimated 16% of familial prostate cancer cases (Xu et al. 1998). Lange et al. (1999) reported positive LOD scores over a 30-cM region containing *HPCX,* with the subset of cases with no evidence of male-to-male transmission and with early onset of disease ($\leqslant$65 years) contributing the greatest evidence for linkage.

Overall, these four loci appear to account for only a minority of familial prostate cancer cases, and none of the putative genes have been identified so far. In an attempt to identify additional loci, we initiated a genomewide search on 162 families affected by prostate cancer. Utilizing both parametric and nonparametric analyses, we present evidence for a genetic-susceptibility locus on chromosome 20, which we have called *HPC20.*

Materials and Methods

Ascertainment of Families

Families were ascertained through the Mayo Clinic radical prostatectomy database. All men who received a radical prostatectomy, in the Department of Urology, for clinically localized prostate cancer or who received radiation therapy, in the Division of Radiation Oncology, were sent a family cancer-history survey (Schaid et al. 1998). A total of 12,675 surveys were sent on two separate occasions: March 1995 and July 1997. From these surveys, 199 high-risk families were identified. More-detailed family histories were obtained over the telephone and 3–4-generation pedigrees were constructed. From this group, a total of 162 families having a minimum of three men affected with prostate cancer were collected for linkage studies. For 118 pedigrees, the minimum criteria was met with the use of first-degree relatives alone, 24 pedigrees with first- and second-degree relatives, and 20 pedigrees with first- and third-degree relatives. All but one of these families are white; the remaining family is Hispanic. There were no black families. For 82 of the families, blood was collected from as many family members as possible, including a minimum of three living affected men and unaffected siblings. The majority of the living affected men come from siblings and cousins, with a smaller proportion from nephews. Rarely were fathers and uncles available for analysis. For the remaining 80 families that met the selection criteria, blood was collected on 73 affected sib pairs and 7 other types of affected relative pairs, since the other affected family members were deceased. All men who contributed a blood specimen and who had prostate cancer had their cancers verified by review of medical records, particularly pathology reports. We were unable to review medical records for deceased individuals. The average age of diagnosis per pedigree was 66.5 years (range 47–77 years), with 73 pedigrees having an average age at diagnosis <66 years. There were 61 pedigrees with $\geqslant$5 affected men. The average number of affected men per pedigree was 4.4 (range 3–11), the average number of affected men with blood specimens per pedigree was 2.8 (range 2–7), and the average number of total blood specimens per pedigree was 3.7 (range 2–12). The characteristics of all families and the three stratified groups used for the linkage analysis (male-to-male vs. no male-to-male transmission, average age at diagnosis <66 years vs. $\geqslant$66 years, and <5 affected men per family vs. $\geqslant$5) are illustrated in table 1. The research protocol and informed consent forms were approved by the Mayo Clinic Institutional Review Board. DNA was isolated from peripheral blood lymphocytes using standard methods.

Genotyping

Markers used for the genotyping were from the ABI Prism Linkage Mapping Set Version 2 (PE Applied Biosystems). Additional markers were derived from the Genome Database (Johns Hopkins University School of Medicine). Forward primers were labeled with phosphoramidite dyes. Each 15-μl reaction contained 25 ng of genomic DNA, 200 mM dNTPs, 8mM each primer, 0.5 U Ampli*Taq* Gold (PE Biosystems), and 1.5–2.5 mM $MgCl_2$. Reactions were cycled in either a Perkin-Elmer GeneAmp PCR System 9600 or an MJR Tetrad Cycler as follows: 10 minutes at 95°C, then 35 cycles of 30 s at 95°C, 30 sec at 58°C or 55°C, 30 s at 72°C; followed by an extension step of 10 min at 72°C. PCR reactions were held at 5°C until analysis. The PCR products were resolved on a 5% denaturing polyacrylamide gel and detected using an ABI 377 DNA sequencer. Genotypes were analyzed using ABI Genescan 2.1 and ABI Genotyper 2.0.

Linkage Analysis

We performed genetic linkage analyses by both parametric and model-free methods. The parametric two-point LOD scores were computed by the LINKAGE package (FASTLINK) using an assumed prostate cancer–susceptibility allele frequency of .003 and an autosomal dominant model. The model used for this analysis is the same as that used by Smith et al. (1996) in the first reported linkage finding for hereditary prostate cancer. In summary, this model assumed a 15% phenocopy rate; affected men had penetrances of .001 and 1.0 for noncarriers and carriers, respectively; the lifetime penetrances for unaffected men of age >75 years were 16%

Table 1

Characteristics of the 162 Families Utilized for the Linkage Analysis in This Study

Families	No. of Families	No. of Affected Individuals Genotyped	No. of Unaffected Individuals Genotyped	Average No. (Range) of Individuals Affected/ Family	Average No. (Range) of Affected Individuals Genotyped/ Family	Average Age at Diagnosis (±SD) of Affected Individuals[a]
All	162	447	160	4.4 (3–11)	2.8 (2–7)	66.5 (±4.7)
Male-to-male transmission	116	325	116	4.7 (3–10)	2.8 (2–7)	66.7 (±4.5)
No male-to-male transmission	46	122	44	3.7 (3–11)	2.7 (2–5)	66.2 (±5.4)
Age at diagnosis <66 years	73	192	62	4.4 (3–10)	2.6 (2–7)	62.4 (±3.2)
Age at diagnosis ⩾66 years	89	255	98	4.5 (3–11)	2.9 (2–7)	69.9 (±2.6)
<5 affected members	101	244	48	3.4 (3–4)	2.4 (2–4)	66.3 (±5.1)
⩾5 affected members	61	203	112	6.2 (5–11)	3.3 (2–7)	67.0 (±4.2)

[a] SD = standard deviation of mean age per pedigree.

for noncarriers and 63% for carriers; and unaffected men of age <75 years and all women were not informative (i.e., unknown phenotype). A similar recessive model was used, except that the susceptibility-allele frequency was set at 0.077, giving the same population frequency of high-risk subjects as the dominant model, and the penetrance for heterozygous carriers was reduced to that of the noncarriers. Linkage in the presence of heterogeneity was assessed using Smith's admixture test for heterogeneity (HOMOG program). Multipoint LOD scores were computed by the GENEHUNTER program. As the inheritance of prostate cancer is complex, we also performed multipoint identical-by-descent model-free linkage analyses for affected pedigree members, using the NPL Z-all statistic in the GENEHUNTER program. Marker-allele frequencies were estimated from the data set.

Results

An analysis of the initial markers used for chromosome 20 provided suggestive evidence of linkage to chromosome 20q13, with two-point parametric LOD scores >1 at multiple markers. The highest two-point LOD score was 2.69 for marker D20S196 at recombination fraction .20. A single marker on 20p, D20S186, had a two-point LOD score >1 (LOD score 1.26 at recombination fraction .20). In an effort to further refine the area of linkage, five additional markers in the 20q13 region (D20S109, D20S120, D20S149, D20S887, and D20S893) were analyzed, creating an average density map interval of 3.8 cM. The two-point parametric LOD scores for all of the markers are shown in table 2. With the additional markers, the peak two-point LOD score remained at D20S196.

Multipoint analyses also provided evidence of linkage to this region on chromosome 20. The maximum multipoint NPL score was 3.02 (P = .002) at D20S887. Assuming homogeneity, multipoint LOD scores were all negative. However, after allowing for linkage heterogeneity, the heterogeneity LOD score (HLOD) was 1.08 (P = .026), with an estimated 12% of the families demonstrating linkage.

Because hereditary prostate cancer is likely a heterogeneous disease, and because prior studies have suggested critical subsets to increase homogeneity (Xu et al. 2000), families were reevaluated for linkage after stratification according to the following criteria: the presence (n = 116) or absence (n = 46) of male-to-male transmission, average age at diagnosis (<66 years, n = 73; ⩾66 years, n = 89), and number of affected individuals (<5, n = 101; ⩾5, n = 61). More detailed characteristics of these stratified groups are illustrated in table 1. Results of the two-point analysis at D20S196 is presented in table 3 while results from the multipoint analysis (both parametric and nonparametric) are presented in table 4. The pedigrees with no male-to-male transmission had a maximum two-point LOD score of 3.38 at a recombination fraction 0.10 for D20S196 (table 3). The families with <5 affected individuals had a maximum two-point LOD score of 2.26 at D20S196, whereas those with a later average age at diagnosis (⩾66 years) had a maximum two-point LOD score of 2.67 at this same marker. The subset (n = 19) that met all three criteria of no male-to-male transmission, <5 affected individuals, and later average age at onset (⩾66 years) had a maximum two-point LOD score of 3.56 at recombination fraction 0 for D20S196.

Multipoint analyses showed evidence for linkage with heterogeneity within the various subsets (table 4): no male-to-male transmission (HLOD 3.61, P = .00005, estimated 56% families linked), <5 affected (HLOD 1.51, P = .0083, estimated 30% families linked), and later average age of diagnosis (HLOD 1.39, P = .011, estimated 22% families linked). The 19 families that met all three criteria had an HLOD of 2.34 (P = .001),

Table 2

Parametric Two-Point LOD Scores for 18 Markers on Chromosome 20 Utilizing Model A for the Analysis

No. of Informative Pedigrees	Marker	LOD Score at Recombination Fraction						
		0	.01	.05	.1	.2	.3	.4
159	D20S117	−71.94	−51.29	−25.54	−13.67	−4.28	−1.17	−.22
153	D20S889	−65.29	−46.91	−23.52	−12.38	−3.52	−.72	−.06
160	D20S115	−36.31	−25.31	−11.51	−5.30	−.83	.16	.11
161	D20S186	−44.13	−29.90	−11.74	−3.70	1.26	1.44	.49
160	D20S112	−41.02	−28.45	−12.23	−4.91	.02	.73	.29
161	D20S195	−58.06	−39.97	−17.57	−7.75	−.93	.46	.26
160	D20S107	−34.14	−22.18	−7.51	−1.50	1.79	1.54	.55
161	D20S119	−45.43	−3.73	−12.54	−4.78	.23	.85	.34
157	D20S178	−44.21	−29.99	−11.73	−3.81	1.00	1.25	.44
156	D20S887	−36.82	−24.71	−9.71	−3.04	1.10	1.24	.41
153	D20S109	−52.78	−35.99	−15.38	−6.28	−.18	.78	.32
160	D20S196	−36.90	−23.98	−7.77	−.92	2.69	2.07	.65
159	D20S893	−55.44	−38.25	−16.58	−6.82	−.17	.89	.36
150	D20S120	−46.24	−33.36	−13.97	−5.40	.25	.97	.37
160	D20S100	−27.75	−17.72	−5.80	−.99	1.64	1.34	.43
159	D20S149	−65.22	−44.83	−19.58	−8.30	−.50	.89	.42
157	D20S171	−33.54	−22.23	−8.40	−2.61	.79	.88	.28
158	D20S173	−47.79	−34.48	−17.01	−8.72	−2.19	−.28	.04

with an estimated 75% of families linked. The nonparametric methods also showed significant evidence for linkage to the 20q13 region in the no male-to-male transmission group (table 4) (figure 1). For this subset, the maximum multipoint NPL score was 3.94 (P = .00007) at D20S887, with a comparable peak (multipoint NPL 3.89) at D20S893. There was also evidence of linkage in the subset with <5 affected (maximum multipoint NPL 3.22, P = .00079) and in the later–age-at-diagnosis subset (maximum multipoint NPL 3.40, P = .0006). When all three of these criteria were used, the maximum multipoint NPL was 3.69 (P = .0001) at D20S893 (table 4) (figure 1). As can also be seen in figure 1, there was another, smaller, multipoint NPL peak on 20p at D20S186. When the groups were stratified, there did not appear to be much difference for this peak among the stratified groups.

The finding of significant evidence for linkage in the no male-to-male transmission group, both by parametric and nonparametric methods, was unanticipated. However, there are several possible explanations for such an observation, including the presence of gene-gene interaction, the presence of recessive rather than dominant inheritance, and the involvement of an imprinted locus. To further explore the potential interactions of genes on the X and 20 chromosomes, we used GENEHUNTER-PLUS, which allows definition of family-specific weights (Cox et al. 1999). The NPL scores from the most likely linked region on the X chromosome (in the vicinity of *HPCX* or, in our population, the marker AFMA113zf5) (Xu et al. 1998) were used to define weights when we examined linkage for chromosome 20, and in a complementary fashion, the NPL scores from the most likely linked region on chromosome 20 (in the vicinity of D20S196) were used as weights to evaluate linkage on chromosome X. We have previously demonstrated the presence of linkage in ~16% of our families at the *HPCX* region (Xu et al. 1998). Two weighting schemes were evaluated: (1) to emphasize interaction of genes on X and 20 chromosomes, a weight of 1 versus 0 used only pedigrees that demonstrated evidence for linkage to the X chromosome (i.e., according to X-NPL >0); and (2) to emphasize linkage heterogeneity between X and 20 chromosomes, a weight of 0 versus 1 used only pedigrees that demonstrated no evidence for linkage to the X-chromosome (i.e., according to X-NPL ≤0). For all analyses, the exponential model was used to compute allele-sharing LOD scores (Kong et al. 1997). These types of analyses were run for the two subsets of pedigrees with male-to-male transmission (80 pedigrees) and the subset of pedigrees without male-to-male transmission (42 pedigrees). Results from these analyses (data not shown), however, did not provide evidence for an interaction of the chromosomal regions around D20S196 and around AFMA113zf5.

To explore the potential overlap between those families linked to *HPCX* and those linked to a locus on chromosome 20, we used the program HOMOG3R to analyze the subset of pedigrees without male-to-male transmission. This programs allows for a test for linkage heterogeneity with two measured loci, as well as a third group of pedigrees not linked to either of the measured loci. If there is no overlap between families linked to

Table 3

Maximum Two-Point LOD Scores at D20S196 for the Entire Data Set and the Four Subsets

Subset	*n*	Dominant LOD	Recessive LOD
Full Group	162	2.69	3.11
Number affected:			
<5	101	2.26	2.74
≥5	61	.63	.63
Average age at diagnosis (years):			
<66	73	.36	.22
≥66	89	2.67	3.64
Male-to-male transmission:			
Yes	116	.78	.92
No	46	3.38	2.58
Combined criteria:			
<5 members affected, age at diagnosis ≥66 years, no male-to-male transmission	19	3.56	3.48
≥5 members affected, age at diagnosis <66 years, male-to-male transmission	25	.15	.16

chromosomes X and 20, then the estimated fraction of pedigrees linked to these two chromosomes should be the same, whether we analyze chromosomes 20 and X together, with the HOMOG3R program, or each chromosome by itself, with the HOMOG program. This, in fact, was the case. Using the dominant model for chromosome 20, an overall HLOD of 2.55 was observed, with an estimate of 45% of the pedigrees linked to chromosome 20, and 10% linked to the X chromosome. Most of this evidence for linkage heterogeneity came from chromosome 20, because an HLOD for chromosome 20 adjusted for X linkage was very similar, 2.20. Results for the recessive model were similar, with an overall HLOD of 2.94 (30% linked to chromosome 20; 15% linked to chromosome X), and an HLOD for chromosome 20 adjusted for chromosome X of 2.59. Additionally, our model-free analyses by the NPL scores were consistent with little overlap of families linked to both the X and 20 chromosomes. Of the 18 families that had an NPL score >1 for either D20S196 or AFMA113zf5, only 1 had an NPL score >1 at both of these loci.

To further explore the reason for linkage in the no male-to-male transmission group, we used a recessive model for the parametric analysis. Overall, the two-point linkage results for each of the markers provided similar results compared to the dominant model. The results of the two-point LOD scores at D20S196 for the entire data set and the four subsets are illustrated in table 3. Although the recessive model gave a higher LOD score than the dominant model for the entire group, the opposite occurred in the subset without male-to-male transmission, suggesting that a recessive mechanism alone does not explain our linkage results for the subset without male-to-male transmission. However, since there is no significant difference in the results for the dominant versus the recessive model, we are unable to distinguish between these two modes of inheritance given our current data.

Discussion

On the basis of the initial two-point parametric linkage analyses, our data suggested the presence of linkage of hereditary prostate cancer to a region on chromosome 20q13 near the marker D20S196, with a two-point LOD score of 2.69. Multipoint model-free allele sharing supported this finding (NPL score 3.02, $P = .002$). On further analysis, significant evidence for linkage was demonstrated in the subset of our 46 families without male-to-male transmission of disease. This subset demonstrated a peak two-point LOD score of 3.38 at D20S196 and a multipoint heterogeneity LOD score of 3.61, with an estimated 56% of the families linked. It is important to note that the model-based analyses can be misleading in terms of the fraction of linked families, because the penetrance of the putative mutation is unknown. However, further support for linkage was provided by the model-free allele sharing statistic with an NPL score of 3.94 ($P = .00007$). Importantly, all of the above mentioned analyses have achieved statistical significance for this particular subgroup.

Although unanticipated, there are several possible explanations for the finding of linkage in the no male-to-male group to a region on chromosome 20. Since such a subgroup would be expected in an X-linked disorder, one possible explanation is the interaction of a gene on the X chromosome and a gene on chromosome 20. Utilizing GENEHUNTER-PLUS and two weighting schemes, we were unable to find evidence for an interaction between the *HPCX* region and the *HPC20* region. However, there are limitations of this approach to testing for gene-gene interactions. First, we have limited power to detect interaction, because of the small number of pedigrees without male-to-male transmission. Second, epistasis is implemented in GENEHUNTER-PLUS as multiplicative penetrance, and, if this is the true model without residual correlation of the phenotypes, then the allele sharing (and NPL scores) between the X and 20 chromosomes are independent—and, hence, epistasis could not be detected without additional factors (such as additional genes) causing residual correlations. Furthermore, our analyses were restricted to a 15-cM region at the *HPCX* locus, which could miss an interaction with a different, yet distant, gene on the X chromosome.

A second possibility is the presence of a recessive rather than a dominant mode of inheritance. Examples

Table 4

Multipoint Nonparametric NPL Scores and Multipoint Parametric LOD Scores Assuming Both Homogeneity and Heterogeneity for the Entire Data Set and Four Subsets

	Nonparametric			Parametric[a]			
Subset	*n*	NPL_{max}	NPL *P* value	Homogeneity LOD_{max}	HLOD	Heterogeneity Alpha	HLOD *P* value
Full Group	162	3.02	.002	−36.31	1.08	.12	.026
Number affected:							
<5	101	3.22	.00079	−13.53	1.51	.30	.0083
⩾5	61	1.26	.107	−18.40	1.36	.19	.012
Average age at diagnosis (years):							
<66	73	1.82	.036	−8.55	1.48	.33	.0089
⩾66	89	3.40	.0006	−21.84	1.39	.22	.011
Male-to-male transmission:							
Yes	116	1.83	.036	−24.57	.17	.05	.306
No	46	3.94	.00007	−2.62	3.61	.56	.00005
Combined criteria:							
<5 members affected, age at diagnosis ⩾66 years, no male-to-male transmission	19	3.69	.0001	1.44	2.34	.75	.001
⩾5 members affected, age at diagnosis <66 years, male-to-male transmission	25	1.40	.085	−2.52	.90	.39	.041

[a] Model A.

of autosomal recessive inheritance of increased cancer risk is provided by the autosomal recessive disorders ataxia telangiectasia and the RecQ helicase disorders (Bloom's syndrome, Werner's syndrome, Rothmund-Thomson syndrome) (Savitsky et al. 1995; Ellis et al. 1995; Gray et al. 1997; Kitao et al. 1999). An autosomal recessive model for hereditary prostate cancer has been suggested (Monroe et al. 1995). Interestingly, both two-point and multipoint parametric analyses demonstrated similar results when compared to the dominant model. Given our current data, therefore, we are unable to distinguish between these two modes of inheritance. Based on simulation studies (not shown), family size will be a critical factor in distinguishing between these two models.

Finally, an alternative explanation is the involvement of an imprinted locus. Of interest, there is both human and mouse data implicating the 20q13 region as an imprinting locus (Hall 1990). Most imprinted genes in the mouse are located within one of nine imprinting regions distributed across six autosomes (Mouse Imprinting Data and References Web site)—one of which is associated with the distal chromosome 2. This region of mouse chromosome 2 shows striking linkage conservation with human chromosome 20 at 20q12-13 (Searle et al. 1989). To date, two imprinted genes have been identified within the distal chromosome 2 region in mice: Gnasxl and Nesp (Peters et al. 1999). Furthermore, there is now clinical and biochemical evidence that GNAS (the human homologue of mouse gene Gnas), which is mutated in patients with Albright hereditary osteodystrophy, is also imprinted (Hayward et al. 1998; Weinstein and Wu 1999). The presence of an imprinting locus at the site of linkage of hereditary prostate cancer provides both challenges and opportunities. Unfortunately, evidence for implicating these alternative explanations will require additional studies.

Although the strongest evidence for linkage occurred in a single subgroup, the definition of the stratification factors were based on prior published reports (Grönberg et al. 1997; Xu et al. 2000) and were not driven by exploratory analyses of our own data. This fact can still inflate the chance of a false-positive finding, and so replication of our results is warranted. It is also notable that significant evidence for linkage to the 20q13 locus was observed in the subsets that are distinct from those most likely to be linked to the *HPC1* and *PCAP* regions. Whereas the families with male-to-male transmission, ⩾5 affected, and an average age at diagnosis <66 years are more likely to be linked to the *HPC1* region (Xu et al. 2000), the strongest evidence for linkage to the 20q13 region was seen in pedigrees meeting the complements of these criteria—that is, those with no male-to-male transmission, <5 affected, and a later average age of diagnosis (⩾66 years). The finding of linkage in the older age at onset might be expected if the penetrance of the susceptibility locus was reduced. However, in spite of the apparent higher age at onset, these families were still selected on the basis of having multiple affected individuals. In this study, we did not perform any comparisons between *HPC20* and any of the loci reported for chromosome 1 (Smith et al. 1996; Berthon

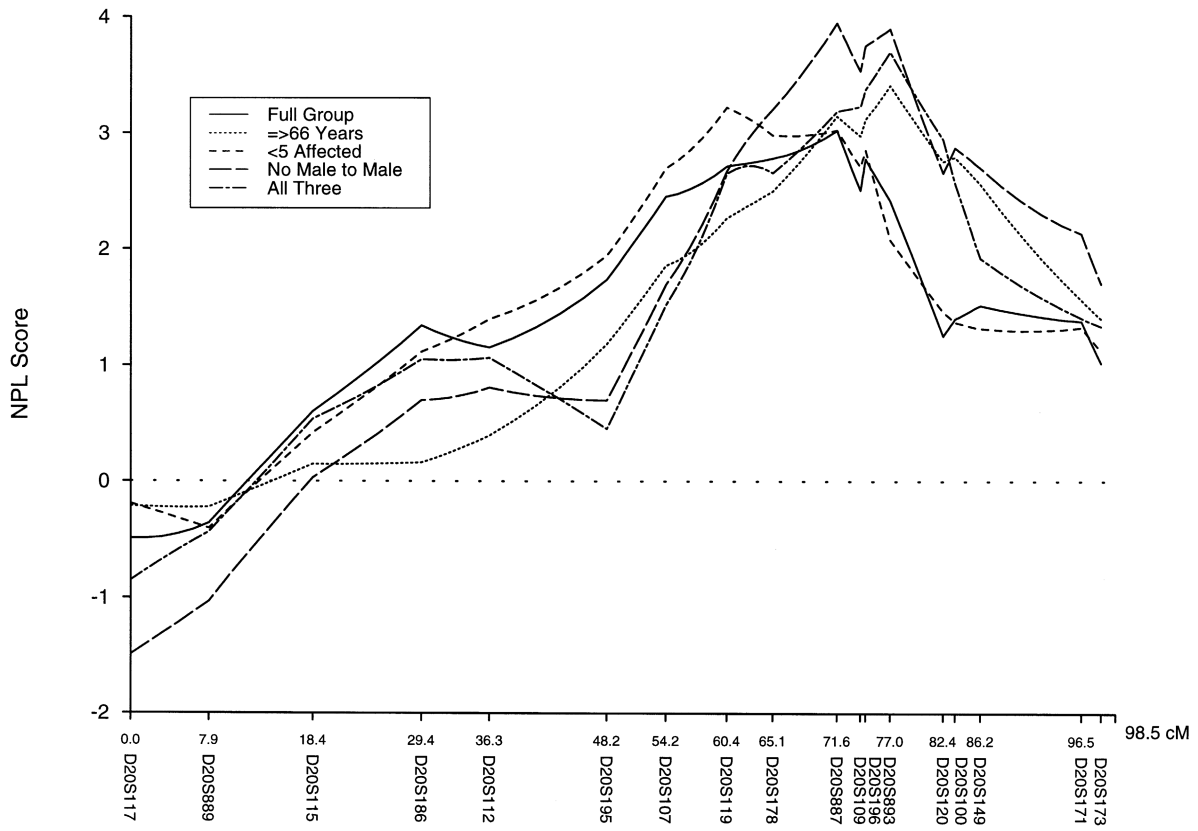

Figure 1 Multipoint NPL scores for the whole data set (n = 162), and four subsets, on an 18-marker map of chromosome 20. The subsets are: no male-to-male transmission (n = 46), average age at diagnosis ⩾66 years (n = 89), <5 affected individuals (n = 101), and the combination (n = 19) of no male-to-male transmission, average age of diagnosis ⩾66 years, and <5 affected individuals.

et al. 1998; Gibbs et al. 1999*b*) because our families demonstrated little to no evidence for linkage at these sites (Berry et al. 2000).

Overall, the two-point parametric LOD scores and the multipoint NPL scores were consistent with each other, both with and without stratification. However, unless heterogeneity was invoked, the parametric multipoint LOD scores were all negative. Although the multipoint LOD scores and the multipoint NPL statistics appear discrepant, it is well known that parametric multipoint LOD scores tend to be spuriously negative when the parameters used for analysis (e.g., allele frequency, mode of inheritance, penetrance) are not correct (Risch and Giuiffra 1992). This is because nonrecombinant offspring tend to be misclassified as double recombinants, a rare event, resulting in the disease locus being "pushed off" the multipoint marker map. In contrast, the parametric two-point LOD scores tend to be more robust to model misspecification. Furthermore, the NPL statistic is ideal for complex traits (Ott 1996). The advantage of the NPL statistic is that it is not based on unknown, yet assumed, genetic models, but rather on the comparison of the observed versus expected sharing of chromosomal regions identical by descent among affected relatives. For these reasons and because of the complex nature of prostate cancer, we have relied on the parametric two-point LOD scores and the multipoint NPL statistics for our main conclusions.

Linkage to chromosome 20 has been examined in two previous reports as part of genomewide scans for genetic susceptibility loci (Smith et al. 1996; Suarez et al. 2000). In the report by Smith et al. (1996), a marker on chromosome 20 (not identified) demonstrated a two-point LOD score of 0.5–1. Although these results support the conclusions of our current study, multiple loci along the genome demonstrated similar results in the Smith et al. (1996) report. The region demonstrating the strongest evidence for linkage in this set of familial was on chromosome 1. In the report by Suarez et al. (2000), on the other hand, there was no evidence for linkage to the chromosome 20q region. However, the ascertainment criteria in the Suarez (2000) report differed from ours. The minimum selection criteria for their families was two or more brothers with documented prostate cancer. Additionally, the strongest evidence for linkage in the Suarez (2000) report, was found on chromosome arm

16q. Clearly, additional studies will be required to confirm the results of our study. A combined analyses, as performed for chromosome 1 (Xu et al. 2000), should provide an ideal mechanism to explore and confirm our findings.

It is well recognized that prostate cancer is heterogeneous and that multiple genetic and environmental factors are very likely to play a role in its etiology. To date, genetic linkage analysis has suggested the presence of four genetic susceptibility loci: *HPC1* (1q24-25), *PCAP* (1q42.2-43), *CAPB* (1p36), and *HPCX* (Xq27-28). In this study, we present evidence for a fifth prostate cancer–susceptibility locus at 20q13, for which we propose the designation *HPC20*. The heterogeneity of this disease is underscored by the finding that these five loci may still account for only a minority (approximately one-third) of the familial cases. *HPC1* and *HPCX* appear to account for ~6% (Xu et al. 2000) and 16% (Xu et al. 1998) of the cases, respectively, whereas *PCAP* and *CAPB* account for a small fraction. In this study, we estimate that ~12% of all our families are linked to *HPC20*. Although the accuracy of the estimated percentage of families linked to these various chromosomal regions is questionable, because misspecification of the genetic model parameters will bias this estimation, it is clear that multiple genetic factors contribute to the etiology of familial prostate cancer.

In addition to data suggesting 20q13 as an imprinting region, DNA amplification of this region has frequently been observed in breast cancer (Kallioniemi et al. 1994), as well as in other tumor types (Iwabuchi et al. 1995; Mohapatra et al. 1995; Schlegel et al. 1995; Solinas-Toldo et al. 1996; Bockmuhl et al. 1997). Increased expression of genes in this region is likely to contribute to the progression—and possibly to the development—of various cancers, including prostate cancer. A number of genes have been mapped to the 20q13 region, resulting in many possible candidates for a prostate cancer–susceptibility gene. We are currently examining these genes for their potential involvement in hereditary prostate cancer.

In summary, we have presented evidence for a prostate cancer susceptibility locus at 20q13, with the strongest evidence among pedigrees without male-to-male transmission. Following confirmation of this linkage, we propose the designation *HPC20* for the locus.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Genome Database, http://gdbwww.gdb.org
Mouse Imprinting Data and References, http://www.mgu.har.mrc.ac.uk/imprinting/implink.html
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nim.nih.gov/omim (for PRCA [MIM 176807], *HPC1* [MIM 601518], *PCAP* [MIM 602759], *CAPB* [MIM 603688] and *HPCX* [MIM 300147])

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