Am. J. Hum. Genet. 69:148–158, 2001

A Genome Screen of Families with Multiple Cases of Prostate Cancer: Evidence of Genetic Heterogeneity

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We conducted a genomewide screen for prostate cancer–susceptibility genes on the basis of data from 98 families from the United States and Canada that had three or more verified diagnoses of prostate cancer among first- and second-degree relatives. We found a statistically significant excess of markers for which affected relatives exhibited modest amounts of excess allele-sharing; however, no single chromosomal region contained markers with excess allele-sharing of sufficient magnitude to indicate unequivocal evidence of linkage. Positive linkage signals of nominal statistical significance were found in two regions (5p-q and 12p) that have been identified as weakly positive in other data sets and in region 19p, which has not been identified previously. All these signals were considerably stronger for analyses restricted to families with mean age at onset below the median than for analyses of families with mean age at onset above the median. The data provided little support for any of the putative prostate cancer–susceptibility genes identified in other linkage studies.

Introduction

Prostate cancer (MIM 176807) is the most common noncutaneous malignancy among North American men: ~198,100 men in the United States will be diagnosed with the disease during the year 2001, and ~31,500 men will die of it (Greenlee et al. 2001). Although the aggregation of prostate cancer in some families is widely recognized, the genetic basis for inherited susceptibility is poorly understood. Data from many studies suggest that men who have a first-degree relative with prostate cancer are two to three times more likely to develop the disease than men in the general population (Woolf 1960; Cannon et al. 1982; Meikle and Stanish 1982; Steinberg et al. 1990; Spitz et al. 1991; Carter et al. 1992; Goldgar et al. 1994; Whittemore et al. 1995). The estimated magnitude of this relative risk does not appear to differ across three racial/ethnic groups (African American, white, and Asian American), despite large differences in incidence across these groups (Whittemore et al. 1995). Moreover, the risk appears to increase with number of affected first-degree relatives (Steinberg et al. 1990).

Received February 15, 2001; accepted for publication May 11, 2001; electronically published June 12, 2001.

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0002-9297/2001/6901-0016$02.00

Further support for inherited prostate cancer susceptibility comes from data suggesting autosomal dominant inheritance within some families (Carter et al. 1992; Gronberg et al. 1997; Schaid et al. 1998), as well as evidence of linkage of the disease to regions on chromosome 1 (Smith et al. 1996; Cooney et al. 1997; Hsieh et al. 1997, Berthon et al. 1998; Gibbs et al. 1999*b*; Neuhausen 1999; Xu 2000), chromosome 17 (Rebbeck et al. 2000; Tavtigian et al. 2001) and chromosome 20 (Berry et al. 2000*a*). There also are data implicating specific genes or regions on the X chromosome. For example, the androgen receptor gene at Xq12 has been studied extensively for its relation to prostate cancer risk. In particular, specific alleles of two trinucleotide repeats (a polyglutamine repeat and a polyglycine repeat) within this gene have been associated with altered prostate cancer incidence (Giovannucci et al. 1997; Ingles et al. 1997; Stanford et al. 1997; Platz et al. 1998) and progression (Eeles 1999). Moreover, a linkage analysis of combined data from the United States, Finland, and Sweden (Xu et al. 1998) revealed evidence of a prostate cancer–susceptibility gene on Xq27-28, ~50 cM from the androgen receptor gene. Evidence supporting this linkage has been provided by Lange et al. (1999).

However, none of these linkage results has been confirmed consistently (McIndoe et al. 1997; Eeles et al. 1998; Whittemore et al. 1999; Berry 2000*b*; Bock 2001; Vesprini et al. 2001; Xu et al. 2001). Thus, despite these provocative clues, the genetic causes of prostate cancer

remain unclear. Like other diseases with late ages at onset, prostate cancer presents formidable obstacles to mapping susceptibility loci: parents of affected men are seldom available for genotyping, and offspring are usually too young for assessment of phenotype. Numbers of informative family members are further restricted by the sex-limited nature of the disease. Moreover it has become increasingly probable that prostate cancer is a genetically heterogeneous disease. Given this likely heterogeneity, it is important to evaluate suggestive leads with several sets of independent families that contain multiple cases of the disease. Accordingly, we present here the results of a genome screen for allele sharing at 382 markers among 98 families from the United States and Canada that contain three or more members diagnosed with prostate cancer.

Families and Methods

Families

The analysis is based on 98 unrelated families, each containing three or more medically verified diagnoses of prostate cancer among first- or second-degree relatives. The families were identified from a multiethnic case-control study conducted in Hawaii, Los Angeles, San Francisco, and Vancouver (Whittemore et al. 1995), as well as from screens of the British Columbia Cancer Registry and the San Francisco–Oakland Cancer Registry and from publicity in the San Jose Mercury News. Eighty-two of these families fulfilled one or more of the proposed criteria for families in which prostate cancer is likely to be hereditary (i.e., three or more affected individuals within one nuclear family, affected individuals in three generations, and/or two or more individuals affected at <55 years of age). Seven families were African American, five were Japanese American, and three were Chinese American.

Table 1 summarizes pertinent characteristics of these 98 families. The mean number of affected and genotyped men per family was 2.6 (range 2–5 men), and the mean age at diagnosis of all affected men was 66.5 years (66.9 years in white families, 63.5 years in African American families, and 69.2 years in Asian American families). We were able to verify 331 (92.2%) of the 359 reported cases of prostate cancer in the families. We did not attempt to obtain systematic information about the stages and grades of the cancers in affected men.

Genotyping

Genotyping was performed by the NHLBI Mammalian Genotyping Service at the Center for Medical Genetics, Marshfield Medical Research Foundation. DNA samples from family members were typed at 382 autosomal and X-linked markers in the Weber screening set 9 (Yuan et al. 1997). Average marker heterozygosity was 77%, and average spacing between markers on sex-equal maps was 9 cM (Broman et al. 1998). Genotyping was implemented using an ABI 377 sequencer to read fluorescence-labeled primers for PCR products. If a marker genotype was missing or ambiguous for an individual, we used the radiolabeling method to retype that marker for that person. To insure interlaboratory comparability, we also retyped the marker in question for at least one relative of the individual and resolved any discrepancies. We genotyped a total of 55 other markers in regions containing markers with evidence suggestive of linkage, either in the present data or in data from other linkage studies. We selected the additional markers from the genome database to achieve ~2–4 cM density in the regions of interest. PCR products of these markers were fractionated using the LiCor Gene Reader 4200 and the SAGA software.

Table 1

Characteristics of 98 Families in the United States and Canada That Contain Three or More Verified Prostate Cancer Cases among First- or Second-Degree Relatives, by Mean Age at Diagnosis

	Mean Age at Diagnosis		
Characteristic	<66.5 Years (N = 49)	⩾66.5 Years (N = 49)	All Families (N = 98)
Mean no. of reported prostate cancer cases per family	3.7	3.6	3.7
Percent of reported cases verified[a]	92.8	91.6	92.2
Mean no. of affected and genotyped cases per family	2.7	2.5	2.6
Percent nonwhite	16.3	14.3	15.3

[a] Verified by medical record or death certificate.

Statistical Methods

Single-point and multipoint parametric LOD scores, nonparametric Z scores and Kong and Cox (KC) Z scores and one-tailed P values were obtained using the software GENEHUNTER-PLUS (Kruglyak et al. 1996; Kong and Cox 1997). For the parametric analyses, we assumed an autosomal dominant mode of inheritance of a disease-susceptibility allele with a frequency of .003 and with penetrances as estimated in the segregation analysis of Carter et al. (1992). For the nonparametric analyses, we report KC Z scores based on the linear model option, the ALL scoring function, and equal weights for each family. Results from the parametric and nonparametric analyses were qualitatively similar. Thus, for brevity, we report only the nonparametric statistics. We estimated marker allele frequencies in family founders, using the software FASTLINK (Cottingham et al. 1993; Schaffer et al. 1994).

Genetic analysis revealed that one pair of twins was

monozygous; we therefore removed one twin from all analyses.

Results

Figure 1 shows the distribution of single-point KC *Z* scores for the 382 markers in the Weber screening set 9. Each *Z* score is plotted as a function of the value $\Phi^{-1}(R/382)$, where R is its rank in order of increasing size, and Φ is the standard Gaussian cumulative distribution function. Under the null hypothesis of no linkage, this curve should fit the line y = x. As evident in the figure, the curve lies above the line y = x for all but two data points. This indicates that the entire distribution of KC *Z* scores is shifted upward. This shift results, in part, from the fact that 262 (69%) of the *Z* scores are positive, in contrast to the number (382/2 = 191) expected under the null hypothesis. There also is an excess of *Z* scores with values of 2.1–2.5. Also shown in figure 1 are 90% and 95% confidence intervals for the curve. These are based on 2,000 replications of 382 independent draws from the null distribution of *Z* scores, which is approximately a 50:50 mixture of the distribution with all its mass at zero and the distribution of the absolute value of a standard Gaussian variable. The nominally significant *Z* scores (i.e., those >1.96) decrease within these bounds, indicating that they do not meet the criteria for statistical significance in a 382-marker screen. However, the excess of positive *Z* scores evident in the figure is statistically significant ($P < .01$). This excess indicates the presence of modest amounts of excess allele sharing among relatives at many different loci in the genome.

Figure 2 shows multipoint and single-point KC *Z* scores for all (382 + 55 = 437) markers on all 22 autosomes and on the X chromosome. Regions with multipoint *Z* scores ⩾1.96 are seen on chromosomes 5q, 12p, and 19p. In addition, single-point *Z* scores >2.0 without correspondingly high multipoint *Z* scores are evident on chromosome 15q (D15S642, 122 cM from 15pter [KC Z = 2.67]), on chromosome 17p (D17S1308, 1 cM from 17pter [KC Z = 2.39], and D17S1303, 24 cM from 17pter [KC Z = 2.44]), and on the X chromosome (DXS2390, 195 cM from pterX; KC Z = 2.57). The X chromosome marker DXS2390 is 1 cM distal to marker DXS1113, which showed strong evidence of linkage in the data of Xu et al. (1998). We also obtained a single-point KC *Z* score of 1.93 for the marker D1S2883, which lies in the region 1q24-25 identified by Smith et al. (1996). Thus, as we noted previously (Hsieh et al. 1997), the single-point analysis provides weak support for the existence of a susceptibility gene in that region. The lack of support for these loci from the multipoint analysis may indicate power loss due to misspecification of the marker locations (Halpern and Whittemore 1999).

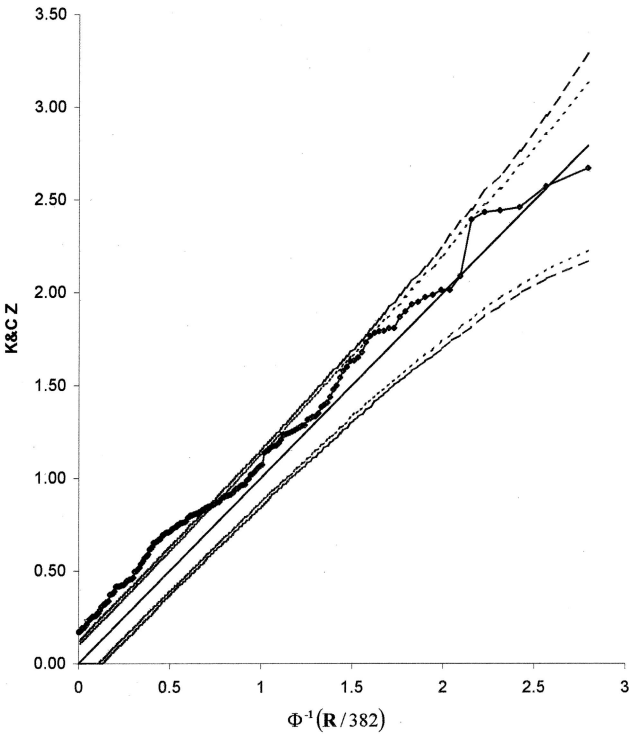

Figure 1 Blackened circles show single-point KC *Z* scores for the 382 markers in the Weber screening set 9, plotted against Φ^{-1} (R/382), where R is the ordered rank of the *Z* score, and Φ is the standard Gaussian cumulative distribution function. The curve contains only the points corresponding to *Z* scores with ranks 191–381. Under the null hypothesis of no linkage, the curve should lie on the line $y = x$. Also shown are 90% (*dotted line*) and 95% (*dashed line*) confidence intervals, based on 2,000 replications of data for 382 independent draws from a 50:50 mixture of the distribution with all its mass at zero and the distribution of the absolute value of a standard Gaussian variable.

Among the pairs of adjacent markers in the Weber screening set 9, we found only one in which both markers had multipoint KC *Z* scores >1.645 (the cutoff for nominal statistical significance based on a single marker). The two markers in this pair are 11 cM apart in region 19p: marker D19S591 (multipoint KC Z = 2.57 = 2.38) and D19S1034 (multipoint KC Z = 2.57 = 2.49). Among all 437 markers (including the additional 55 from suggestive regions), the number of such pairs was 10: one pair in region 5q, three pairs in region 12p, and six pairs in region 19p. The specific markers and their multipoint KC *Z* scores are shown in table 2.

To further investigate the suggestive evidence of linkage in regions 5q, 12p, and 19p, we subdivided the families according to numbers of affected men (3 vs. 4+) and according to mean age at prostate cancer diagnosis among the affected men (<66.5 years vs. ⩾66.5 years), where 66.5 years was the median value for all

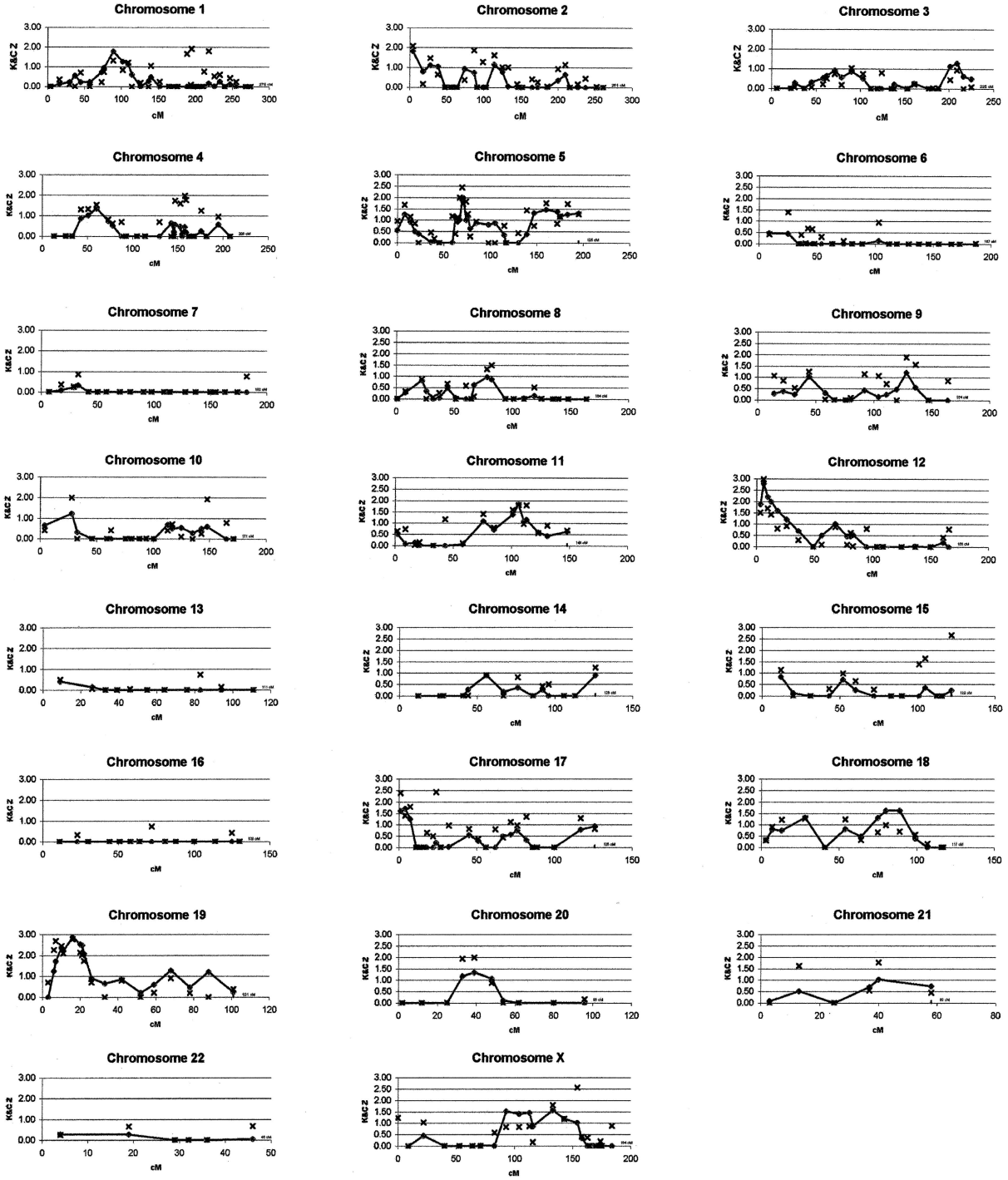

Figure 2 Single-point (×) and multipoint (♦) KC *Z* scores for the 437 markers genotyped in 98 families that contain three or more members with prostate cancer.

families. Table 2 shows multipoint KC *Z* scores for each of these subgroups and for all families combined. Virtually without exception, the *Z* scores are higher for families whose affected members were diagnosed at early ages than for those whose members were diagnosed at later ages. Because cancers due to mutations of susceptibility genes tend to occur at earlier ages than do sporadic cancers, these observations lend support to the possibility that these regions may contain susceptibility loci. For regions 5q and 12p, but not 19p, the

Table 2

Multipoint KC *Z* Scores for Regions Containing a Marker with Multipoint KC *Z* Score >1.96, According to Mean Age at Diagnosis and Number of Affected Family Members

Region and Marker	Position (cM)	*Z* Scores in Families with Mean Age at Diagnosis of <66.5 Years (N = 49)	*Z* Scores in Families with Mean Age at Diagnosis of ≥66.5 Years (N = 49)	*Z* Scores in Families with ≥4 Men Affected (N = 39)	*Z* Scores in Families with 3 Men Affected (N = 59)	Overall *Z* Scores (N = 98)
5p13.3-5q13.1						
D5S2076	62	1.21	.35	.44	1.07	1.12
D5S407	65	1.15	.11	.68	.67	.94
D5S2507	67	.96	.44	.44	.94	1.02
D5S2500	69	1.54	1.14	1.77	1.12	1.92
D5S2858	70	1.54	1.25	2.04	1.02	1.98
D5S647	74	1.45	≤0	1.82	≤0	1.01
D5S629	76	1.29	.21	1.53	.36	1.14
D5S1988	78	.84	.02	1.06	.02	.64
12p13.3-12.3						
D12S1694	3	1.18	1.47	1.71	.97	1.87
D12S372	6	2.07	1.86	2.41	1.57	2.78
D12S1725	10	1.97	1.12	2.30	.89	2.20
D12S93	13	2.08	.68	2.04	.85	2.00
D12S2395	18	2.18	≤0	2.18	.22	1.60
D12S391	26	1.06	.60	1.52	.25	1.20
D12S373	36	1.13	≤0	1.97	≤0	.70
19p13.3						
D19S883	6	1.25	.50	.66	1.05	1.24
D19S878	7	1.85	.56	.91	1.45	1.71
D19S591	10	2.39	.99	1.38	1.95	2.38
D19S209	11	2.65	.54	1.41	1.75	2.21
D19S894	16	2.67	1.38	1.71	2.33	2.87
D19S177	20	2.81	.80	1.73	1.89	2.54
D19S1034	21	2.68	.85	1.68	1.87	2.49
D19S427	22	2.13	.83	.94	1.88	2.08

Z scores tend to be higher for families with four or more affected men than for those with only three affected men.

We also evaluated evidence of linkage to markers on the X chromosome after classifying the families according to presence or absence of evidence of male:male transmission (MMT), as defined by Xu et al. (1998). Table 3 shows single-point and multipoint KC *Z* scores in the region Xq25-27 for the two subgroups of families and for all families. The single-point *Z* scores at two of the markers were elevated for families without evidence of MMT. However, the multipoint *Z* scores in the region were unremarkable.

Table 3

KC *Z* Scores for Region Xq25-27 According to Presence or Absence of Evidence for Male:Male Transmission (MMT)

Marker	Position (cM)	Scores in Families Without MMT (N= 6)		Scores in Families With MMT (N = 42)		Overall *Z* Scores (N = 98)	
		Z_1	Z_M	Z_1	Z_M	Z_1	Z_M
GATA165B12	133	2.10	.33	.55	2.06	1.79	1.58
DXS1047	143	1.02	.53	.70	1.23	1.19	1.19
DXS2390	154	2.07	.83	1.62	.59	2.57	1.02
DXS6751	158	.07	.60	.47	≤0	.39	.35
DXS8106	163	≤0	.55	1.08	≤0	.38	.00

Note.—Z_1 = single-point KC *Z* score; Z_M = multipoint KC *Z* score.

Table 4 summarizes the strength of evidence in the present data for chromosomal regions identified by other linkage analyses as possibly containing a prostate cancer–susceptibility gene. The table shows multipoint KC *Z* scores for markers within 10 cM of those identified by others as having a *Z* score >1.96 or a LOD score >0.834. The multipoint *Z* scores shown in table 4 provide no support for any of the three regions identified on chromosome 1 (1p36, 1q24-25, or 1q42-43). Indeed, only two of the regions identified by others are supported by the present data: region 5p12-q13 (identified by Smith et al. [1996]), and region 12p13-14 (identified by Suarez et al. [2000]). There is need for further evaluation of these two regions in additional linkage data.

Because of the likely genetic heterogeneity, it is pos-

Table 4

Multipoint KC *Z* Scores for Markers in Regions Reported to Contain Possible Prostate Cancer–Susceptibility Genes

Region	Marker	Position (cM)	Reference	Nearest Marker(s)	Position (cM)	Multipoint KC *Z*
1p32-41	D1S1656	245	Gibbs et al. 1999*b*	D1S549	240	.28
1q24-25	D1S218	191	Smith et al. 1996	D1S452	189	≤0
				D1S1589	192	≤0
				D1S212	194	≤0
1q42-43	D1S235	255	Smith et al. 1996	D1S3462	247	.14
				D1S235	255	≤0
	D1S2785	266	Berthon et al. 1998	D1S547	268	≤0
				D1S1609	275	≤0
2q37-38	D2S2228	224	Suarez et al. 2000	D2S1363	227	≤0
4q26-31	D4S430	126	Smith et al. 1996	D4S2623	114	≤0
				D4S2394	130	≤0
				D4S1644	143	.64
5p12-q13	D5S407	65	Smith et al. 1996	D5S2076	62	1.12
				D5S407	65	.94
				D5S2507	67	1.02
				D5S2500	69	1.92
				D5S2858	70	1.98
				D5S647	74	1.01
5q31-33	D5S1480	147	Witte et al. 2000	D5S1480	147	1.33
	D5S820	160		D5S820	160	1.47
7p21	D7S507	29	Smith et al. 1996	D7S3051	29	.23
7q32	D7S3061	128	Witte et al. 2000	D7S3061	128	≤0
	D7S1804	137		D7S1804	137	≤0
8q21	D8S2324	94	Gibbs et al. 2000	D8S1136	82	.87
				D8S2324	94	≤0
9p22-23	D9S925	32	Gibbs et al. 2000	D9S921	22	.39
				D9S925	32	.25
10q25-26	D10S1223	156	Gibbs et al. 2000	D10S1213	148	.61
				D10S1248	165	≤0
11p13-15	ATA34E08	33	Gibbs et al. 2000	ATA34E08	33	≤0
12p13-14	D12S1685	8	Suarez et al. 2000	D12S1694	3	1.87
				D12S372	6	2.78
				D12S1725	10	2.20
				D12S93	13	2.00
12q24-25	D12S1045	161	Gibbs et al. 2000	D12S1045	161	.20
				D12S392	166	≤0
13q32	D13S159	79	Smith et al. 1996	D13S793	76	≤0
				D13S779	83	≤0
14q24	D14S588	76	Gibbs et al. 2000	D14S588	76	.38
15q13	D15S1010	24	Suarez et al. 2000	D15S165	20	.14
16p13-14	D16S748	23	Gibbs et al. 2000	D16S748	23	≤0
16p13	D16S3103	32	Suarez et al. 2000	D16S748	23	≤0
				D16S764	30	≤0
16q23-24	D16S3096	99	Suarez et al. 2000	D16S2624	88	≤0
				D16S516	100	≤0
17P	D17S947	32	Tavtigian et al. 2001	D17S947	32	.04
19q12	D19S433	52	Witte et al. 2000	D19S433	52	.22
				D19S245	59	.61
20q13	D20S196	75	Berry et al. 2000*a*	D20S480	80	≤0
Xq27-28	DXS1193	175	Smith et al. 1996; Xu et al. 1998	DXS1193	175	≤0

sible that families with elevated *Z* scores in one candidate region have only slightly positive, or even negative, *Z* scores in other regions. In addition, if mutations at two different loci interact epistatically to cause disease, restriction of analysis to families with positive *Z* scores in one candidate region may substantially elevate the overall *Z* score at another candidate region (Cox et al. 1999). To investigate these possibilities, we examined correlations between family-specific *Z* scores in regions that may harbor a susceptibility locus. Table 5 shows correlation coefficients for family-specific nonparametric multipoint *Z* scores at representative markers in regions 1q24-25, 1q42-43, 5p12-q13, 12p13-14, 19p13, and 20q13. *Z* scores at the markers on chromosome 12p are negatively correlated with those in all the other regions. This suggests that families with elevated *Z* scores in this region are not segregating deleterious alleles at other candidate loci. Separate analysis of the two subgroups of families determined by mean ages at prostate cancer diagnosis produced similar correlation coefficients (data not shown). We recalculated the KC multipoint *Z* score at marker D1S452 in the *HPC1* region 1q24-25, weighting each family according to its *Z* score at D12S372, as proposed by Cox et al. (1999). Specifically, families with positive *Z* scores at D12S372 were excluded from analysis, and families with negative *Z* scores were weighted in proportion to the absolute values of their *Z* scores. This analysis did not increase the *Z* score at D1S452, as would be expected if one subset of families were linked to D1S452, with the remaining families linked to D12S372. Conversely, the multipoint *Z* score at D12S372 with families weighted according to their *Z* scores at D1S452 also failed to support such a partition of families. Furthermore, we did not find any strong indications from similar analyses at other pairs of loci of statistically significant positive or negative correlations (table 5).

Discussion

We have reported the results of a linkage screen, using a total of 437 polymorphic markers, of 98 families with multiple cases of prostate cancer. Although four chromosomal regions showed suggestive evidence of linkage, no single region showed excess allele sharing of a magnitude sufficient to exclude chance as an explanation (Kruglyak and Lander 1996). In contrast, the number of loci exhibiting small-to-moderate amounts of excess allele sharing was significantly greater than that expected by chance. This extensive excess allele sharing suggests the presence of many susceptibility loci, each having small to moderate effect, and acting either additively or synergistically to increase risk. The possibility of synergism among two or more genes is supported by prostate cancer occurrence in the co-twins of World War II veterans from the United States who had prostate cancer (Page et al. 1997). The disease was approximately four times more prevalent in monozygous (MZ) co-twins than in dizygous (DZ) co-twins of the affected men. This fourfold increase is larger than that expected under a model in which one or more genes act independently to increase risk. Instead, it suggests the synergistic action of two or more genes, all of whose deleterious alleles are more likely to be shared by an MZ co-twin than a DZ one.

We have summarized the strength of support in these data for chromosomal regions identified in previous linkage studies of prostate cancer. Overall, the level of support is weak. Three regions on chromosome 1 have been implicated as containing a potential prostate cancer–susceptibility gene: 1p36, 1q24-25, and 1q42-43. On the basis of 70 multiple-case families in a genomewide screen and 71 additional families, Gibbs et al. (1999*b*) presented evidence of a rare susceptibility locus at 1p36. This region appears to be important only for families with primary brain cancer: the authors found a LOD score of 3.22 in 12 of the families that contained at least one individual with primary brain cancer. However, an analysis of 13 families with prostate cancer that also included one or more member with a diagnosis of brain cancer did not find evidence of linkage in this region (Berry et al. 2000*b*). Cancers of

Table 5

Pearson-Product Correlation Coefficients for Family-Specific Multipoint Nonparametric *Z* Scores

		Coefficient at						
Marker	Region	D1S549	D1S452	D1S235	D5S2500	D12S372	D19S894	D20S480
D1S549	1p32-41	1.0	.25[a]	.51[b]	.12	−.11	.18	.19
D1S452	1q24-25		1.0	.20[a]	.22[a]	−.20[a]	.15	.15
D1S235	1q42-43			1.0	.05	−.20[a]	.21[a]	.11
D5S2500	5p12-q13				1.0	−.10	.13	−.03
D12S372	12p13-14					1.0	−.01	−.08
D19S894	19p13						1.0	−.01
D20S480	20q13							1.0

[a] $P < .05$
[b] $P < .01$

the CNS were reported in only six of the families included in the present analysis, and we were able to verify only one of them as being a primary cancer of the brain. Thus, we have little power to confirm or refute the linkage.

Smith et al. (1996) presented evidence, obtained from 91 families from the United States and Sweden that included multiple cases of prostate cancer, of a locus in region 1q24-25 (designated "HPC1"). A subsequent reanalysis of an expanded collection of these families suggested that the strongest linkage evidence derived from families with early mean ages at onset (Gronberg et al. 1999). This finding has received modest support from the following three studies. (1) Cooney et al. (1997) reported a nonparametric Z score of 1.58 ($P = .06$) at D1S466 that was based on analysis of 59 multiplex families. (2) We reported equivocal evidence of linkage (Hsieh et al. 1997) that was based on a subset of 92 of the 98 families described here; however, other data sets have failed to exhibit linkage in this region (McIndoe et al. 1997; Berthon et al. 1998; Eeles et al. 1998; Suarez et al. 2000). (3) A combined analysis of 772 families with multiple cases of prostate cancer that was conducted by the International Consortium for Prostate Cancer Genetics (Xu 2000) found weak evidence overall, suggesting that HPC1 may account for, at most, a small fraction of families with multiple cases of prostate cancer. We did not find additional support from the slightly expanded collection of the present families, even in that subset of families ($N = 21$) with early ages at onset and with evidence of MMT. This absence of additional support suggests that, if there is a prostate cancer–susceptibility gene in this region, it segregates only in a small subset of families with multiple cases of prostate cancer.

On the basis of findings in 47 French and German families, Berthon et al. (1998) reported a nonparametric Z score of 3.1 for marker D1S2785 in region 1q42-43. This finding is interesting because, although D1S2785 is considerably distal to the region 1q24-25 identified by Smith et al. (1996), this marker is only 14 cM away from D1S235, which also produced an elevated Z score in the scan by Smith et al. (1996). However, the multipoint KC Z scores for all three of the nearest markers in the present analysis were negative, as we have reported elsewhere (Whittemore et al. 1998). Moreover, Gibbs et al. (1999*a*) reported negative LOD scores for four markers in this region.

In addition to these three regions on chromosome 1, linkage studies have implicated regions on chromosomes 16 (Gibbs et al. 2000; Suarez et al. 2000), 17 (Tavtigian et al. 2001), and 20 (Berry et al. 2000*a*). Using data from 162 North American families that contained three or more members with prostate cancer, Berry et al. (2000*a*) reported evidence of linkage to region 20q13, with a maximum multipoint nonparametric Z score of 3.02 at D20S887 and a maximum single-point LOD score of 2.69 for marker D20S196. However, the linkage study of Bock et al. (2001) did not provide statistically significant support for this locus. Moreover, in the present data, none of the multipoint KC Z scores for markers near these regions were elevated. Similarly, these data did not show strong evidence of linkage to markers near the gene *HPC2* identified by Tavtigian et al. (2001), and reports of altered prostate cancer risk associated with two relatively common polymorphisms of these genes have been equivocal (Rebbeck et al. 2000; Xu et al. 2001; Vesprini et al. 2001).

Instead, we found elevated KC Z scores in three regions: 5p13.3-5q13.1, 12p13.3-12.3, and 19p13.3. There is modest evidence suggesting that chromosome 5q may harbor a prostate cancer–susceptibility gene (e.g., reports by Lin et al. [2000] and Bova et al. [1996]). Nonstatistically significant LOD scores of ~1.0 were noted in the centromeric region 5p13.3–5q13.1 among the 91 families from the United States and Sweden described by Smith et al. (1996). The long arm of chromosome 5 contains the gene encoding α-catenin (CTNNAI), which is part of the E-cahedrin pathway that has been implicated extensively in prostate carcinogenesis. Associations between LOH on 5q and prostate cancer stage at diagnosis have been noted (Cher et al. 1996, 1998; Cunningham et al. 1996; Latil et al. 1996; Brothman et al. 1997; Dong et al. 1997; Ozen et al. 1998).

We found a large region on chromosome 12p containing markers with elevated Z scores. Moreover, the elevated Z scores were restricted to families in which prostate cancers were diagnosed when affected family members were relatively young. Suarez et al. (2000) also found elevated KC Z scores in this region, and these authors found stronger signals when analysis was restricted to families with relatively early ages at onset. Kibel et al. (1998, 1999, 2000) used representational difference analysis to identify 12p12-13 as a region of frequent deletion in prostate cancer (see also Azar et al. [1997]).

In the present data, the strongest evidence of linkage occurred in the region 19p. The evidence was consistently stronger when analysis was restricted to families in which prostate cancers were diagnosed when affected family members were relatively young, compared with families that had older ages at onset. To our knowledge, this region has not been reported by other investigators as harboring a putative susceptibility locus, and it warrants further investigation.

In conclusion, in these 98 families with three or more confirmed cases of prostate cancer, we found statistically significant evidence of excess allele sharing among af-

fected relatives throughout the genome. However, in no single chromosomal region did the excess allele sharing meet the criteria for statistical significance in a genomewide linkage scan. The regions with the highest *Z* scores (on chromosomes 5, 12, and 19) need confirmation or refutation in other data sets. Conversely, the present data fail to support strong roles for the loci identified by other investigators. This failure is consistent with the general lack of confirmation of rather strong initial linkage signals that has plagued the study of inherited susceptibility to prostate cancer. The strength of these initial signals suggests that some rather penetrant mutations are segregating in some families and that the studies reporting signals have ascertained by chance, a substantial fraction of such families.

The probable multiplicity of prostate cancer–susceptibility genes, their probably varied modes of inheritance, and their low penetrances complicate the task of gene identification. An additional complication is the variability within and between families in prostate cancer aggressiveness, as measured by the stage and grade of disease at diagnosis. This variability has been increased by the introduction of prostate-specific antigen (PSA) screening in the mid-1980s. If a gene causes only aggressive cancer and if PSA-detected prostate cancers tend to be less aggressive, then the presence of PSA-screened cancers in families studied for linkage could substantially decrease power. These complications, when coupled with other barriers to research in prostate cancer etiology (e.g., the high prevalence of subclinical cancer in older men) provide formidable challenges to our further progress in attempts to understand the genetic basis of this disease. A full discussion of these issues and of suggested strategies for dealing with them has recently been provided by Ostrander and Stanford (2000).

Acknowledgments

This research was supported by National Institutes of Health grants R01 CA49446 and R35 CA47448. We are grateful for the genotyping support of James L. Weber, Director of the National Heart, Lung and Blood Institute Genotyping Service at the Center for Medical Genetics, Marshfield Medical Research Foundation.

Electronic-Database Information

Accession number and URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for AHC [MIM 176807]).

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