Linkage Analysis of 153 Prostate Cancer Families Over a 30-cM Region Containing the Putative Susceptibility Locus HPCX

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ABSTRACT

Several genetic epidemiological studies have provided data to support the hypothesis that there are genes on the X chromosome that may contribute to prostate cancer susceptibility. A recent linkage study of 360 prostate cancer families described evidence for a prostate cancer predisposition gene, termed HPCX, which maps to Xq27–28. To confirm this potential contribution of this locus to prostate cancer susceptibility in an independent dataset, we studied 153 unrelated families who are participants in the University of Michigan Prostate Cancer Genetics Project. Families selected for this analysis have at least two living family members with prostate cancer that are related in a way that they could potentially share a common ancestral copy of an X chromosome. DNA samples were genotyped using a panel of seven polymorphic markers spanning 30 cM and containing the HPCX candidate region. The resulting data were analyzed using both nonparametric and parametric linkage methods. Analysis of all 153 families using multipoint nonparametric linkage (NPL) methods resulted in positive NPL Z-scores across the entire candidate interval (NPL Z-scores of 0.23–1.06, with corresponding one-sided Ps of 0.41 and 0.15, respectively). The 11 African-American families had negative NPL Z-scores across the same 30-cM interval. Analysis of the 140 Caucasian families produced a maximal NPL Z-score of 1.20, with a corresponding one-sided P of 0.12 at marker DXS1113. The subset of families with no evidence of male-to-male disease transmission and with early-onset prostate cancer (average age at diagnosis within a family ≤ 65 years) contributed disproportionately to the evidence for linkage for the entire dataset in the HPCX candidate region (near marker DXS1113). In conclusion, this study of 153 families, each with two or more living members with prostate cancer, provides additional support for the existence of a prostate cancer susceptibility gene at Xq27–28.

INTRODUCTION

Prostate cancer is the most common cancer affecting American men, with an anticipated 180,000 new cases expected in 1999 (1). Despite the high frequency of this disease, relatively little is known about the potential predisposing genetic risk factors. Many epidemiological studies have observed clustering of prostate cancer cases within families (see reviews in Refs. 2 and 3). These studies include both hospital-based and case-control designs and have been performed in countries with a variety of approaches to the clinical diagnosis and management of prostate cancer. Several complex segregation analyses have been completed using different study populations (4–6). Each of these analyses has concluded that clustering of prostate cancer in families is best explained by autosomal dominant inheritance of a susceptibility allele(s) with a population frequency that varies between 0.3% and 1.7% in different studies. Recognition of this potential inherited predisposition to prostate cancer has led to the search for prostate cancer susceptibility genes. To date, three autosomal loci have been proposed to contribute to hereditary prostate cancer: (a) HPC1 at 1q24–25 (7–9); (b) PCAP at 1q42.2–43 (10, 11); and (c) CAPB at 1p36 (12). HPC1 has been implicated in site-specific prostate cancer (13), and CAPB has been proposed to contribute to both prostate and primary brain cancers (12).

Two recent epidemiological studies have supported the hypothesis that genes on the X chromosome may also contribute to prostate cancer susceptibility. This evidence is based on the observation that affected brothers confer a higher risk of prostate cancer compared to affected fathers. For example, Narod et al. (14) observed that men participating in the Laval University Prostate Cancer Detection Program with one or more brothers with prostate cancer had a 2.6-fold greater risk of having prostate cancer compared to 1.7-fold increased risk if their father was affected with the disease. Similarly, in a population-based cohort of adults residing in Los Angeles and Hawaii, Monroe et al. (15) observed a 2-fold higher age-adjusted risk of prostate cancer in brothers of probands compared to sons of probands. This finding was confirmed in each of four racial groups (African American, Hispanic, Japanese, and Caucasian). In contrast, the risk of breast cancer in this study was similar in women.
with an affected sister or mother and is consistent with an autosomal dominant pattern of hereditary breast cancer.

In 1998, Xu et al. (16) reported linkage evidence for a prostate cancer susceptibility gene at Xq27–28; this locus has been tentatively called HPCX. In this study, family members from 360 prostate cancer pedigrees from the United States, Finland, and Sweden were genotyped using a panel of markers that mapped to Xq27–28. This region had been previously implicated in a genome scan of high-density prostate cancer families reported in 1996 by Smith et al. (7). The maximum two-point LOD score in the 360 prostate cancer families was 4.60 (θ = 0.26) at marker DXS1113 (153 cM from Xpter). Heterogeneity was assumed in this analysis, and the estimate for the proportion of linked families (α) in the entire dataset was 0.16. However, the estimate of α varied from a low of 0.15 in families from the Johns Hopkins University to a high of 0.41 in Finnish families. Stratification by whether or not families exhibited evidence of male-to-male transmission demonstrated increased evidence for linkage in families consistent with X-linked inheritance (i.e., without male-to-male transmission).

The University of Michigan Prostate Cancer Genetics Project was established in 1995; the primary goal of this research project is to define the molecular basis for the inherited predisposition to prostate cancer. A set of families from the Prostate Cancer Genetics Project provided the first evidence supporting prostate cancer linkage to the putative susceptibility locus HPC1 at 1q24–25 (8). We now report the analysis of 153 prostate cancer pedigrees using a panel of markers that map to the HPCX candidate region using both parametric and nonparametric methods.

MATERIALS AND METHODS

Patient Selection. All individuals described in this report are participants in the University of Michigan Prostate Cancer Genetics Project. Current enrollment criteria for this research project include families with two or more living men affected with prostate cancer. Written consent was obtained from all participants, and research protocols were approved by the Institutional Review Boards of the University of Michigan and the Ann Arbor Veteran’s Affairs Medical Center. DNA was isolated from whole blood using the Puregene kit (Genta Systems, Inc., Plymouth, MN).

The diagnosis of prostate cancer was confirmed by review of pathology records in 352 of 358 (98.3%) affected men who were genotyped. In the remaining 6 cases, the medical records referred to the diagnosis of prostate cancer, and these men were considered to be affected. For those affected men who were not available for genotyping, the diagnosis of prostate cancer was confirmed by pathological and/or medical records or by report from two family members. Age at diagnosis was determined from the date of the first biopsy that demonstrated prostate cancer. Average age of prostate cancer diagnosis was calculated using data only from those family members who were available for genotype analysis.

Genotype Analysis. Affected and unaffected individuals were genotyped using a panel of seven polymorphic markers that span approximately 30 cM and include the proposed HPCX candidate interval: (a) DXS1047; (b) DXS294; (c) DXS1205; (d) DXS1200; (e) DXS1113; (f) DXS1193; and (g) DXS1108. Genotype information using markers DXS1200 and DXS1113 was obtained for a subset of families using high-throughput fluorescent genotyping as described by Smith et al. (7). All other genotypes were obtained using radioactive methods as described previously (8). Due to technical limitations, genotype information was available for only 279 of 402 (69%) study participants at marker DXS1113; however, complete genotype data were obtained at marker DXS1193, which is approximately 0.3 cM distal to DXS1113.

Statistical Methods. Two-point and multipoint parametric and mode-of-inheritance free NPL analyses were performed using the computer linkage program GENEHUNTER, version 1.3 (17). For the parametric analyses, a similar model was used as described by Xu et al. (16), which assumes an X-linked predisposing locus with a disease allele frequency of 0.003 and a fixed penetrance rate of 0.15 for affected males. Women and unaffected men were assumed to have an unknown phenotype. Consistent with the analyses reported by Xu et al. (16), we used a genetic admixture model to account for the likely genetic heterogeneity of prostate cancer susceptibility. This model assumes that there are two types of families, with α representing the proportion of families linked to the putative X-linked susceptibility locus, and 1 − α representing the proportion of families not linked to this locus. The parameter α was estimated from the data. For the NPL analyses, the score function “pairs” was used. Allele frequencies were estimated from our pooled sample of patient and unaffected family members using an iterative approach that accounts for the correlation in the distribution of alleles among family relatives (18). Marker distances used in the multipoint analyses were provided by Xu et al. (16).

The marker map is as follows: DXS1047—8.7 cM—DXS294—4.8 cM—DXS1205—8.1 cM—DXS1200—2.6 cM—DXS1113—0.3 cM—DXS1193—5.5 cM—DXS1108.

Methods for Stratification. All 153 families in the complete dataset had at least two affected family members who could share a common ancestral copy of an X chromosome (i.e., omitting isolated father/son and paternal uncle/nephew pairs). Families were divided according to whether or not there was evidence of male-to-male prostate cancer transmission. For this stratification, we modified the definitions of male-to-male transmission proposed by Xu et al. (16) to include families in which all prostate cancer cases within a pedigree could not be explained by the transmission of a single (not necessarily fully penetrant) X-linked disease allele. This definition would include families with (a) an affected father and affected son combination, and/or (b) prostate cancer on the paternal side of the family, and/or (c) two consecutive generations of affected men on the maternal side. Bilineal families are defined as those families with evidence of prostate cancer on both sides of the family (note: these families are also classified as having evidence of male-to-male transmission). The families without evidence of male-to-male transmission were further stratified ac-

3 The abbreviation used is: NPL, nonparametric linkage.

4 J. Xu, personal communication.
Table 1  Characteristics of the 153 families analyzed for linkage to the putative prostate cancer susceptibility gene HPCX

The clinical features of the 153 families as well as the 140 Caucasian families and the 11 African-American families are illustrated. Families were also stratified according to whether evidence of male-to-male transmission of prostate cancer was present as described in “Materials and Methods.” Early-onset families are defined as those families with an average age of prostate cancer onset of ≤65 years. The second column refers to number of families; the next four columns indicate the number of individuals.

<table>
<thead>
<tr>
<th></th>
<th>No. of families</th>
<th>No. of affected individuals genotyped</th>
<th>No. of unaffected individuals genotyped</th>
<th>Average no. of individuals affected/family (range)</th>
<th>Average no. of affected individuals genotyped/family (range)</th>
<th>Average age (yr) of affected individuals genotyped (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All families</td>
<td>153</td>
<td>358</td>
<td>44</td>
<td>2.8 (2–9)</td>
<td>2.3 (2–5)</td>
<td>64.0 (± 8.4)</td>
</tr>
<tr>
<td>African-American</td>
<td>11</td>
<td>29</td>
<td>9</td>
<td>4.2 (2–9)</td>
<td>2.6 (2–4)</td>
<td>60.4 (± 10.0)</td>
</tr>
<tr>
<td>Caucasian families</td>
<td>140</td>
<td>325</td>
<td>35</td>
<td>2.7 (2–7)</td>
<td>2.3 (2–5)</td>
<td>64.3 (± 8.2)</td>
</tr>
<tr>
<td>Families with male-to-male transmission</td>
<td>43</td>
<td>116</td>
<td>24</td>
<td>3.8 (2–9)</td>
<td>2.7 (2–5)</td>
<td>62.7 (± 10.0)</td>
</tr>
<tr>
<td>Families without male-to-male transmission</td>
<td>110</td>
<td>242</td>
<td>20</td>
<td>2.4 (2–7)</td>
<td>2.2 (2–5)</td>
<td>64.6 (± 7.5)</td>
</tr>
<tr>
<td>Families without male-to-male transmission and with early-onset prostate cancer</td>
<td>56</td>
<td>124</td>
<td>11</td>
<td>2.4 (2–5)</td>
<td>2.2 (2–5)</td>
<td>60.2 (± 6.5)</td>
</tr>
</tbody>
</table>

According to whether the mean age of prostate cancer diagnosis in genotyped individuals within each pedigree was less than or equal to 65.0 years.

RESULTS

DNA samples were obtained from 358 affected and 44 unaffected members of 153 prostate cancer families. Fifty-nine of the 153 families (38.6%) fulfilled one or more of the clinical characteristics of hereditary prostate cancer as proposed by Carter et al. (19). These criteria include: (a) three or more affected individuals within one nuclear family; and/or (b) affected individuals occurring in three successive generations (maternal or paternal lineage); and/or (c) a cluster of two or more relatives each affected before the age of 55 years. The majority of the families are Caucasian; however, 11 African-American and 2 Hispanic families were also included in this study (7.2% and 1.3% of families, respectively). The clinical characteristics of the prostate cancer families are illustrated in Table 1. Compared to the Caucasian families, the African-American families had more affected men/family (4.2 versus 2.7 men/family) and an earlier average age of prostate cancer diagnosis (60.4 versus 64.3 years). The youngest age of prostate cancer diagnosis in any family was 43 years.

Multipoint NPL Z-scores using all 153 families ranged between 0.23 and 1.06 (corresponding one-sided $P = 0.41$ and 0.15, respectively) over the 30-cM map, and the maximum NPL Z-score was observed at marker DXS1047 (Table 2). This marker is approximately 24.2 cM centromeric to DXS1113 (Fig. 1), which was the marker at which the most significant evidence for linkage (using two-point LOD scores) was observed in the 360 prostate cancer families described by Xu et al. (16). Analysis of the 11 African-American prostate cancer families produced negative NPL Z-scores across the same 30-cM interval (Fig. 1). Removal of these 11 African-American families as well as the 2 Hispanic families from the dataset increased the NPL Z-score at DXS1113 from 0.71 to 1.20 (corresponding one-sided $P = 0.24$ and 0.12, respectively).

We hypothesized that the strongest evidence for prostate cancer linkage to an X chromosomal susceptibility locus would occur in families having two specific features: (a) no evidence of father-to-son disease transmission; and (b) early-onset prostate cancer. To classify families as having evidence of male-to-male transmission, we modified the definitions proposed previously by Xu et al. (Ref. 16; see “Materials and Methods”). Two of our families did not fulfill any of these three proposed criteria, yet all of the cases of prostate cancer within these pedigrees could not be explained by the inheritance of a single X-linked gene. One such family has a proband with an affected maternal cousin, who is the son of his mother’s unaffected brother. Using our modified definition, 43 of the 153 families (28%) demonstrated evidence of male-to-male transmission (Table 1). Six of these 43 families demonstrated evidence of bilineal inheritance. Fifty-six of the families without evidence of male-to-male transmission had early-onset prostate cancer, which was defined as an average age at prostate cancer diagnosis of all affected family members available for genotyping that was less than or equal to 65 years.

When we divided all families according to whether or not there was evidence of male-to-male transmission, we observed maximum NPL Z-scores at two distinct regions. The maximum multipoint NPL Z-score in families with evidence for male-to-male transmission was 1.89 (corresponding one-sided $P = 0.03$) at marker DXS294 (Table 2). In contrast, the maximum multipoint NPL Z-score in the families without evidence for male-to-male transmission was 0.88 (corresponding one-sided $P = 0.19$), which was observed at markers DXS1113 and DXS1193. The 56 families without evidence of male-to-male transmission and with early-onset prostate cancer contributed disproportionately to the evidence for linkage at DXS1113. This marker is the same marker at which the maximum two-point LOD score was observed in the previous report of prostate cancer linkage to HPCX (Ref. 16; Table 2). In our study, the subset of families without evidence of male-to-male transmission and with early-onset prostate cancer had a maximum NPL Z-score of 1.51 at DXS1108 (corresponding one-sided $P = 0.07$). A similar set of linkage results was obtained when all 140 Caucasian families were stratified according to the presence or absence of male-to-male transmission and early-onset prostate cancer (data not shown). The NPL Z-scores at marker DXS1113 were 0.49 (one sided $P = 0.32$) in the 36 Caucasian families with evidence of...
The NPL Z-scores and corresponding one-sided $P$-values are indicated at each X chromosome marker for all 153 families, the 140 Caucasian families, and the 11 African American families. NPL Z-scores are also provided for all families after stratification according to the presence or absence of male-to-male disease transmission (male-to-male) within a pedigree. The families without male-to-male transmission occurred at marker $D XS 1108$ in families without apparent male-to-male transmission. Evidence for linkage was marginally higher in the subset of families without male-to-male disease transmission and early-onset prostate cancer at marker $D XS 1108$ (HLOD = 0.47; $\alpha = 0.21$) and at marker $D XS 1113$ (HLOD = 0.18; $\alpha = 0.12$). Multipoint analysis is sensitive to estimated distances between markers; therefore, two-point parametric HLOD scores were also calculated (Table 4). The maximum two-point HLOD score in all families and in the families with male-to-male transmission was at marker $D XS 1205$ (HLOD = 0.27 and $\alpha = 0.11$ and HLOD = 1.09 and $\alpha = 0.37$, respectively). By comparison, the maximum HLOD for the families with no evidence of male-to-male transmission and early-onset prostate cancer was 0.73 at marker $D XS 1108$ ($\alpha = 0.29$).

### DISCUSSION

The recognition that prostate cancer has a strong familial component has led to the search for prostate cancer susceptibility genes. Localization of these genes through linkage studies, however, has become a particularly formidable task. Prostate cancer is the most common cancer affecting American men; this high background rate of sporadic cancer obscures the ability to identify families with true genetic predisposition. Prostate cancer is also a late-onset disease. Thus, it is often difficult to identify and collect samples from individuals in several generations. Despite these problems, linkage studies have identified three autosomal putative prostate cancer susceptibility loci, namely, $H P C 1$, $P C A P$, and $C A P B$. The likelihood of locus heterogeneity further complicates prostate cancer linkage studies and necessitates large sample sizes.

To localize $H P C X$, Xu et al. (16) studied 360 prostate cancer families with an average of 4.3 affected men/family obtained from research studies from two institutions in the United States (the Johns Hopkins University and the Mayo Clinic), Finland, and Sweden. Whereas all groups demonstrated two-point parametric LOD scores that were slightly positive across a broad 19-cM region, disparate peaks were observed in the different populations. Given that the potential localization of $H P C X$ may lie anywhere within a broad region at Xq27–28, we decided to select markers that would allow us to cover a somewhat larger candidate interval in our confirmatory study. Thus, in addition to using five markers that were used in the Xu study (16), we selected two additional markers, $D XS 1047$ and $D XS 294$, which are located approximately 13.5 and 4.8 cM centromeric to $D XS 1205$. In our entire set of 153 families, there was evidence of excess allele sharing across the entire interval, although the maximal NPL Z-score occurred outside of the candidate interval proposed by Xu et al. (Ref. 16; NPL

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**Table 2: Multipoint NPL results, NPL Z-scores and associated $P$-values**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Caucasian families (n = 140)</th>
<th>African-American families (n = 11)</th>
<th>All families (n = 153)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$D XS 1047$</td>
<td>1.06 (0.15)</td>
<td>1.12 (0.12)</td>
<td>1.07 (0.16)</td>
</tr>
<tr>
<td>$D XS 1106$</td>
<td>0.28 (0.39)</td>
<td>0.05 (0.91)</td>
<td>0.18 (0.55)</td>
</tr>
<tr>
<td>$D XS 1205$</td>
<td>1.32 (0.90)</td>
<td>0.96 (0.83)</td>
<td>1.24 (0.11)</td>
</tr>
<tr>
<td>$D XS 1294$</td>
<td>0.25 (0.60)</td>
<td>0.37 (0.36)</td>
<td>0.38 (0.27)</td>
</tr>
<tr>
<td>$D XS 1113$</td>
<td>1.57 (0.95)</td>
<td>1.63 (0.95)</td>
<td>1.09 (0.03)</td>
</tr>
</tbody>
</table>

* In the initial report describing localization of $H P C X$, the maximum two-point LOD score for prostate cancer linkage occurred at $D XS 1113$. The NPL Z-scores and corresponding one-sided $P$-values are indicated at each X chromosome marker for all 153 families, the 140 Caucasian families, and the 11 African American families. NPL Z-scores are also provided for all families after stratification according to the presence or absence of male-to-male disease transmission (male-to-male) within a pedigree. The families without male-to-male transmission occurred at marker $D XS 1108$ in families without apparent male-to-male transmission. Evidence for linkage was marginally higher in the subset of families without male-to-male disease transmission and early-onset prostate cancer at marker $D XS 1108$ (HLOD = 0.47; $\alpha = 0.21$) and at marker $D XS 1113$ (HLOD = 0.18; $\alpha = 0.12$). Multipoint analysis is sensitive to estimated distances between markers; therefore, two-point parametric HLOD scores were also calculated (Table 4). The maximum two-point HLOD score in all families and in the families with male-to-male transmission was at marker $D XS 1205$ (HLOD = 0.27 and $\alpha = 0.11$ and HLOD = 1.09 and $\alpha = 0.37$, respectively). By comparison, the maximum HLOD for the families with no evidence of male-to-male transmission and early-onset prostate cancer was 0.73 at marker $D XS 1108$ ($\alpha = 0.29$).
Exclusion of families with evidence of male-to-male transmission should enrich our dataset for families that may have prostate cancer due to a mutation in \textit{HPCX}. Because sons receive their copy of the X chromosome from their mothers, transmission of prostate cancer from father to son cannot occur if the disease locus is on the X chromosome. Accordingly, the maximal NPL Z-score in the 110 families with no evidence for male-to-male transmission was 0.88 at marker \textit{DXS1113} and \textit{DXS1193} (corresponding one-sided \( P = 0.19 \)). \textit{DXS1113} is the same marker that provided the maximum two-point LOD score in the report by Xu \textit{et al.} \textit{(16)}. The NPL Z-score continued to increase at \textit{DXS1113} when only the families without male-to-male transmission and early-onset prostate cancer were examined (Table 2), consistent with the hypothesis that inherited forms of prostate cancer may occur 5 or perhaps 10 years earlier than their sporadic counterparts.

Interestingly, we observed two distinct, nonoverlapping, small linkage peaks when stratifying our data by pedigree patterns of potential disease transmission. The 43 families with apparent evidence for male-to-male prostate cancer transmission provided the strongest overall evidence for linkage with a peak HLOD and NPL-Z score that was centromeric to the previously described \textit{HPCX} candidate region. However, these 43 families provide no evidence for linkage near or at marker \textit{DXS1113}. We do not believe there is evidence here for two distinct \textit{HPCX} genes. The most likely explanation for this result is the relatively modest power in our data to detect \textit{HPCX} linkage. Typically, a one or two LOD support interval is constructed to delimit the boundaries of a candidate region. Given the relatively modest peak LOD scores in the various strata, constructing such support intervals would not exclude any of these regions. The observation of excess X chromosome sharing among affected family members in pedigrees with apparent paternally transmitted prostate cancer may be a false positive result (i.e., type 1 error). Alternatively, errors in classification of inheritance pattern may have contributed to this unexpected finding. Notably, Xu \textit{et al.} \textit{(16)} did not observe disparate linkage peaks when stratifying by apparent mode of transmission.

African-American men have the highest age-adjusted incidence rate of prostate cancer in the world \textit{(20)}. Despite this observation, African-American prostate cancer families have not been well-represented in prostate cancer genetic studies. We previously reported that six African-American families contributed disproportionately to the evidence of prostate cancer linkage to \textit{HPC1} at 1q24–25 that was observed in a set of 59 prostate cancer families \textit{(8)}. The 11 families reported here include these original 6 families; 7 of the 11 African-American families exhibited evidence of male-to-male disease transmission. African-American prostate cancer families will likely exhibit locus heterogeneity, as suggested in Caucasian prostate cancer families; the lack of evidence to support \textit{HPCX} linkage in this subset may be attributed to small sample size.

In this report, we present the results of linkage analyses using both parametric and mode-of-inheritance free NPL methods \textit{(17)}. Our strategy of collecting DNA samples primarily from affected family members was designed for the use of NPL analysis. This type of approach has a number of advantages in that it assumes no prior knowledge of mode of inheritance or disease allele frequency. Parametric linkage analysis incorporates the phenotype information of the entire pedigree under consideration, and it uses this information to calculate a LOD score. However, prostate cancer is a late-onset disease with a high background of sporadic prostate cancer, and the disease is only manifested in men. This latter point is particularly relevant when studying potential X-linked prostate cancer susceptibility, given that disease alleles must be transmitted through women.

To compare the level of significance of linkage observations using parametric and nonparametric analyses, one can...
Table 3  Multipoint parametric linkage results, HLOD scores and associated admixture coefficients (α)

The HLOD and corresponding admixture coefficients (α) are indicated at each X chromosome marker for all 153 families, the 140 Caucasian families, and the 11 African American families. HLOD are also provided for all families after stratification according to the presence or absence of male-to-male disease transmission (male-to-male) within a pedigree. The families without male-to-male transmission were further subdivided according to whether there was early-onset prostate cancer within a family, which was defined as an average age of diagnosis of ≤65 years. The bold values indicates the highest HLOD score for each category.

<table>
<thead>
<tr>
<th>A. Family groups</th>
<th>DXS1047</th>
<th>DXS294</th>
<th>DXS1205</th>
<th>DXS1200</th>
<th>DXS1113α</th>
<th>DXS1108</th>
</tr>
</thead>
<tbody>
<tr>
<td>All families (n = 153)</td>
<td>0.22 (0.08)</td>
<td>0.06 (0.04)</td>
<td>0.00 (0.00)</td>
<td>0.02 (0.03)</td>
<td>0.04 (0.04)</td>
<td>0.03 (0.04)</td>
</tr>
<tr>
<td>Caucasian families (n = 140)</td>
<td>0.28 (0.10)</td>
<td>0.12 (0.06)</td>
<td>0.01 (0.02)</td>
<td>0.16 (0.08)</td>
<td>0.22 (0.09)</td>
<td>0.19 (0.08)</td>
</tr>
<tr>
<td>African-American families (n = 11)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>B. All families</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male-to-male (n = 43)</td>
<td>0.62 (0.23)</td>
<td>0.77 (0.27)</td>
<td>0.37 (0.18)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>No male-to-male (n = 110)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.06 (0.05)</td>
<td>0.11 (0.07)</td>
<td>0.09 (0.06)</td>
</tr>
<tr>
<td>No male-to-male and age of diagnosis &gt;65 years (n = 54)</td>
<td>0.06 (0.08)</td>
<td>0.04 (0.06)</td>
<td>0.00 (0.00)</td>
<td>0.01 (0.04)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>No male-to-male and age of diagnosis ≤65 years (n = 56)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.05 (0.07)</td>
<td>0.18 (0.12)</td>
<td>0.15 (0.11)</td>
</tr>
</tbody>
</table>

α In the initial report describing localization of HPCX (16), the maximum two-point LOD score for prostate cancer linkage occurred at DXS113.

Table 4  Two-point HLOD scores and associated admixture coefficients (α)

The HLODs and corresponding admixture coefficients (α) are indicated at each X chromosome marker for all 153 families and after stratification according to the presence or absence of male-to-male disease transmission (male-to-male) within a pedigree. The families without male-to-male transmission were further subdivided according to whether there was early-onset prostate cancer within a family, which was defined as an average age of diagnosis ≤65 years. The bold values indicate the highest HLOD score for each category.

<table>
<thead>
<tr>
<th>A. Family groups</th>
<th>DXS1047</th>
<th>DXS294</th>
<th>DXS1205</th>
<th>DXS1200</th>
<th>DXS1113α</th>
<th>DXS1108</th>
</tr>
</thead>
<tbody>
<tr>
<td>All families (n = 153)</td>
<td>0.18 (0.08)</td>
<td>0.01 (0.03)</td>
<td>0.27 (0.11)</td>
<td>0.00 (0.00)</td>
<td>0.12 (0.09)</td>
<td>0.15 (0.07)</td>
</tr>
<tr>
<td>Male-to-male (n = 43)</td>
<td>0.54 (0.24)</td>
<td>0.82 (0.31)</td>
<td>1.09 (0.37)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.03 (0.06)</td>
</tr>
<tr>
<td>No male-to-male (n = 110)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.40 (0.20)</td>
<td>0.12 (0.08)</td>
</tr>
<tr>
<td>No male-to-male and age of diagnosis &gt;65 years (n = 54)</td>
<td>0.01 (0.04)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.05 (0.11)</td>
<td>0.00 (0.00)</td>
</tr>
<tr>
<td>No male-to-male and age of diagnosis ≤65 years (n = 56)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.00 (0.00)</td>
<td>0.04 (0.11)</td>
<td>0.45 (0.30)</td>
<td>0.20 (0.15)</td>
</tr>
</tbody>
</table>

α In the initial report describing localization of HPCX (16), the maximum two-point LOD score for prostate cancer linkage occurred at DXS1113.
calculate the associated $P$ for each HLOD statistic according to methods proposed by Faraway (21). For example, the maximum multipoint HLOD in families exhibiting male-to-male transmission was 0.77 at marker DXS294 and has a corresponding $P$ of 0.06 that can be compared to a multipoint NPL Z-score of 1.89 with a corresponding $P$ of 0.03 at the same marker. However, in the families without apparent male-to-male transmission, the nonparametric approach to linkage analysis produces more significant results compared to the parametric methods. To illustrate, in the subset of families without evidence for male-to-male transmission and with early-onset prostate cancer, the observed NPL Z-score was 1.24 with an associated $P$ of 0.11 at DXS1113, whereas the HLOD at this marker was 0.18 ($P = 0.30$). This result suggests that this parametric model, incorporating a rare, highly penetrant disease allele, may not be the optimal model to analyze families with prostate cancer due to HPCX.

Multipoint parametric and NPL analyses incorporate genotype information on multiple genetic markers simultaneously. The presence of information at a nearby marker compensates for the lack of information at a particular marker. The results of these analyses are sensitive to both marker order and distances between markers. Furthermore, a genotype error at one marker would not only affect the results at that marker location but would also impact the results around the nearby surrounding markers. This is particularly true when analyzing sibling pairs with missing parental data. For this reason, two-point HLOD scores were generated at each of the markers (Table 4). The magnitudes of the two-point results were consistent with the multipoint results. However, the overall maximum HLOD scores shifted from DXS1047 in all families and DXS294 in families consistent with male-to-male transmission to marker DXS1205 in the two-point analysis (Tables 3 and 4). The maximum two-point HLOD score was observed at marker DXS1108 in families without apparent male-to-male transmission of prostate cancer. Thus, these two-point results appear to corroborate our multipoint results but further demonstrate the difficulty in conclusively narrowing the candidate region using linkage analysis.

It is interesting to speculate about the potential function of a prostate cancer susceptibility gene on the X chromosome. Because men have only one copy of the X chromosome, an X-linked prostate cancer predisposition gene is unlikely to be a classical tumor suppressor gene (TSG). Families with cancer due to an inherited mutation in a TSG, for example RB1 or VHL, exhibit autosomal dominant transmission of cancer predisposition (22); however, these genetic defects are recessive at the cellular level. Dominant mutations in proto-oncogenes have more recently been implicated in inherited cancer syndromes (e.g., MET and papillary renal cell carcinoma; see a review in Ref. 23). The most likely candidate for a prostate cancer susceptibility gene on the X chromosome is the androgen receptor gene. However, this gene is approximately 50 cm centromeric to the most likely location for HPCX, and prostate cancer linkage to this gene has not been observed by us or others (24).  


Hopefully, continued efforts to narrow the candidate interval through linkage mapping combined with transcript identification and analysis will lead to the identification of HPCX in the future.

In summary, this study of 153 families with two or more living members with prostate cancer provides some additional evidence in support of an X-linked prostate cancer predisposition gene. Although the level of significance associated with our findings is not high, we are encouraged that our most significant evidence for linkage in the HPCX candidate region proposed by Xu et al. (16) was observed in the families without male-to-male prostate cancer transmission and with early-onset prostate cancer. The addition of similar families to future studies should increase the power to detect linkage to HPCX and help to narrow the large candidate region.

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Linkage Analysis of 153 Prostate Cancer Families Over a 30-cM Region Containing the Putative Susceptibility Locus *HPCX*

Ethan M. Lange, Hong Chen, Kristin Brierley, et al.


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