Localization of a Prostate Cancer Predisposition Gene to an 880-kb Region on Chromosome 22q12.3 in Utah High-Risk Pedigrees

Nicola J. Camp, James M. Farnham, and Lisa A. Cannon-Albright

Division of Genetic Epidemiology, Department of Biomedical Informatics, University of Utah School of Medicine, Salt Lake City, Utah

Abstract

Chromosome 22q has become recently a region of interest for prostate cancer. We identified previously a logarithm of odds (LOD) of 2.42 at chromosome 22q12.3. Additionally, this region has been noted by eight other studies, with linkage evidence ranging from LOD of 1.50 to 3.57. Here, we do fine mapping and localization of the region using a pedigree-specific recombinant mapping approach in 14 informative, high-risk Utah pedigrees. These 14 pedigrees were chosen because they were either "linked" or "haplotype-sharing" pedigrees or both. "Linked" pedigrees were those with significant pedigree-specific linkage evidence (LOD, >0.588; $P < 0.05$) to the 22q12.3 region, regardless of the number of prostate cancer cases sharing the segregating haplotype. "Haplotype-sharing" pedigrees were those with at least five prostate cancer cases sharing a segregating haplotype in the 22q12.3 region, regardless of the linkage evidence. In each pedigree, the most likely haplotype configuration (in addition to the multipoint LOD graph for linked pedigrees) was used to infer the position of recombinant events and delimit the segregating chromosomal segment in each pedigree. These pedigree-specific chromosomal segments were then overlaid to form a consensus recombinant map across all 14 pedigrees. Using this method, we identified a 881,538-bp interval at 22q12.3, between D22S1265 and D22S277, which is the most likely region that contains the 22q prostate cancer predisposition gene. The unique Utah extended high-risk pedigree resource allows this powerful localization approach in pedigrees with evidence for segregating predisposition to prostate cancer. We are mutation screening candidate genes in this region to identify specific genetic variants segregating in these pedigrees. (Cancer Res 2006; 66(20): 10205-12)

Introduction

Prostate cancer is the most common noncutaneous cancer in men in the United States, with an estimated 234,460 new cases and 27,350 deaths in the United States in 2006 (1). Although dietary and environmental factors are clearly involved in the etiology of prostate cancer, family history has been uniformly found to be the best predictor (2). To date, multiple genome-wide linkage scans have been published using different ascertainment criteria, linkage approaches, and disease definitions. It has become clear from the general inconsistency of results that there is not only significant genetic heterogeneity for prostate cancer predisposition but also problems with power and high rates of sporadic cases, which hinder gene localization efforts. Our approach here was to focus on a region with good linkage evidence across several, although by no means all, linkage studies and to do statistical recombination mapping in Utah high-risk pedigrees to define the most likely region containing the prostate cancer predisposition gene.

Chromosome 22q has become recently a region of interest for prostate cancer. In our own genome-wide linkage scan, the second best linkage peak [hetereogeneity-adjusted logarithm of odds (HLOD), 2.42] was identified on chromosome 22q in pedigrees with a maximum of three generations (3). This region has also been noted in several other studies. Lange et al. (4) observed a LOD of 2.35 in African American prostate cancer pedigrees. Janer et al. (5) observed a HLOD of 2.21 in pedigrees with five or more affected men. Cunningham et al. (6) reported LODs between 1.0 and 2.0 in subset analyses for late age at diagnosis and five or more affected relatives. Xu et al. (7) identified a LOD of 1.5 in 188 prostate cancer families having at least three first-degree relatives with prostate cancer. This linkage signal increased to 2.06 when the same resource was analyzed for clinically significant prostate cancer (8). Stanford et al. (9) observed a HLOD of 1.90 among men with aggressive disease. This region was also noted in a two-locus multigroup analysis in conjunction with chromosome 21q22 ($P = 1.9 \times 10^{-5}$; ref. 10). Finally, chromosome 22q was the most compelling peak and only significant finding in the combined genome-wide scan from 10 groups done by the International Consortium for Prostate Cancer Genetics (ICPCG), with a HLOD of 3.57 in an analysis of 269 pedigrees with five or more affected men (11). This combined ICPCG analysis included overlapping pedigrees from some of the above-mentioned studies, but in some cases, disease definition and, for Utah, pedigree structure differed from the original studies. Six of the 10 ICPCG groups had at least 10 pedigrees in the "five or more affected men" subset analysis and four indicated LOD of >1.0 in the same chromosome 22q region [113 pedigrees from Johns Hopkins University (LOD, 1.22), 10 pedigrees from Mayo Clinic (LOD, 2.05), 16 pedigrees from the University of Michigan (LOD, 1.57), and 49 pedigrees from the University of Utah (LOD, 1.31)].

The Utah high-risk prostate pedigree resource has been particularly notable for its ability to significantly confirm previously reported prostate cancer loci (Neuhausen et al. (12) for HPC1; Farnham et al. (13) for HPCX) and for the localization and identification of HPC2/ELAC2 (14). This is perhaps because the Utah pedigrees are ascertained for significant excess disease and multiple cases, both of which reduce the chance of only sampling clusters of sporadic disease. The unique nature of the extended, informative pedigrees, which are typically powerful enough to provide

Requests for reprints: Nicola J. Camp, Division of Genetic Epidemiology, Department of Biomedical Informatics, University of Utah School of Medicine, 391 Chipeta Way, Suite D, Salt Lake City, UT 84108. Phone: 801-587-9351; Fax: 801-581-6052; E-mail: nicola.camp@utah.edu.

doi:10.1158/0008-5472.CAN-06-1233

pedigree-specific linkage or compelling haplotype segregation evidence in a region, provides an advantage for gene localization. Genetic heterogeneity can be extremely problematic for localizing a linkage region because the noise from unlinked pedigrees can shift the linkage evidence and be misleading for fine mapping. Therefore, the ability to focus on linked pedigrees and pedigrees with substantial haplotype sharing is beneficial. Here, we report our fine mapping and localization of the chromosome 22q region using 14 extended, high-risk prostate cancer pedigrees from Utah.

Subjects and Methods

Utah resource. The Utah high-risk pedigrees were ascertained for excess prostate cancer using the Utah Population Database (UPDB; 15), which combines an extensive genealogy consisting of Utah pioneers and up to 10 generations of their descendants, with information from the Utah Cancer Registry. Fifty-nine pedigrees containing at least four prostate cancer cases and for which all prostate cancer cases were no more distantly related than by two meioses were studied in our previous genome-wide scan (3). The chromosome 22q region identified was at chromosome 22q12.1 to 22q13.1 (D22S689 to D22S445), with D22S685 being the marker position with the highest heterogeneity LOD (2.42).

This study was approved by the appropriate University of Utah institutional review board and informed consent was obtained from all human subjects.

Fine-mapping pedigrees. In our previous genome-wide analysis (3), we used a pedigree splitting approach to reduce the effect of intrafamilial heterogeneity. In brief, the approach iteratively removes the top founders creating smaller pedigrees. The details of this approach may be found in Camp et al. (3). Using that approach, the best linkage evidence was in the maximum three-generation splits of the full Utah pedigrees. Nine such "three-generation" pedigrees were identified to be linked to the region (individual pedigree multipoint LOD, >0.588; $P < 0.05$). In addition, in the full pedigree structures, six pedigrees were identified with substantial haplotype sharing in the absence of significant linkage evidence (5 or more prostate cancer cases sharing a segregating haplotype). These 15 pedigrees were initially selected for our fine-mapping efforts. For linked pedigrees that were splits of larger pedigrees, fine-mapping markers were added to the four-generation pedigree (extending upward one generation and down to all additional descendants). This was done so that we could assess whether the linked haplotype segregated beyond the three-generation subpedigree. For haplotype-sharing pedigrees, fine-mapping markers were added to all descendants below the hypothesized entry point of the shared segregating haplotype.

Genotyping. Genotyping for the seven genomic search markers was done previously by the Center for Inherited Disease Research (CIDR). Laboratory methods used by CIDR are described in detail at the CIDR Web site.[1] An additional 14 fine-mapping microsatellite markers were genotyped, resulting in resolution of 1.1 cM across the region of interest (Table 1). Puregene DNA isolation kits (Gentra Systems, Minneapolis, MN) were used to extract DNA from blood buffy coats. PCR products were analyzed on ABI 377 fluorescent sequencers. We used CheckErrors, which assesses Mendelian inheritance of alleles to detect inheritance incompatibilities and assign probabilities to each involved individual.[2] Samples with a high probability of causing the observed inheritance incompatibility were set to missing. All cM positions, cytogenetic bands, and bp positions for the genetic markers were taken from the University of California Santa Cruz Genome Bioinformatics Web site.[3]

Linkage analysis. Linkage analysis was done using MCLINK, (16), a Markov chain Monte Carlo method. The most likely haplotype configuration for each pedigree was also determined using MCLINK. Marker allele frequencies were estimated using genotype data for all typed subjects (17). The prostate cancer inheritance model used was that originally presented in Smith et al. (18). This is a strict dominant model with a disease allele frequency of 0.003. The penetrance for affected men who carry the disease allele is 50% and for those that do not carry is 0.05%. Unaffected men under 75 years and women are considered unknown. Unaffected men >75 years are considered 2.27 times more likely not to carry the disease allele.

Classic parametric multipoint LOD scores under the dominant "Smith" model were calculated for all 15 pedigrees. In addition, the most likely haplotype configurations for each pedigree were examined to identify haplotype sharing. Pedigrees exhibiting haplotype sharing in the absence of linkage evidence were also studied because, although the "Smith" model has been proven empirically over many studies to be powerful, it is extremely intolerant to non-haplotype-sharing prostate cancer cases, especially close prostate cancer relatives. We considered pedigrees with at least five prostate cancer cases sharing a segregating haplotype to represent substantial sharing.

Recombinant mapping. For each pedigree with a LOD of >0.588, the profile of the multipoint LOD graph was used to infer recombinant events. These statistically inferred recombinant events were determined as follows: a sharp change in multipoint LOD of >0.5 LOD units was used to indicate a loss in sharing (recombination). With perfect information content, the LOD will dramatically decrease at the recombinant event. However, in the more likely scenario of imperfect information content, the LOD may decrease over several cM and across several markers. The innermost (high) position before the change in LOD indicates the innermost position of the recombinant, and the outermost (low) position after the LOD change indicates the outmost position of the recombinant event. To remain conservative, we report the outermost recombinant positions. For each haplotype-sharing pedigree, the shared chromosomal segment is identified from the most likely haplotype configuration as estimated from MCLINK. The first nonshared marker at each end of the shared region indicates the outermost position of the recombinant event(s). Multiple recombinant events can occur in a pedigree, or the recombinant haplotype may be

Table 1. Marker characteristics

Marker	deCODE cM	Start site (bp)	Cytogenetic band
D22S420	2.96	16,233,835	22q11.1
D22S686	15.46	21,393,070	22q11.22
D22S345*	18.80	22,813,141	22q11.23
D22S310	24.38	24,952,777	22q12.1
D22S1144	29.41	26,007,487	22q12.1
D22S689	32.92	27,180,894	22q12.1
D22S273*	34.81	30,576,610	22q12.3
D22S1686	36.74	31,289,840	22q12.3
D22S1172	37.45	31,998,946	22q12.3
D22S281	38.55	32,661,262	22q12.3
D22S685	38.79	32,920,033	22q12.3
D22S1265	39.06	33,714,462	22q12.3
D22S424	39.85	34,023,567	22q12.3
D22S277*	40.73	34,596,000	22q12.3
D22S683*	41.17	34,838,191	22q12.3
D22S283*	41.59	35,075,205	22q12.3
D22S692	42.27	35,450,045	22q12.3
D22S1045	44.75	35,860,785	22q13.1
D22S445	45.22	35,890,398	22q13.1
D22S423	49.14	38,706,686	22q13.1
D22S1169	68.82	47,722,917	22q13.32

*Decode cM not available. cM position estimated from an available physically close mapped marker or the relative physical bp position.

[1] http://www.cidr.jhmi.edu.
[2] http://bioinformatics.med.utah.edu/~alun/software/docs/CheckErrors.html.
[3] http://www.genome.ucsc.edu.

Table 2. Pedigree characteristics

Pedigree	LOD	Total prostate cancer cases	Average age at diagnosis (minimum, maximum)	Aggressive prostate cancer*	Other cancers in pedigree
Linked pedigrees					
14342	1.18	6	61.3 (46, 78)	3	None
17675	1.04	5	63.8 (50, 73)	3	None
14016	0.89	5	65.5 (57, 71)	3	None
14343	0.86	7	63.6 (52, 75)	3	None
14358	0.61	9	64.2 (50, 73)	1	1†
14019	0.60	4	69.3 (60, 77)	3	None
17688	0.59	4	68.3 (61, 87)	3	None
14315a‡	0.58	4	63.0 (54, 76)	1	2§
Haplotype-sharing pedigrees					
14143	—	20	76.2 (57, 91)	10	9‖
14315b	—	12	69.3 (54, 88)	4	4¶
14102	—	8	60.6 (43, 88)	3	None
14305**	—	25	72.2 (51, 91)	10	5††
14357	—	8	66.3 (53, 75)	3	1‡‡

*Aggressive disease determined as PSA >20 ng/mL, high stage (regional or distant) or grade (poorly or undifferentiated).
† Skin melanoma.
‡ Pedigree 14315a is a three-generation split of 14315b, which is the four-generation pedigree.
§Colon and lip cancers.
‖Breast, kidney, lip, liver, non-Hodgkin's lymphoma, oral, small cell lung, squamous cell lung, and urinary/bladder cancers.
¶Colon, lip, kidney, and esophageal cancers.
**14305 is the full pedigree that contains haplotype-sharing pedigrees 14305a and 14305b (see Table 3).
†† Colon cancer (×2), leukemia, non-Hodgkin's lymphoma, and lentigo melanoma.
‡‡ Uterine cancer.

inherited to many prostate cancer cases. We therefore inspected the segregating haplotypes in all pedigrees, assessed all recombinants and the number of prostate cancer that share the recombinant haplotype.

Consensus region. For each pedigree, the shared chromosomal segment is identified and overlaid to create the consensus region. The minimal one-recombinant consensus region is the region defined by at least one recombinant at each side. Similarly, two- and three-recombinant consensus regions can be defined. We define these larger multirecombinant regions to provide a more conservative identification of our region of interest. If we consider a sporadic rate of 15%, the probability that two recombinants occur by chance in sporadic prostate cancer cases is low (2.3%) and that three recombinants occur by chance is very low (0.3%).

Results

Linkage evidence was reassessed for all 15 pedigrees using the additional 14 fine-mapping markers. One of the previously linked pedigrees no longer indicated a LOD of >0.588 ($P < 0.05$) with the

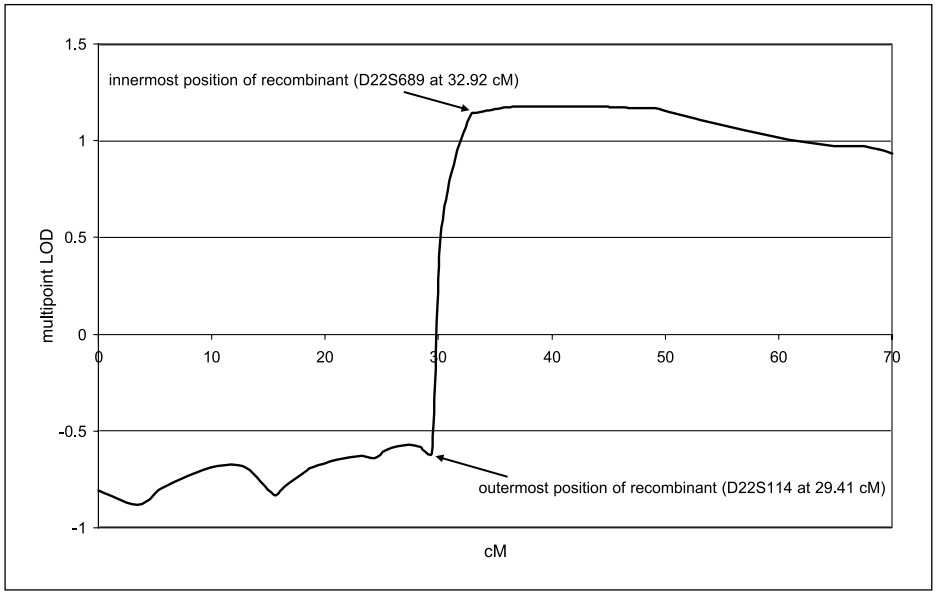

Figure 1. LOD graph for linked pedigree 14342. *Arrows,* the outermost (D22S1144; 29.41 cM) and the innermost (D22S689; 32.92 cM) position of the recombinant event in pedigree 14342.

additional markers and also did not have at least five haplotype sharers and hence was dropped from further consideration. All of the haplotype-sharing pedigrees maintained at least five haplotype sharers, with increased resolution for the shared segment. Table 2 describes pedigree characteristics for the 14 fine-mapping pedigrees.

To show the determination of recombinant events from a LOD graph profile, Fig. 1 indicates an example of a pedigree-specific

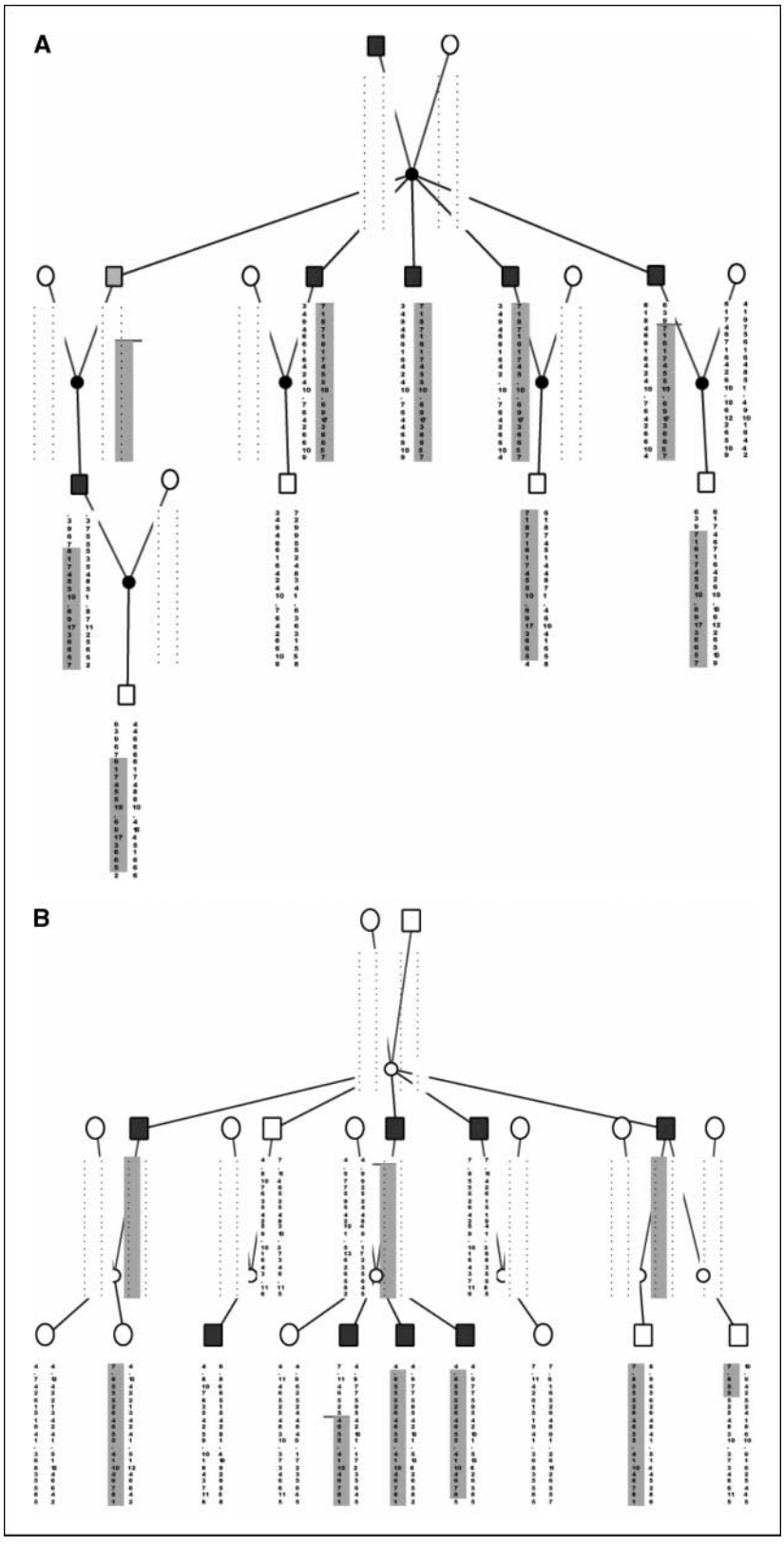

Figure 2. *A,* linked pedigree 14342; *B,* haplotype sharing pedigree 14102. *Squares,* males; *circles,* females. *Filled squares,* men diagnosed with prostate cancer; *gray squares,* unknown disease status. *Gray shading,* segregating haplotype. *Horizontal lines,* recombinant events on the segregating haplotype.

Table 3. Fine-mapping results indicating left and right recombinants by pedigree

Pedigree	LOD	Haplotype sharers (meioses*)	Left outermost recombinant	cM	Right outermost recombinant	cM
Linked pedigrees						
14342	1.18	5 (6)	D22S1144	29.41	q-ter	end
		4 (4) †	D22S420	2.96		
17675	1.04	5 (5)	D22S686	15.46	D22S1169	68.82
		5 (5)	D22S686	15.46		
14016	0.89	5 (5)	D22S686 (i2) ‡	15.46	D22S445 (i2)	45.22
		3 (3)	D22S420	2.96		
14343	0.86	5[7]§, 4[6]	D22S345	18.80	D22S423	49.14
14358	0.61	7 (8)	D22S1265	39.06	D22S1169	68.82
14019	0.60	4 (4)	D22S420	2.96	D22S1169	68.82
17688	0.59	4 (3)	D22S281	38.55	D22S423	49.14
14315a‖	0.58	4 (4)	D22S1144	29.41	q-ter	end
Haplotype-sharing pedigrees						
14143	—	7 (11)	D22S1144	29.41	D22S1045 (i5)	44.75
		7 (11)	D22S1144	29.41		
		5 (7)	D22S420 (i2)	2.96		
		5 (7)	D22S420	2.96		
14315b	—	7 (9)	p-ter	0	D22S277	40.73
		6 (7)			D22S423	49.14
14102	—	6 (6)	D22S1686	36.74	D22S1169	68.82
14305a	—	6 (9)	D22S1144	29.41	q-ter	end
14305b	—	5 (7)	D22S685	38.79	q-ter	end
		4 (6)	D22S686	15.46		
14357	—	5 (7)	D22S420 (i2)	2.96	D22S1169 (i2)	68.82

*Number of meioses separating the haplotype sharers.
†Multiple rows for a pedigree indicate additional recombinants.
‡(ix) inherited to x prostate cancer cases.
§In the three-generation pedigree (for which the LOD corresponds), there are five haplotype sharers, but in the full four generation pedigree, there are seven sharers.
‖Pedigrees with a and b suffices have a common ancestor (but different segregating haplotypes).

LOD graph for linked pedigree 14342 (pedigree-specific LOD, 1.18) with the inferred recombinant events labeled. Figure 2*A* indicates the pedigree drawing for pedigree 14342, which indicates the segregating haplotype that corresponds to the recombinant events indicated in Fig. 1. To show an example of a haplotype-sharing pedigree, Fig. 2*B* shows pedigree 14102 where six of eight prostate cancer cases share a segregating haplotype, but no significant linkage evidence exists (pedigree-specific LOD, 0.215).

Table 3 shows the summary of linkage evidence, haplotype sharing, and chromosomal sharing for all 14 fine-mapping pedigrees. Each pedigree-specific chromosomal segment shared has been graphed in Fig. 3 such that the consensus regions can be seen. The minimal one-recombinant region is at chromosome 22q12.3 between D22S1265 (39.06 cM) and D22S277 (40.73), a 1.67-cM region measuring 881,538 bp in size. Eleven genes reside in this region: *RAXLX, HMG2L1, TOM1, HMOX1, MCM5, RASD2, MB, LOC284912, APOL6, APOL5*, and *RBM9* (listed centromeric to telomeric). The two-recombinant region was found to be between D22S685 (38.79 cM) and D22S1045 (44.75 cM), a 5.96-cM region measuring 2,940,752 bp, and the three-recombinant region was between D22S281 (38.55 cM) and D22S1045 (44.75 cM).

In an attempt to elucidate factors that may have led to the linkage evidence to chromosome 22q, we investigated characteristics of the 76 prostate cancer cases who were haplotype carriers, in addition to general characteristics of the pedigrees. No specific prostate cancer characteristics of the haplotype carriers were evident. Chang et al. (8) and Stanford et al. (9) identified their best linkage evidence with clinically significant and aggressive disease, respectively. However, in the 66 haplotype carriers for whom we had stage, grade, or prostate-specific antigen (PSA) data, less than one third (21 haplotype carriers) showed evidence of severe disease by either high PSA (PSA, >20 ng/mL) or high stage or grade. Cunningham et al. (6) observed their best evidence in a late onset subgroup (prostate cancer onset, >65 years). However, in our study, approximately half (36 of 75) indicated earlier onset (onset, ≤65 years) and half (39 of 75) later onset (onset, >65 years), with one missing value. Survival also did not seem to distinguish the haplotype carriers. The observed 5-year survival of the haplotype carriers (both effects of cancer-related and noncancer deaths) was 72.6%, which closely matches the Surveillance, Epidemiology, and End Results (SEER) observed 5-year survival of 74% (19). Finally, we considered multiple cancers. Five of the 76 haplotype carriers were additionally diagnosed with at least one other cancer (one prostate cancer case had two other cancers); however, all six of these cancers were different (oral, lip, hepatic, non-Hodgkin's lymphoma, esophagus, and lentigo melanoma). Pedigree characteristics were a little more concordant with prior studies. Janer et al. (5), Cunningham et al. (6), and Xu et al. (11) all identified linkage evidence to chromosome 22q in pedigrees with at least five prostate cancer cases. Thirteen of the 14 pedigrees

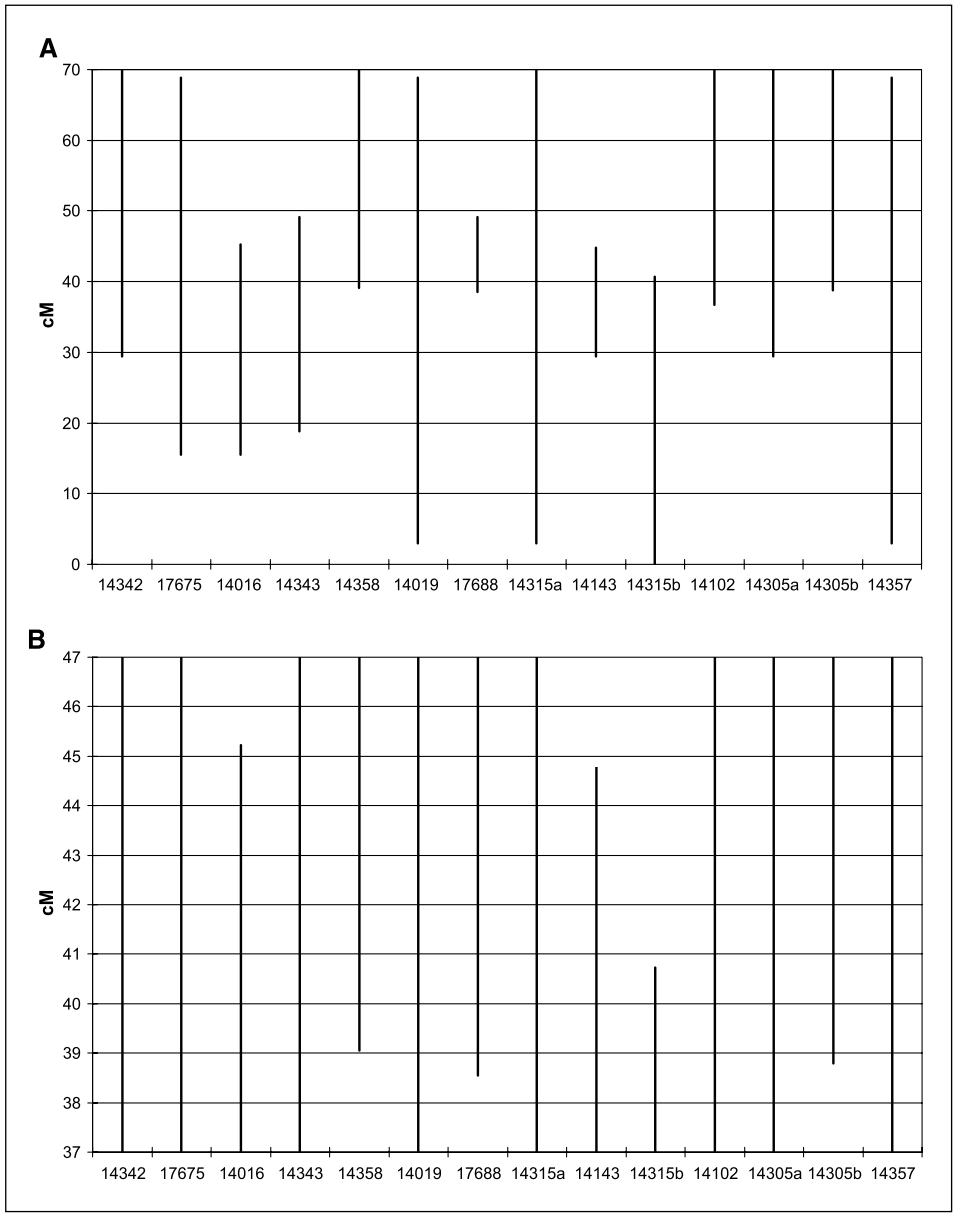

Figure 3. Recombinant map. *A,* whole chromosome view; *B,* focus on chromosome 22 from 37 to 47 cM. *Vertical solid line,* chromosomal segment that is shared in the pedigree. The minimum consensus region across pedigrees is the region of overlap of all these lines.

considered in this fine-mapping effort contained at least five prostate cancer cases. Xu et al. (7) identified their chromosome 22q evidence in pedigrees with at least three first-degrees prostate cancer cases. All pedigrees considered here also contained at least three first-degree relatives. Finally, in a two-locus epistatic analysis, Chang et al. (10) found evidence for interaction of chromosome 22q with chromosome 21q22. Our results for this are less compelling. Only 2 of the 14 pedigrees (one linked and one haplotype sharing) attained nominal linkage evidence (LOD, >0.58) at 21q22. Hence, the most consistent result for identification of linkage is in heavily loaded and dense prostate cancer pedigrees of moderate size.

Discussion

We have used 14 high-risk Utah pedigrees and identified 76 predisposition haplotype carriers in the 22q12.3 region. Thirty-one recombinant events were inferred in the haplotype carriers, some of which were inherited by up to five prostate cancer cases. All recombinants taken together identified a consensus region with no conflicts. The one-recombinant consensus region defined a 881,538-bp interval containing 11 genes (*RAXLX, HMG2L1, TOM1, HMOX1, MCM5, RASD2, MB, LOC284912, APOL6, APOL5*, and *RBM9*). Of these 11 candidate genes, *HMOX1* and *MCM5* have been the most studied with respect to cancer (20–36), with some studies specific to prostate cancer (34–36). Several studies focused on the expression of the *HMOX1* gene [heme oxygenase-1 (HO-1)], with overexpression of HO-1 repeatedly shown to provide a cell growth advantage. The *MCM5* gene is a cell cycle regulator and a reasonable candidate gene. The minichromosome maintenance (Mcm) proteins are essential for DNA replication, and expression of Mcm proteins implies the potential for cell proliferation. The *HMG2L1, APOL6*, and *RBM9* genes have also been studied with cancer (37–40).

If we consider the 22q12.3 candidate locus as the larger three-recombinant region, the physical size is still relatively

small (3,229,136 bp) and contains only an additional 20 genes (*APOL3, APOL4, APOL2, APOL1, MYH9, TXN2, FLJ23322, EIF3S7, CACNG2, RABL4, PVALB, FLJ90680, NCF4, CSF2RB, MGC35206, TST, MPST, KCTD17, TMPRSS6*, and *IL2RB*). All of these additional genes are telomeric of the one-recombinant region as there is a gene desert centromeric of D22S1265. In terms of prior evidence of involvement of any of these genes in cancers, studies of *MYH9, PVALB*, and *IL2RB* appear in the literature (41–44).

The next step of our research in the Utah pedigrees is to test these candidate genes; we are currently mutation screening several of these genes in linked pedigrees. Our mutation screening set comprises two haplotype carriers from each pedigree. One haplotype carrier from each pedigree is screened for mutations, and if a putative variant is identified, segregation is established by screening the second haplotype carrier. For this particular locus, we anticipate that the underlying predisposition variants may be reasonably common. Multiple lines of evidence support this suggestion. First, the evidence in the Utah resource for the chromosome 22q12.3 region was in the split pedigrees such that three generations was the maximum structure. This indicates that, in the full extended pedigree, structures that intrafamilial heterogeneity exists for 22q. This is borne out in the fine-mapping results. In each linked pedigree, we extended all three-generation subpedigrees to four generations, where possible, to locate the entry of the segregating gene in to the pedigree. Only in one of the eight linked pedigrees did the segregating haplotype appear to enter the pedigree higher than the three-generation piece analyzed, indicating that marry-in founders brought the gene into subpedigree. In addition, in two pedigrees (those denoted with the "a" and "b" suffixes in Table 3), we observed two independent segregating haplotypes in the same larger pedigree. Further, in 9 of the 14 pedigrees (5 linked and 4 haplotype sharing), we observed pedigree-specific recessive linkage evidence (LOD, >0.5) to the region. This indicates that, in sib-ships, the prostate cancer brothers often shared two alleles identical-by-descent, suggesting that the alleles are likely more common. Finally, this region has good replication evidence from multiple studies, which again indicates a more common gene frequency. In particular, one replication sample was identified under a recessive model (9) and another was of African descent (4), suggesting that this prostate cancer locus may have an extensive history.

This study shows the continued high utility of an extended high-risk pedigree approach. For this specific locus, three-generation pedigrees were found to be most powerful, although smaller structures may not necessarily be the rule for all loci. Large, extended pedigrees can always be split into smaller structures to locate more common underlying variants, and the full pedigree structure can be maintained for rarer segregating variants. It must also be noted that the three-generation Utah high-risk pedigrees are larger than those usually seen in other linkage studies. These three-generation pedigrees remain powerful enough to be informative in a pedigree-specific approach, allowing for linkage evidence and haplotype sharing to be used to infer recombinant events and to delimit the region containing the putative disease gene. Such a pedigree approach becomes reasonable once substantial evidence exists for a region. The ability to do detailed analyses in the individual pedigrees, which are driving the resource-wide linkage evidence, is a unique and valuable approach that has largely been overlooked, but which provides a significant advantage to gene localization for the Utah resource.

In conclusion, there exists substantial evidence from multiple independent studies for a predisposition gene for prostate cancer at chromosome 22q12.3. We analyzed 14 high-risk pedigrees and identified an 881,538-bp interval, between D22S1265 and D22S277, which is the most likely region containing the 22q prostate cancer predisposition gene. The identification of segregating variants that explain prostate cancer predisposition in these pedigrees is our necessary next step.

Acknowledgments

Received 4/6/2006; revised 7/12/2006; accepted 8/17/2006.

Grant support: NIH National Cancer Institute (NCI) grant R01 CA90752 (L.A. Cannon-Albright), subcontract from Johns Hopkins University with funds provided by NIH NCI grant R01 CA89600 (L.A. Cannon-Albright), and NIH grant K07 CA 98364 (N.J. Camp). Genomic search genotyping was provided by the CIDR. CIDR is fully funded through a federal contract from the NIH to the Johns Hopkins University, contract number N01-HG-65403. Data collection was assisted by the Utah Cancer Registry, which is funded by contract number N01-PC-35141 from the NIH SEER Program with additional support from the Utah State Department of Heath and the University of Utah. Partial support for all data sets within the UPDB was provided by the University of Utah Huntsman Cancer Institute. This investigation was also supported by the Public Health Services research grant number M01-RR00064 from the National Center for Research Resources.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

We thank the Utah high-risk prostate pedigree members; Study Coordinators, Marguerite Jost and Lewis Ershler; laboratory specialist Kim Nguyen; and Dr. Jeffrey Swensen for discussions on candidate gene screening.

References

1. Jemal A, Siegal R, Ward E, et al. Cancer Statistics, 2006. CA Cancer J Clin 2006;56:106–30.
2. Ostrander EA, Stanford JL. Genetics of prostate cancer: too many loci, too few genes. Am J Hum Genet 2000;67:1367–75.
3. Camp NJ, Farnham JM, Cannon Albright LA. Genomic search for prostate cancer predisposition loci in Utah pedigrees. Prostate 2005;65:365–74.
4. Lange EM, Gillanders EM, Davis CC, et al. Genome-wide scan for prostate cancer susceptibility genes using families from the University of Michigan prostate cancer genetics project finds evidence for linkage on chromosome 17 near BRCA1. Prostate 2003;57:326–34.
5. Janer M, Friedrichsen DM, Stanford JL, et al. Genomic scan of 254 hereditary prostate cancer families. Prostate 2003;57:309–19.
6. Cunningham JM, McDonnell SK, Marks A, et al. Genome linkage screen for prostate cancer predisposition loci: results from the Mayo Clinic Familial Prostate Cancer Study. Prostate 2003;57:335–46.
7. Xu J, Gillanders EM, Isaacs SD, et al. Genome-wide scan for prostate cancer susceptibility genes in the Johns Hopkins hereditary prostate cancer families. Prostate 2003;57:320–5.
8. Chang BL, Isaacs SD, Wiley KE, et al. Genome-wide screen for prostate cancer predisposition genes in men with clinically significant disease. Prostate 2005; 64:356–61.
9. Stanford JL, McDonnell SK, Friedrichsen DM, et al. Prostate cancer and genetic susceptibility: a genome scan incorporating disease aggressiveness. Prostate 2006;66:317–25.
10. Chang BL, Lange EM, Dimitrov L, et al. Two-locus genome-wide linkage scan for prostate cancer predisposition genes with an interaction effect. Hum Genet 2006;118:716–24.
11. Xu J, Dimitrov L, Chang BL, et al. A combined genomewide linkage scan of 1,233 families for prostate cancer-susceptibility genes conducted by the international consortium for prostate cancer genetics. Am J Hum Genet 2005;77:219–29.
12. Neuhausen SL, Farnham JM, Kort E, Tavtigian SV, Skolnick MH, Cannon-Albright LA. Prostate cancer susceptibility locus HPC1 in Utah high-risk pedigrees. Hum Mol Genet 1999;8:2437–42.
13. Farnham JM, Camp NJ, Swensen J, Tavtigian SV, Albright LA. Confirmation of the HPCX prostate cancer predisposition locus in large Utah prostate cancer pedigrees. Hum Genet 2005;116:179–85.
14. Tavtigian SV, Simard J, Teng DH, et al. A candidate prostate cancer susceptibility gene at chromosome 17p. Nat Genet 2001;7:172–80.

15. Skolnick M, The Utah genealogical data base: a resource for genetic epidemiology. In: Cairns J, Lyon JL, Skolnick M, et al., editors. Banbury Report 4: cancer incidence in defined populations. New York: Cold Spring Harbor Laboratory; 1980. p. 285–97.
16. Thomas A, Gutin A, Abkevich V, Bansal A. Multipoint linkage analysis by blocked Gibbs sampling. Statistics and Computing 2000;10:259–69.
17. Boehnke M. Allele frequency estimation from data on relatives. Am J Hum Genet 1991;48:22–5.
18. Smith JR, Freije D, Carpten JD, et al. Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. Science 1996;274: 1371–4.
19. Gloeckler Ries LA, Reichman ME, Lewis DR, Hankey BF, Edwards BK. Cancer survival and incidence from the Surveillance, Epidemiology, and End Results (SEER) program. Oncologist 2003;8:541–52.
20. Nishie A, Ono M, Shono T, et al. Macrophage infiltration and heme oxygenase-1 expression correlate with angiogenesis in human gliomas. Clin Cancer Res 1999;5:1107–13.
21. Takahashi S, Takahashi T, Mizobuchi S, et al. CYP2E1 overexpression up-regulates both non-specific δ-aminolevulinate synthase and heme oxygenase-1 in the human hepatoma cell line HLE/2E1. Int J Mol Med 2003;11:57–62.
22. Sunamura M, Duda DG, Ghattas MH, et al. Heme oxygenase-1 accelerates tumor angiogenesis of human pancreatic cancer. Angiogenesis 2003;6:15–24.
23. Liu ZM, Chen GG, Ng EK, Leung WK, Sung JJ, Chung SC. Upregulation of heme oxygenase-1 and p21 confers resistance to apoptosis in human gastric cancer cells. Oncogene 2004;23:503–13.
24. Mayerhofer M, Florian S, Krauth MT, et al. Identification of heme oxygenase-1 as a novel BCR/ABL-dependent survival factor in chronic myeloid leukemia. Cancer Res 2004;64:3148–54.
25. Sasaki T, Yoshida K, Kondo H, Ohmori H, Kuniyasu H. Heme oxygenase-1 accelerates protumoral effects of nitric oxide in cancer cells. Virchows Arch 2005;446: 525–31.
26. Berberat PO, Dambrauskas Z, Gulbinas A, et al. Inhibition of heme oxygenase-1 increases responsiveness of pancreatic cancer cells to anticancer treatment. Clin Cancer Res 2005;11:3790–8.
27. Chang KW, Lee TC, Yeh WI, et al. Polymorphism in heme oxygenase-1 (HO-1) promoter is related to the risk of oral squamous cell carcinoma occurring on male areca chewers. Br J Cancer 2004;91:1551–5.
28. Kikuchi A, Yamaya M, Suzuki S, et al. Association of predisposition to the development of lung adenocarcinoma with the heme oxygenase-1 gene promoter polymorphism. Hum Genet 2005;116:354–60.
29. Williams GH, Romanowski P, Morris L, et al. Improved cervical smear assessment using antibodies against proteins that regulate DNA replication. Proc Natl Acad Sci U S A 1999;95:14932–7.
30. Murphy N, Ring M, Heffron CC, et al. Quantitation of CDC6 and MCM5 mRNA in cervical intraepithelial neoplasia and invasive squamous cell carcinoma of the cervix. Mod Pathol 2005;18:844–9.
31. Williams GH, Swinn R, Prevost AT, et al. Diagnosis of oesophageal cancer by detection of minichromosome maintenance 5 protein in gastric aspirates. Br J Cancer 2004;91:714–9.
32. Korkolopoulou P, Givalos N, Saetta A, et al. Minichromosome maintenance proteins 2 and 5 expression in muscle-invasive urothelial cancer: a multivariate survival study including proliferation markers and cell cycle regulators. Hum Pathol 2005;36:899–907.
33. Guida T, Salvatore G, Faviana P, et al. Mitogenic effects of the up-regulation of minichromosome maintenance proteins in anaplastic thyroid carcinoma. J Clin Endocrinol Metab 2005;90:4703–9.
34. Stoeber K, Swinn R, Prevost AT, et al. Diagnosis of genito-urinary tract cancer by detection of minichromosome maintenance 5 protein in urine sediments. J Natl Cancer Inst 2002;94:1071–9.
35. Porterfield H. Us Too PC-SPES surveys: review of studies and update of previous survey results. Mol Urol 2000;4:289–91.
36. Lu X, Guo J, Hsieh TC. PC-SPES inhibits cell proliferation by modulating p21, cyclins D, E, and B and multiple cell cycle-related genes in prostate cancer cells. Cell Cycle 2003;2:59–63.
37. Yamada M, Ohkawara B, Ichimura N, et al. Negative regulation of Wnt signalling by HMG2L1, a novel NLK-binding protein. Genes Cells 2003;8:677–84.
38. Liu Z, Lu H, Jiang Z, Pastuszyn A, Hu CA. Apolipoprotein l6, a novel proapoptotic Bcl-2 homology 3-only protein, induces mitochondria-mediated apoptosis in cancer cells. Mol Cancer Ther 2005;3:21–31.
39. Li C, Kato M, Shiue L, Shively JE, Ares M, Jr., Lin RJ. Cell type and culture condition-dependent alternative splicing in human breast cancer cells revealed by splicing-sensitive microarrays. Cancer Res 2006;66: 1990–9.
40. Norris JD, Fan D, Sherk A, McDonnell DP. A negative coregulator for the human ER. Mol Endocrinol 2002;16: 459–68.
41. Lamant L, Gascoyne RD, Duplantier MM, et al. Non-muscle myosin heavy chain (MYH9): a new partner fused to ALK in anaplastic large cell lymphoma. Genes Chromosomes Cancer 2003;37:427–32.
42. Chen YT, Tu JJ, Kao J, Zhou XK, Mazumdar M. Messenger RNA expression ratios among four genes predict subtypes of renal cell carcinoma and distinguish oncocytoma from carcinoma. Clin Cancer Res 2005;11: 6558–66.
43. Iida A, Kurose K, Isobe R, et al. Mapping of a new target region of allelic loss to a 2-cM interval at 22q13.1 in primary breast cancer. Genes Chromosomes Cancer 1998;21:108–12.
44. Lasota J, Wozniak A, Kopczynski J, et al. Loss of heterozygosity on chromosome 22q in gastrointestinal stromal tumors (GISTs): a study on 50 cases. Lab Invest 2005;85:237–47.