Results of a Genome-Wide Linkage Analysis in Prostate Cancer Families Ascertained Through the ACTANE Consortium†

[I]The International ACTANE Consortium*

BACKGROUND. The aggregation of prostate cancer within families suggests a major inherited component to the disease. Genetic linkage studies have identified several chromosomal regions that may contain prostate cancer susceptibility loci, but none has been definitively implicated.
METHODS. We performed a genome-wide linkage search based on 64 families, 63 with at least 3 cases of prostate cancer, ascertained in five countries. The majority of cases from these centers presented with clinically detected disease. Four hundred and one polymorphic markers were typed in 268 individuals. Multipoint heterogeneity analysis was conducted under three models of susceptibility; non-parametric analyses were also performed.
RESULTS. Some weak evidence of linkage, under at least one of the genetic models, was observed to markers on chromosomes 2 (heterogeneity LOD (HLOD) = 1.15, $P = 0.021$), 3 (HLOD = 1.25, $P = 0.016$), 4 (HLOD = 1.28, $P = 0.015$), 5 (HLOD = 1.20, $P = 0.019$), 6 (HLOD = 1.41, $P = 0.011$), and 11 (HLOD = 1.24, $P = 0.018$), and in two regions on chromosome 18 (HLOD = 1.40, $P = 0.011$ and HLOD = 1.34, $P = 0.013$). There were no HLOD scores greater than 1.5 under any model, and no locus would be predicted to explain more than half of the genetic effect. No evidence in favor of linkage to previously suggested regions on chromosomes 1, 8, 17, 20, or X was found.
CONCLUSIONS. Genetic susceptibility to prostate cancer is likely to be controlled by many loci, with no single gene explaining a large fraction of the familial risk. Pooling of results from all available genome scans is likely to be required to obtain definitive linkage results. *Prostate 57: 270–279, 2003.* © 2003 Wiley-Liss, Inc.

KEY WORDS: **prostate cancer; linkage; genome search; ACTANE**

INTRODUCTION

Prostate cancer is one of commonest cancers in the developed world. The etiology of the disease is, however, poorly understood. One of the few established risk factors for prostate cancer is having a family history of the disease, suggesting that there may be an inherited component of susceptibility to this disease. This hypothesis is supported by the strength of concordance for disease observed in twin pairs, that is higher within monozygotic pairs than within dizygotic pairs [1,2]. Overall, the risk of prostate cancer is increased approximately two-fold in men with a first degree family history of the disease, but the relative risk is greater in men with relatives affected at a younger age or with multiple affected relatives [3]. Segregation

†See Appendix A for Authorship Information.

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analyses have suggested that this familial aggregation may be attributable to one or more, relatively rare, susceptibility alleles conferring a dominantly-inherited high lifetime risk of the disease [4–6]. Other authors have, however, suggested a role for recessive or X-linked models [7,8], or a mixture of dominant and recessive/X-linked components [9].

Several studies have attempted to map prostate cancer susceptibility genes by linkage analysis using multiple case families. Smith et al. [10] found evidence of linkage to markers on chromosome 1q24-25 (*HPC1*), with a multipoint LOD score of 5.34 and an estimated 34% of families being linked. Confirmatory evidence of linkage to this region was reported by some groups [11–16], but not others [17–22]. A meta-analysis by the International Consortium for Prostate Cancer Genetics (ICPCG) in 772 families found only marginally significant evidence of linkage, with a LOD score of 1.40 and an estimated 6% of families being linked to this locus. Evidence for linkage was stronger among families with five or more cases of prostate cancer and an average age of onset of <65 years [23]. Subsequent mutation analysis of candidate genes found mutations in the *RNASEL* gene, which lies in the *HPC1* region [24]. Other groups have, however, failed to confirm an association between *RNASEL* variants and prostate cancer [25,26].

A second putative susceptibility locus was suggested by Tavtigian et al. [27], who found evidence of linkage to markers on chromosome 17p, and subsequently reported sequence variants in *HPC2* (*ELAC2*) in familial prostate cancer cases. Again, however, other groups have found few deleterious variants in this gene and little evidence that they segregate with disease in families. Some association studies have suggested that there is an increased risk of prostate cancer associated with two common polymorphisms in this gene, but it now appears that this risk is, at most, moderate [28,29].

Subsequent genome-wide searches have indicated several additional regions of interest. Although some regions have been identified by more than one study, none has yet been definitively replicated. The regions suggested include a further locus on chromosome 1q42 (*PCAP*) [18], and regions on Xq27 [22,30], 8p22-23 [31,32], and 20q13 (*HPC20*) [33]. Goddard et al. [15] found linkage to markers on 4q (LOD 2.80) and Xq12-13 (LOD 3.06) using covariates determined by cluster size, age at onset, and tumour characteristics. An affected sibling pair study found suggestive evidence of linkage to regions on 2q, 12p, 15q, 16p, and 16q [34]. Gibbs et al. [35] have suggested linkage to a locus on 1p36 (*CAPB*) in families with cases of primary brain tumour in addition to prostate cancer. Additional regions on 19q12-13.11, 5q31, and 7q32-34 have been suggested to contain loci associated with aggressive disease as defined by a higher stage and grade [36–38].

Sequence variants have been reported in familial prostate cancer cases in *MSR1*, a gene in the 8p linked region [39], but this association has not been replicated. No definite susceptibility genes in any of the other possible linkage regions have yet been reported.

In order to evaluate further the evidence for linkage to previously reported regions, and to identify new linked regions, we have performed a genome-wide linkage search based on 65 multiple case families ascertained through the Anglo/Canadian/Texan/Australian/Norwegian/EU Biomed (ACTANE) consortium.

MATERIALS AND METHODS

Family Collection

Multiple case prostate cancer families contributed by the ACTANE consortium were collected through five centers, as follows:

UK. Families were ascertained through a national study of familial prostate cancer. Families with at least three cases of prostate cancer at any age or affected relative pairs, at least one of whom was diagnosed at <65 years, were eligible for inclusion. Approximately 97% of cases within these families were clinically detected. Cases of prostate cancer were confirmed by pathology reports, medical records, or death certificates.

Canada. The criterion for entry into this study was at least two affected men with prostate cancer. Family history, DNA, and pathological records were collected from probands and affected relatives with prostate cancer in Montreal over the years 1992–1999. Families were identified through a prostate cancer clinic associated with McGill University (3 families); through referral by urologists elsewhere in Canada (5 families); and through the Patient Advocates for Advanced Cancer Treatment (PAACT; 10 families), a not-for-profit patient group established in the USA by Mr. Lloyd Ney. At least 50% of the cases were detected as a result of PSA screening, and in several cases, the prostate cancer diagnoses subsequent to the first one were as a direct result of targeted screening following the proband's diagnosis.

Texas. Index cases were patients referred to the UTMD Anderson Cancer Centre, Houston, Texas. Diagnoses of prostate cancer were subsequently confirmed by pathological review. Approximately 40% were clinically detected.

Australia. Families were ascertained from a population-based case-control study conducted in Melbourne, Sydney, and Perth between 1994 and 1998 [9]. All probands had histopathological confirmation of prostate cancer with Gleason scores of >4. Affected family members' diagnoses were verified on the National Cancer Registry or by medical record where possible. Approximately 65% of all cases were clinically detected.

Norway. Families were ascertained through the Department of Medical Genetics at the Norwegian Radium Hospital. Diagnoses of prostate cancer were confirmed by medical records or the Norwegian Cancer Registry. Families were also ascertained via urology clinics in Ullevaal hospital. All Norwegian cases presented symptomatically.

Eu Biomed. These families were collected from major urological centers over Europe. For this study, none were large enough to include in this genome search and will be included in future confirmatory analyses.

For the genome-wide search, we restricted analyses to families in which DNA was available from at least two affected members in each cluster. We then selected the 65 most informative families, on the basis of the expected LOD score under the rare dominant inheritance model of Carter et al. [4] (see below), given a highly informative marker in tight linkage with the susceptibility locus. Details of these families are given in Table I. All but one of these families includes at least three cases of prostate cancer. Samples were available from 184 men with prostate cancer (mean age at diagnosis 62 years, range 36–83 years) and 85 unaffected ones (mean age 64 years, range 38–96 years; 33 men and 52 women). All sampled individuals were Caucasian. Samples were analyzed in 3 × 96-well trays, each of which also included control DNAs for quality control and lane tracking, and a negative control.

Marker Genotyping

Four hundred and one microsatellite markers were genotyped. The marker set was based on Research Genetics, Inc., version 9 (http://www.resgen.com) which spans the genome at approximately 10 cM density. We included additional markers to cover previously described candidate regions at greater density. Genotypes were generated following PCR with 5′ fluorescently labelled primers (labelled with FAM, HEX, or NED; ABI). Fragments were post PCR mixed in 45 panels and run on Applied Biosystems 377 genetic analyzers. Alleles were assigned in ABI genotyper.

PCR

PCR and thermocycling was conducted in 15 μl reactions using proprietary 1× buffer (AbGene final conc. 75 mM Tris-HCl pH 8.8, 20 mM ammonium sulphate, 0.01% v/v Tween 20) and magnesium chloride (1.5 mM), dNTP (AbGene 1.25 mM total), fragment primers 0.1 OD (absorbance units A_{260} nm), Taq (AbGene Thermoprime Plus DNA Polymerase 0.35 U), 5 μl DNA (25 ng per reaction at the start of project, then lowered to 12.5 ng). For weak or difficult fragments a magnesium and temperature titration was conducted. Thermocycling was conducted using a 57–50°C touchdown protocol and was conducted on a ThermoHybaid multiblock system and consisted of 96°C for 2 min, 94°C for 45 sec, 57°C for 45 sec, 72°C for 60 sec (8 cycles with decreasing annealing temp 1°C per cycle), 94°C for 45 sec, 50°C for 45 sec, 72°C for 60 sec (22 cycles), and 72°C for 10 min.

After PCR, representative products were run on an ABI 377 genetic analyzer to obtain an approximate fluorescence intensity ('Intensity Checks'). The volume of each product required for the mix was then calculated to obtain an approximate intensity of 1,000 fluorescent units on the ABI 377. The PCR products were analyzed using standard methods on an ABI 377

TABLE I. Summary of 65 Families Used in the ACTANE Genome-Wide Search*

Group	Total number of families	Number of cases of prostate cancer				
		2	3	4	5	>5
Anglo (UK)	16	0	3	9	3	1
Canada	18	1	6	4	5	2
Texas (USA)	3	0	2	1	0	0
Australia	26	0	11	12	3	0
Norway	2	0	1	1	0	0
EU Biomed	0	0	0	0	0	0
Total	65	1	23	27	11	3

*One family was excluded (see methods).

genetic analyzer, using a modification; a small volume of sample was loaded by multichannel pipette on to a 'comb dipper' (a device to enable a membrane comb to be dipped and absorb samples). A membrane comb (Web Scientific CAE96 then subsequently CAM96) was loaded as per manufacturer's instructions and then placed on the gel (Hamoudi et al., personal communication). Samples were pre run onto the gel and then subjected to electrophoresis at 1,650 V for upto 5 hr. Data obtained from the ABI 377 were collected by Genescan and then submitted for more rigorous size standard checking in an in-house program GSQC ('GeneScan Quality Control,' Hamoudi et al., personal communication). This program ensured the presence of the requisite size standard peaks (Rox 500) in each sample lane of the gel. Error messages would highlight problems such as weak intensity or high baseline. Such problems could then be corrected by manual adjustment and were a rare occurrence.

After quality control, data were imported into Genotyper software package ver. 2.1 (ABI) for automatic allele sizing. Control samples included Ceph DNA 1347-02 (http://www.cephlib.fr) for which genotype information was available for over 90% of the markers. Subsequently, all samples were checked by the operator for correct assignment and then rechecked by a second operator.

After constructing linkage files, all marker typings inconsistent with Mendelian inheritance were rechecked. We also performed two further consistency checks. Firstly, we checked for pedigree inconsistencies both manually and using the program PREST [40]. This program estimates the mean IBD sharing across all markers, and hence identifies potential errors in pedigree structures. Using this check, we identified one family that erroneously included multiple individuals unlikely to be related; this family was excluded from all analyses. Three further families were found to have more minor inconsistencies, and these families were included in the analysis after appropriate corrections to the pedigree structures.

Statistical Analysis

Multipoint linkage analyses were performed using the program Genehunter (ver. 2.1) [41]. We computed multipoint HLOD scores under three genetic models. First we used a model based on the segregation analysis of Carter et al. [4] that has been used in previous linkage analyses. Under this dominant inheritance model, susceptibility to prostate cancer is conferred by alleles in one or more genes with an overall population frequency of 0.3%, such that the cumulative risk of prostate cancer is 85% by age 85 in carriers as compared to 6.5% in non-carriers. This model is referred to as the ''rare dominant (RD)'' model. To allow for the possibility that susceptibility may be conferred by commoner alleles conferring lesser risks, we also performed analyses based on the segregation analysis by Grönberg et al. [5], in which the frequency of the susceptibility allele (or alleles) was 1.67%. This allele was assumed to confer a lifetime risk (to age 85) of 63%. We refer to this as the ''common dominant (CD)'' model. To allow for the possibility of recessive inheritance, we performed analyses based on a recessive susceptibility allele with population frequency 7.7% (chosen so that the frequency of susceptible individuals was equal to that under the RD model). In this model (the ''RR'' model), the penetrances were taken to be the same as in the RD model. Analysis of X chromosome markers were undertaken using xgh (ver. 1.3), using models analogous to the RD and CD models.

Age-specific penetrances were implemented by assigning individuals to 1 of 11 liability classes, with separate liability classes for affected individuals by age at diagnosis (<50, 50–59, 60–69, 70–79, 80+ years) and unaffected individuals by age at last observation (females and males <40, 40–49, 50–59, 60–69, 70–79, 80+ years). The penetrances assigned to these classes were the estimated cumulative risks to the mid-point of the age-interval, for unaffected, and the disease risk (density) over the interval for affected [42].

All analyses were performed under genetic heterogeneity to allow for multiple disease loci, using the standard admixure model of Smith [43]. Thus, results are presented in terms of the maximum HLOD scores over the proportion of families linked (α), for each chromosomal location. Non parametric linkage (NPL) scores were also computed [40].

For markers in the pseudoautosomal regions, we calculated LOD scores adjusting for linkage between these markers with the non-recombining Y. In this analysis the null hypothesis allowed the recombination fraction in males, θ_m, to be the recombination fraction between the marker and Y specific markers (estimated as $\theta_m = 0.02$ for DXYS154 and $\theta_m = 0.2$ for DXYS6814 [44].

The order or marker loci and distances between them were compiled using Marshfield and Research Genetics maps (http://www.marshfieldclinic.org/research/genetics). Sex averaged recombination fractions were used. Allele frequencies were estimated empirically based on the observed frequencies in this dataset. The marker positions in the figure are given as the distance in centimorgans from the most telomeric marker on the short arm of the relevant chromosome.

RESULTS

Multipoint HLOD scores across the genome under the rare dominant model are illustrated in Figure 1. All

HLOD scores over one under any model are given in Table II. Under the rare dominant model the highest HLOD scores were on chromosomes 6 (HLOD = 1.41, $P = 0.011$ at position 11.0) and 18 (HLOD = 1.40, $P = 0.011$ at position 102.0). A second peak on chromosome 18 was observed 44 cM proximal to the first (HLOD = 1.34, $P = 0.014$). Two further peaks with LOD scores in excess of one were found on chromosomes 3 (1.19, $P = 0.019$) and 4 (1.10, $P = 0.024$), respectively.

Analysis under the common dominant model identified the same linkage peaks as the rare recessive model, with minor differences in the HLOD scores (Table II). One further HLOD in excess of one was identified on chromosome 2 (HLOD = 1.01, $P = 0.031$).

Under the recessive model, HLOD scores over one were obtained at the peak on chromosome 2 and the two peaks on chromosome 18. Two further scores greater than one on chromosomes 5 (HLOD = 1.20, $P = 0.019$) and 11 (HLOD = 1.24, $P = 0.017$) were identified.

Using the NPL approach, significant evidence of linkage (as defined by a Z-score nominally significant at the 5% level) was found for the peaks on chromosomes 4 ($P = 0.008$), 5 ($P = 0.031$), and 6 ($P = 0.04$), and for the two peaks on chromosome 18 ($P = 0.048$ and $P = 0.012$). No additional nominally significant regions were identified.

Table II also summarizes the linkage results subdivided by the number of affected individuals in the family. The evidence for linkage on chromosome 4 is entirely due to families with less than five cases of prostate cancer (HLOD = 2.21). DXYS154 achieved an HLOD of 1.59 for families with fewer than five cases under the RD model, although the overall HLOD for this marker was 0.60.

To evaluate the evidence that any of the observed loci were specifically linked to clinically detectable disease, we performed additional analyses excluding the families from Canada, where the rate of screen detected disease was highest. In this analysis, the HLOD score for the chromosome 6 peak increased to

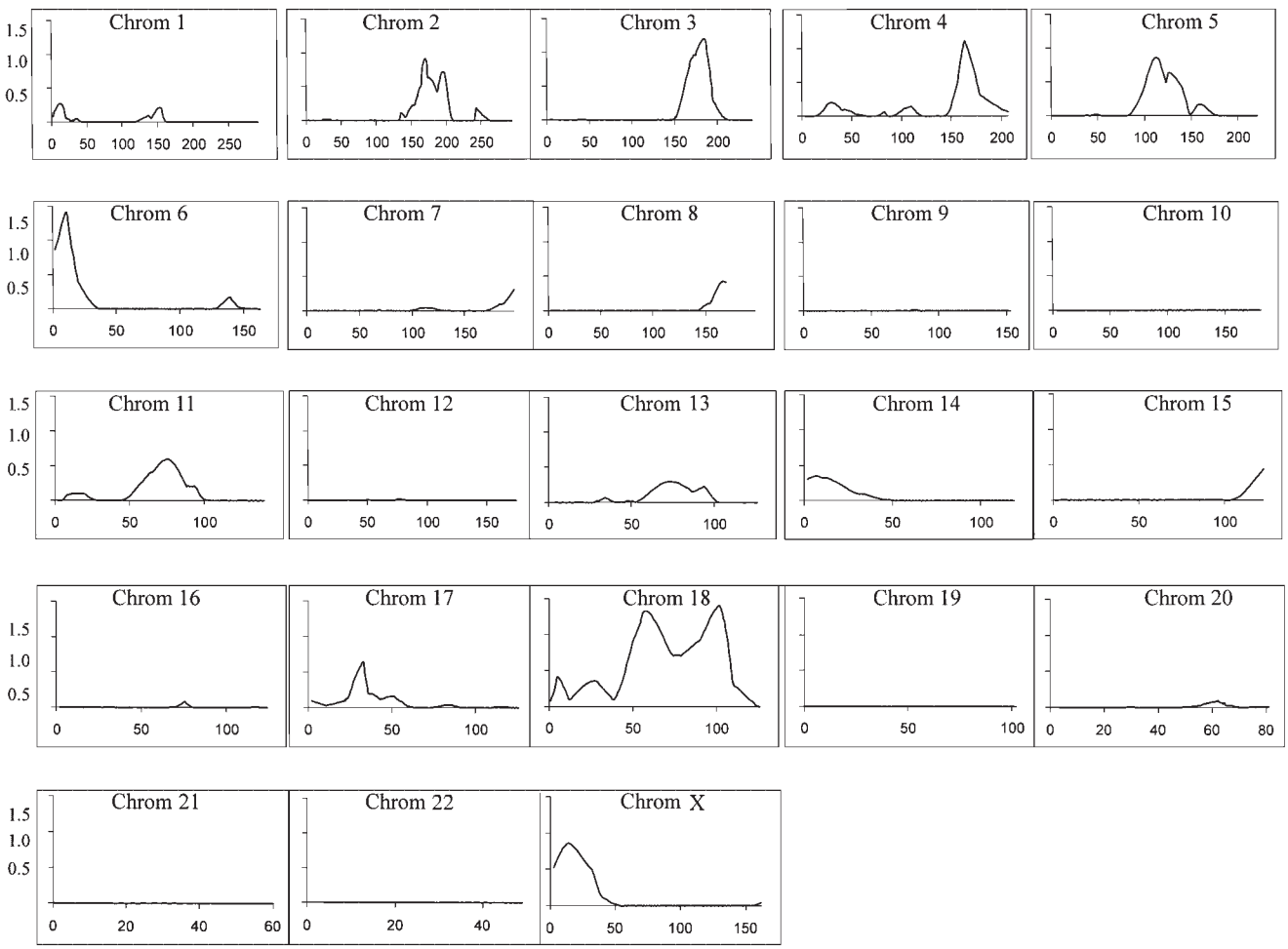

Fig. 1. Heterogeneity LOD scores by chromosomal position (in centimorgans from the most telomeric short arm marker) under the rare dominant (RD) model. Chrom, chromosome.

TABLE II. Summary of Heterogeneity LOD Scores Greater than 1.0 in 64 Prostate Cancer Families

		All families			Families with <5 cases of prostate cancer			Families with 5+ cases of prostate cancer		
Chromosome	Model[a]	HLOD	α^b	Nearest marker	HLOD	α^b	Nearest marker	HLOD	α^b	Nearest marker
2	RD	0.91	0.33	D2S1399	1.07	0.37	D2S1399	0.67	0.38	D2S434
	CD	1.01	0.43	D2S1776	1.15	0.44	D2S1399	0.54	0.42	D2S434
	RR	1.15	0.31	D2S1399	0.87	0.29	D2S1399	0.58	0.94	D2S1353
3	RD	1.19	0.32	D3S1763	0.62	0.31	D3S1763	0.49	0.36	D3S1763
	CD	1.25	0.36	D3S1763	0.68	0.37	D3S1763	0.50	0.42	D3S1763
	RR	0.61	0.16	D3S1763	0.73	0.20	D3S1763	0.76	0.73	D3S1766
4	RD	1.10	0.34	D4S2368	2.12	0.58	D4S2368	0.07	0.01	D4S408
	CD	1.28	0.41	D4S2368	2.21	0.67	D4S2368	0.01	0.12	D4S408
	RR	0.84	0.30	D4S2417	0.94	0.34	D4S2417	0.12	0.31	D4S1652
5	RD	0.85	0.34	D5S1725	1.07	0.36	D5S2501	0.24	0.44	D5S1725
	CD	0.83	0.39	D5S1725	1.11	0.42	D5S2501	0.16	0.43	D5S1725
	RR	1.20	0.29	D5S1453	1.21	0.31	D5S2501	0.19	0.40	D5S820
6	RD	1.41	0.35	GATA163B10	0.77	0.32	GATA163B10	0.58	0.40	GATA163B10
	CD	1.32	0.38	GATA163B10	0.65	0.33	GATA163B10	0.65	0.50	GATA163B10
	RR	0.96	0.29	D6S1959	0.54	0.23	D6S1959	0.45	0.50	D6S1959
11	RD	0.60	0.21	D11S2371	0.75	0.30	D11S1986	0.54	0.45	D11S2371
	CD	0.67	0.26	D11S2371	0.67	0.32	D11S1986	0.45	0.50	D11S2371
	RR	1.24	0.32	D11S2371	1.25	0.33	D11S2002	0.26	0.49	D11S1981
18	RD	1.34	0.35	D18S877	1.16	0.45	D18S877	0.86	0.64	D18S481
	CD	1.30	0.44	D18S877	1.20	0.54	D18S877	1.16	0.88	D18S481
	RR	1.01	0.29	D18S877	0.83	0.33	D18S877	0.54	0.57	D18S481
	RD	1.40	0.30	D18S878	1.04	0.41	GATA82B02	0.81	0.24	D18S1357
	CD	1.38	0.37	D18S878	1.09	0.48	GATA82B02	0.69	0.31	D18S1357
	RR	1.25	0.27	D18S878	0.64	0.26	D18S878	0.93	0.42	D18S851

[a]For definitions, see ''Materials and Methods.''
[b]Estimated proportion of linked families.

1.94 and the chromosome 4 peak increased to 1.33, while the scores for the chromosome 18 peaks reduced (HLODs 0.64 and 0.41).

Table III summarizes the HLOD scores for some of the regions suggested by previous linkage analyses of prostate cancer families, under the three models. There was no evidence for linkage to any of the regions under any model. In no case did the estimated proportion of linked families exceed 7%.

DISCUSSION

In our genome-wide linkage search, we identified eight chromosomal regions with some evidence of

TABLE III. Heterogeneity LOD Scores at Specified Candidate Loci

			Rare dominant		Rare recessive		Common dominant	
Arm	Linkage to	Nearest marker	HLOD	α	HLOD	α	HLOD	α
1q24-25 (10)	*HPC1:* D1S422	D1S518	0.00	0.00	0.00	0.00	0.00	0.00
1q42.2-43 (18)	*PCAP:* D1S2785	D1S547	0.00	0.00	0.00	0.00	0.00	0.00
1p36 (34)	*CAPB:* D1S1597	D1S1612	0.04	0.06	0.00	0.00	0.03	0.06
8p (31)	D8S1130	D8S1130	0.00	0.00	0.00	0.00	0.00	0.00
17p (27)	*ELAC2/HPC2:* D17S1289	D17S1289	0.10	0.07	0.00	0.00	0.05	0.05
20q13 (33)	*HPC20:* D20S887	D20S887	0.00	0.01	0.00	0.00	0.00	0.00
Xq27-28 (30)	*HPCX:* DXS1200-DXS297	GATA31E08	0.03	0.05	0.02	0.04	0.02	0.05

linkage, as defined by HLOD scores in excess of one in the total dataset, but no scores in excess of two. The number of ''linkage peaks'' could be consistent with what would be expected by chance, so it is possible that none represents a real susceptibility locus. Asymptotic arguments suggest that the expected number of such peaks for a single analysis with an infinitely dense map is of the order of five [45], though the expected number of peaks may be less for analyses based on finite numbers of markers and the arguments may not apply accurately to HLODs in specific datasets [45,46]. Nonetheless, these loci would be worthy of more detailed investigation in other family sets. We did observe an HLOD score of 2.21 on chromosome 4 in families with less than five cases of prostate cancer. However, at least one LOD score of this magnitude in a subgroup might be expected, given that three subgroups were examined. One of the chromosome arms identified by our search, 2q, was also identified by Suarez et al. [34] in their analysis based on 230 multiplex sibships. They reported a Z-score of 2.78 at D2S2228, but this is approximately 60 cM distal to our observed peak. None of the other regions suggested by our search coincided with those suggested to be of interest by other searches. Our HLOD scores at each of the chromosomes 1, 8, X, and 20 loci were close to zero.

The failure to replicate initial linkage results has been a persistent feature in prostate cancer family studies. This is most clearly illustrated in the case of the *HPC1* locus, where an initial LOD score of greater than 5 (generally considered strong evidence for linkage), could not be replicated in a combined analysis of 772 families [23]. The other regions have followed a similar history. Genetic heterogeneity between populations has been suggested as a possible explanation for the differences between studies, but this seems unlikely given that most studies, including our own, are based largely on individuals of Western European ancestry.

A more plausible explanation for the non-replication is clinical heterogeneity. Most of the previously published linkage studies are based on families from the USA, where the majority of prostate cancer is detected by PSA screening whilst asymptomatic. In contrast, PSA screening is still relatively uncommon in most Western European countries, including the UK and Norway, and is less common in Australia than in the USA. As a result, the majority of cases in our families were detected as a result of symptomatic disease. The incidence of the disease in screened populations is far greater than in non-screened populations, indicating that the great majority of screen detected cases would not have progressed to symptomatic disease. If the genetic basis of symptomatic disease were distinct from that underlying non-aggressive asymptomatic disease, this might explain the failure of our study to replicate the positive linkage results from other groups, and vice versa. Witte et al. [36] have studied disease aggressiveness as a quantitative trait, as defined by grade and stage in families, and found linkage of this trait to markers on chromosomes 5, 7, and 19. However, we did not find any hints of linkage to these regions except to chromosome 5 under a recessive model.

Another difficulty is the choice of an appropriate genetic model. Several segregation analyses have suggested a dominant model of susceptibility, but the apparently greater risk to brothers of cases than to fathers indicated by studies suggests a recessive (or X-linked) component. Moreover, given that there are several susceptibility loci involved, the penetrances and allele frequencies relevant to a particular locus cannot be known in advance. We have attempted to deal with this problem by analyzing our data under a recessive model and two dominant models with different allele frequencies, incorporating locus heterogeneity, as well as the allele sharing method implemented in Genehunter. Although the true model will undoubtedly be more complex than any that we have used, use of this variety of models should have given reasonable robustness against model misspecification.

Perhaps the most likely explanation for the discrepancies between studies is that there are, in fact, many prostate cancer susceptibility loci, but none explain a substantial fraction of the familial effect. If this is the case, none of the studies will have sufficient power to detect these loci by linkage. This phenomenon is illustrated by *BRCA2*. Studies of both breast cancer families and early onset prostate cancer cases have shown clearly that germline mutations in the *BRCA2* gene are associated with a markedly increased risk of prostate cancer [47–49]. Despite this, none of the genome searches, including this study, have shown any evidence of linkage to the *BRCA2* region in prostate cancer families, presumably because the mutations are too infrequent to give a detectable signal.

Our study has identified several new chromosomal regions that may contain genes of particular relevance to clinically presenting disease in particular, and that would warrant investigation in other family sets. Definitive identification of prostate susceptibility loci is likely to require pooling of data from genome scans, and direct analysis of candidate genes.

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Conflicts of interest: none

APPENDIX: ACTANE INVESTIGATORS

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