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ARTICLE

Linkage of aggressive prostate cancer to chromosome 7q31-33 in German prostate cancer families

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It has been suggested that chromosome 7q32 contains genes that influence the progression of prostate cancer from latent to invasive disease. In an attempt to confirm this linkage to prostate cancer aggressiveness, 100 German prostate cancer families were genotyped using a panel of eight polymorphic markers on chromosome 7q. We used a multipoint allele sharing method based upon a likelihood ratio test implemented in GENEHUNTERPLUS v1.2 in order to calculate the nonparametric Z_{lr} and the associated LOD scores. We applied the aggressiveness of prostate cancer given by the pathological tumour grade of each individual, and the mean age of onset of a family as covariates, and constructed two weighted models. The first (weight$_{0-1}$ model) puts weights on families with at least two cases of GIII prostate cancer. The second (weight$_{0-2}$ model) also adds weights to families with early and late onset cancer respectively. The unweighted analysis gave no evidence of linkage to chromosome 7q. The Z_{lr} scores increased when including the covariates, to 2.60 (P=0.005) using the weight$_{0-1}$ and to 3.02 (P=0.001) using the weight$_{0-2}$ model for late onset prostate cancer. The associated LOD scores were respectively 1.47 (P=0.009) and 1.98 (P=0.002). The markers that gave most evidence for linkage were exactly in the range of the published prostate cancer aggressiveness region. Our results support a widespread relevance of this locus and suggest that aggressive and late onset prostate cancer is linked to chromosme 7q31-33 in the German population.
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Introduction

Prostate cancer is a common disease, with multifactorial and complex etiology. The mechanisms of tumour initiation and progression are poorly understood. Epidemiological data, including case control studies and complex segregation analyses, clearly demonstrated a genetic background of the disease. Two susceptibility genes have been proposed so far,[1,2] but the associated mutations segregate only in a very small number of families.[3] In addition multiple susceptibility loci have been suggested, with most of them being specific to the sample they were first detected in. The varying results of different linkage analyses and association studies give evidence of the genetic heterogeneity and complex nature of prostate cancer.[4,5]

Recently a genome wide sib pair analysis used the Gleason score, a measure of prostate cancer aggressiveness, as a quantitative trait for linkage, suggesting chromosomes 5, 7 and 19 may contain genes that influence the aggressiveness of prostate cancer.[6] The strongest signals were detected on chromosome 5q31-33, chromosome 7q32 and chromosome 19q12. Several cytogenetic and LOH studies showed an association of 7q with aggressive prostate cancer.[7–11] Furthermore several potential tumour suppressor genes associated with PCA are localized in the region of 7q31.[12,13] Therefore we focused on chromosome 7 in this analysis.

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The goal of the current study was to examine linkage to chromosome 7q in a set of 108 German prostate cancer families. To account for the known genetic heterogeneity we incorporated the information on 7q32 being linked to aggressive prostate cancer[6] in our analysis. Instead of equal weighting of every family when performing traditional linkage analysis, we specified individual weights for each family based on covariates such as prostate cancer aggressiveness and age of onset.

Materials and methods

Patient selection

All individuals described in this report are participants in the University of Ulm Prostate Cancer Genetics Project. The collection of prostate cancer families has been described in detail previously.[14] Briefly, the probands' self-reported family history of prostate cancer has been used as a guide for recruitment of affected and relevant unaffected relatives. Families with at least two relatives with histologically confirmed prostate cancer, and of whom DNA was available, have been selected for this study. All probands gave informed consent and the research protocol was approved by the ethics committee of the University of Ulm.

The families were all caucasian and originated from all over Germany, with the majority resident in the Southern part. One hundred and eight families with 273 affected individuals (2.53 cases/family) were genotyped. Sufficient genotype data for linkage analysis were available in 216 affected men from 100 families (2.16 cases/family).

Clinical data

In the 216 affected men that were genotyped and informative the diagnosis of prostate cancer and the age of onset were confirmed by review of the histopathological report and detailed clinical data were obtained by the review of suitable medical records. For those affected men who were not available for genotyping, the diagnosis of prostate cancer and age of onset was confirmed by pathological or medical records.

PSA-screening is not very popular in Germany. Most patients in this study were detected due to urological complaints. The 216 affected men genotyped had a mean PSA at diagnosis of 15.5 ng/ml (3.0–138). One hundred and sixty-one (74.6%) patients were treated by radical prostatectomy (RP), five (2.3%) by radiation therapy, and 50 (23.1%) by primary androgen deprivation. The pathological TNM stages of the 161 RP patients and the clinical TNM stages of the patients treated by irradiation or androgen deprivation are given in Table 1.

Table 1 pT, pN and Grading in 161 patients as determined following Radical Prostatectomy and Clinical T stage and Grading as determined by needle biopsy or TURP in 55 patients with radiation or hormonal therapy

	Radical prostatectomy (n=161)		*Radiation/hormonal therapy (n=55)*	
T stage	pT2	88	T1	10
	pT3	66	T2	38
	pT4	7	T3	7
N stage	pN0	141	—	—
	pN+	20	—	—
Grading	GI	24	GI	14
	GI-II/GII	107	GI-II/GII	27
	GII-III/GIII	30	GII-III/GIII	14

Tumour grade

In Germany two prostate cancer grading systems are currently in use. Until recently, the common practice for the measurement of PCA aggressiveness has been limited to the assessment of the tumour grade according to a system[15] which is nearly equivalent to the American Joint Committee on Cancers grade I–IV system.[16] Only lately has the Gleason grading system[17] been used additionally. As Gleason scores were available in only 69 cases they were not used in this study.

Genotype analysis

We selected a set of eight polymorphic markers (D7S657, D7S515, D7S486, D7S530, D7S640, D7S661, D7S684, D7S798) from the Applied Biosystems (Foster City, CA, USA) marker panels spanning a region of approximately 64.12 cM on chromosome 7q. The physical and genetic positions of the markers were determined from the STS and Marshfield gene maps (Table 2). Recombination fractions between markers were determined from the Marshfield Genetic Map (Table 2).

The markers D7S3061, D7S1804 and D7S1824 that gave the strongest evidence for linkage to PCA aggressiveness in the report of Witte *et al*[6] are not included in the marker set purchased from Applied Biosystems. However, markers D7S530, D7S640 and D7S684 are located within that region (Table 2) and we can report linkage results comprising the proposed locus.[6]

Marker allele frequencies were estimated from the genotyped individuals of the complete dataset using FBATv1.2.[18] The derived frequencies closely correspond to the allele frequencies communicated by CEPH (data not shown).

DNA preparation was performed according to standard techniques. Fluorescence labelled primers were used and the PCR products were analysed on ABI PRISM^{TM} 310 Genetic Analyzer (PE Applied Biosystems, Foster City, CA, USA). Allele lengths and the number of repeats were determined by genotyping the DNA from a member of a CEPH family (1347-2).

Table 2 Position of markers on 7q used in the present study and the study by Witte *et al*[6]

Markers Present study	*Genetic distance cM*[a]	*Markers Witte* et al[6]	*Position Genetic map cM*[b]	*Position Physical map kbp from pter*[c]
D7S657	7.46		0.00	94504
D7S515	11.76		7.46	101946
D7S486	4.33		19.22	116186
	6.14	D7S3061	23.55	123542
D7S530	2.40		29.69	133223
	0.88	D7S1804	32.09	135923
D7S640	9.39		32.97	136283
D7S684	2.68		42.36	142524
	5.20	D7S1824	45.04	143520
D7S661	13.88		50.24	147872
D7S798			64.12	157183

[a]Determined from the Mashfield Genetic Map; [b]Marshfield Genetic Map; [c]STS Physical Map.

Statistical methods

Altogether 136 affected relative pairs (ARP) were analysed. The sample included 88 families with two genotyped affected relatives; eight families with three genotyped affected relatives and four families with four genotyped affected relatives. Additionally 115 non-affected sibs from 63 families were included in order to estimate the genotypes of the parents. Founders could not be genotyped in most cases. In five families marker data were available from a mother or a father but never from both.

For complex traits, such as prostate cancer, model free linkage analysis is considered most appropriate, especially when small pedigrees are available only. Traditional NPL-Z-scores were calculated using GENEHUNTER v1.3.[19] An extension of this software is provided in GENEHUNTER-PLUS v1.2[20], which is based upon a one-parameter allele-sharing model (ASM) and allows the exact calculation of likelihood ratios and LOD scores even when marker data of founders are missing. When IBD sharing is ambiguous due to incomplete data, just as in our study, the likelihood ratio test computing the Z_{lr} statistic in GENEHUNTER-PLUS v1.2 is less conservative than the score test that is applied to calculate the traditional NPL-Z-score.[20,21] The LOD-score can be derived from the Z_{lr} score using

$$\mathrm{LOD} = \frac{(Z_{lr})^2}{2.0 * \ln(10.0)} \qquad (1)$$

The *P*-values associated with this test can be determined either from the Z_{lr} or from the LOD score.[20] The asymptotic distribution of the Z_{lr} statistic is standard normal and the nominal *P*-values associated with the Z_{lr} can be calculated using a one-sided z-test. The *P*-value associated with the LOD can be determined using a χ^2-test with 1-d.f ($2\ln(10)\ \mathrm{LOD} \sim \chi^2_1$). GENEHUNTER-PLUS v1.2[20,21] allows the introduction of covariates by assigning individual weights to each family. In our study the two covariates used to derive the weights were prostate cancer aggressiveness and mean age of onset of the individual family. Prostate cancer aggressiveness was classified by the tumour grade from prostatectomy pathological reports, if available, and from biopsy pathological reports otherwise.

To account for prostate cancer aggressiveness we used a weight_{0-1} family weighting by assigning weight one to 10 families (31 ARP's) with at least two cases of GIII prostate cancer and weight 0 otherwise. In order to put an additional weight on the age of onset we constructed a weight_{0-2} family weighting for early and late onset disease respectively. As in the weight_{0-1} model all families that did not have at least two cases of GIII cancer received weight 0 irrespective of their mean age of onset. For the early onset model, we assigned weight one to four families (11 ARP's) with at least two cases of GIII cancers and late onset disease (mean age of onset >65 years) and weight two to six families (20 ARP's) with at least two cases of GIII cancers and early onset disease (mean age of onset $\leqslant 65$ years). In constructing the late onset model, we assigned weight one to the six families (20 ARP's) with at least two cases of GIII cancers and early onset disease and weight two to the four families (11 ARP's) with at least two cases of GIII cancers and late onset disease. In all of these tests a multipoint analysis, the S_{all}-option and the exponential model option[20] were used in order to calculate the Z_{lr}- and associated LOD-scores.

Results

With no weights applied to the complete set of 100 families there was no evidence of linkage between a prostate cancer susceptibility locus and markers on chromosome 7q. The traditional NPL-Z-score using GENEHUNTER v1.3 and the Z_{lr}-scores calculated in GENEHUNTERPLUS v1.2 were negative throughout all markers (Figure 1).

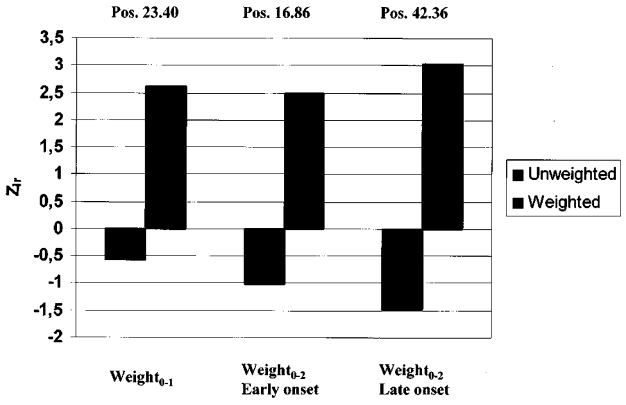

Figure 1 Increase of Z_{lr} by weighting models using prostate cancer aggressiveness and age of onset as covariates. The description of the $weight_{0-1}$, and $weight_{0-2}$ models for early and late onset prostate cancer is given in the text.

Given the information that chromosome 7q32 is linked to prostate cancer aggressiveness genes[6] we expected linkage in families with high grade prostate cancer. Putting the weights on families with at least two cases of GIII prostate cancer ($weight_{0-1}$ model) increased the Z_{lr} score to 2.60 (P=0.005) at position 23.40 (Figure 1). This site is located between markers D7S486 and D7S530 which are seperated by a distance of 10.47 cM (Table 2). The position at which the maximum Z_{lr} occurred is almost identical to marker D7S3061 (at 23.55 cM) which gave the most significant results in the study of Witte.[6] The associated LOD was 1.47 at this position (one LOD support interval 14.92–53.01 cM). For a disorder with a genetic predisposition early manifestation of the disease is assumed to be more frequent. To account for early manifestation we constructed the $weight_{0-2}$ model for early onset cancer, which put maximal weights on families with high grade prostate cancer and a mean age of onset <65 years. Applying this model, the Z_{lr} score did not change significantly. The maximum Z_{lr} was 2.48 (P=0.006) and the derived LOD was 1.34 (one LOD support interval 0–55.79 cM) at position 16.86 corresponding to a site between markers D7S515 and D7S486.

We also constructed a $weight_{0-2}$ model for late onset cancer which put maximal weights on families including at least two cases of late onset (mean age >65 years) and aggressive (GIII) prostate cancer. With these weights multipoint allele-sharing analysis showed a Z_{lr} score of 3.02 (P=0.001) which is associated with a LOD score of 1.98 (1 LOD support interval 21.31–53.01 cM) at position 42.36 (Figure 1). This site is 2.68 cM proximal of marker D7S1824 (Table 2) reported to be linked to prostate cancer aggressiveness by Witte *et al*.[6]

We also used aggressive prostate cancer as the phenotype for the disease trait and assigned affection status 2 to all patients with GIII tumours and affection status 1 to all patients with low grade cancer as well as to all unaffected individuals. With this model we observed a NPL-Z-score of 2.39 at position 32.97 corresponding to marker D7S640.

Discussion

Several molecular and cytogenetic approaches including LOH and CGH studies suggested that chromosome 7q is associated with aggressive prostate cancer.[7–11] In a recent study by Witte *et al*[6] the Gleason score was used as a measure of prostate cancer aggressiveness and applied to a multipoint generalised Haseman-Elston (HE) linkage test as a quantitative trait. The mean-corrected cross product of the Gleason scores between brothers was regressed on the estimated proportion of alleles at a particular marker that are shared among brothers identical by descent (IBD). The results suggested chromosomes 5q, 7q and 19q as candidate regions for prostate cancer aggressiveness genes. A confirmation of these findings in an independent population has not yet been published.

Our study attempted to confirm linkage of prostate cancer aggressiveness to chromosome 7q in a set of 108 German prostate cancer families. For linkage analyses, we preferred to use a one parameter allele sharing model which is implemented as the ASM program in GENEHUNTERPLUS v1.2[20,21] for several reasons. First, model free analysis is considered to be the best approach to linkage studies of complex disorders.[4] Model based methods are powerful when estimations of genetic parameters as mode of transmission and mutation frequencies and penetrances of the disesase gene are reliable and extended pedigrees with several affected relatives are available for genotyping. In the case of complex diseases such as prostate cancer estimations of genetic models are complicated by factors such as genetic heterogeneity, a late onset and a high phenocopy rate of the disease. While several complex segregation analyses suggested genetic models for prostate cancer susceptibility genes, there are no models proposed for the transmission of genes that are responsible for the aggressiveness of prostate cancer. Furthermore, most families of our sample were comprised of affected relative pairs contributing almost no information to model based linkage analysis.

Second, the likelihood ratio method applied in the ASM program of GENEHUNTERPLUS v1.2 is the preferred test when genotype information on the nuclear families is incomplete.[20,21] In our sample marker data of founders were missing in the majority of families and we expected the allele sharing method of GENEHUNTERPLUS v1.2 to be more powerful.

Third, GENEHUNTERPLUS v1.2 allows the introduction of covariates by specifying individual weights to each family.[21] This additional information on the sample can be included in order to increase power of the linkage analysis.

The unweighted analysis of the complete set of the 100 informative families revealed negative NPL-scores at all markers on chromosome 7. However, given the information that chromosme 7q32 may contain prostate cancer aggressive genes we expected linkage in families with aggressive prostate cancer. This information was incorporated in our analysis by assigning weights to families with aggressive prostate cancer.

It is straightforward to score prostate cancer aggressiveness by the degree of tumour differentiation, as carried out by Witte *et al*.[6] The assessment of the tumour grade is standardised, reproducible and can be obtained whenever prostate cancer is diagnosed. Other measures of prostate cancer aggressiveness might be considered but are difficult to assess. We preferred not to use parameters as tumour stage, the presence of metastases or outcome for the scoring of prostate cancer aggressiveness because the validity of these parameters depends on too many unreliable factors including the amount of diagnostics performed, the mode of treatment, the delay of treatment and the quality of follow up.

In North America and some European countries the most common measure of prostate cancer aggressiveness is the Gleason score[17] that is assigned to the tumour by the pathologist. In Germany Gleason scores have not been assessed routinely until lately. Instead pathologists use a grading system which is similar to the American Joint Committee on Cancers grade I–IV system.[16] This grading system considers two morphologic criteria, the histologic growth pattern and the degree of cytologic atypia. The growth pattern is given a score between 0–3, 0, for highly differentiated glandular carcinoma, 1, for poorly differentiated glandular carcinoma, 2, for cribriform carcinoma and 3, for solid carcinoma. The cytologic atypia is given a score between 0–2, 0, for low atypia, 1, for moderate atypia and 2, for high atypia. The scores of both morphologic criteria are summed yielding GI at a sum of 0–1, GII at a sum of 2–3 and GIII at a sum of 4–5. In heterogeneous carcinomas the worst differentiated and not the most predominant tumour is considered.[15]

We included tumour grade as a covariate by weighting families with at least two cases of GIII prostate cancer. The Z_{lr} and LOD increased significantly (Figure 1) at a site that is located between markers D7S486 and D7S530 and is almost identical to the position of D7S3061, one of the linked markers reported by Witte *et al*.[6] Putting additional weights on the mean age of onset showed no effect for early onset cancer. When using the $weight_{0-2}$ model for late onset cancer the Z_{lr} score increased to 3.02 (P=0.001) and the associated LOD to 1.96 (P=0.002) at marker D7S684 (Figure 1). Apparently most evidence for linkage is contributed by families with aggressive and late onset prostate cancer. D7S684 is located in between markers D7S1804 and D7S1824 that gave the strongest evidence of linkage in the study of Witte *et al*.[6] The one LOD support intervals overlap using all three models suggesting that all subgroups are linked to the same locus. Our findings are consistent with the results described in that study and support the evidence for linkage of chromosome 7q31-33 to aggressive prostate cancer.

The use of covariates is a powerful tool in linkage studies of complex traits. It accounts for the genetic heterogeneity of most complex disorders by focusing on families that share certain genetic or non-genetic factors and therefore are likely to be linked to the same disease gene. Covariates have been shown to increase power of linkage analyses by including phenotypic variables or linkage information from previous studies.[22] However, the interpretation of the significance level of such findings requires care. Secondary analyses using covariates as performed in our study may also increase the overall false positive rate because they are designed to strengthen the support for regions that do not meet genome-wide criteria for significance by themselves. To account for these problems a P-value <0.01 has been suggested for determining significance.[21] This criterion is clearly met by all our weighting models.

The identification of families with a clustering of aggressive and late onset prostate cancer is extremely difficult. In our study this is reflected by the small size of the total sample and especially of the subset of weighted families combining aggressive and late onset disease. There are marked differences between the US and German population concerning the epidemiological aspects of familial prostate cancer.[14] The rate of familial predisposition is lower and the size of prostate cancer families is smaller in Germany. Extended families with >5 affected relatives are rare and most families are restricted to affected brothers.[14] Although we did not apply any selection criteria to family sampling there is a significant ascertainment bias in our collection. Most families were contributed by Urologic Surgical Departments from Southern Germany, and most index patients were treated by radical prostatectomy. Patients with poorly differentiated cancer presenting with extended disease at the time of diagnosis are no candidates for radical prostatectomy. Such patients will be transferred to a Urological Surgical Department less frequently resulting in a preponderance of low-grade prostate cancer in our collection. When interpreting our linkage results the limited power of our sample size must be taken into consideration.

In conclusion our study supports linkage of chromosome 7q31-33 to aggressive prostate cancer in the German population. Most evidence for linkage is contributed by families with aggressive and late onset disease. Larger samples including such families are needed in order to characterise this candidate region more precisely.

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