Genome-Wide Linkage Analysis of *TMPRSS2-ERG* Fusion in Familial Prostate Cancer

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Abstract

Fusion of the 5′-untranslated region of androgen-regulated *TMPRSS2* promoter with ETS transcription factor family members is found frequently in prostate cancers, and recent work suggests that the most common *TMPRSS2-ERG* fusion is associated with an aggressive clinical phenotype compared with fusion-negative prostate cancer. Thus far, analysis of the fusion has been limited to sporadic cases of prostate cancer. In the current study, we explore for an enrichment of *TMPRSS2-ERG* fusion in familial prostate cancer. *TMPRSS2-ERG* fusion was identified using a break-apart fluorescence *in situ* hybridization assay on tissue microarrays. Presence of *TMPRSS2-ERG* fusion was associated with higher Gleason scores (P = 0.027). Of 75 patients with established history of prostate cancer, we detected the *TMPRSS2-ERG* fusion in 44 (59%) patients. Almost three quarters (73%) of fusion-positive patients accumulated within 16 specific families whereas only 27% were single fusion-positive cases within one family. Based on reported prevalence rates, we calculated a sibling recurrence risk ratio of up to 18.9. A subset (63%) of families with uniformly *TMPRSS2-ERG*–positive prostate cancer underwent a genome-wide linkage scan at 500 markers. This revealed several loci located on chromosomes #9, #18, and X that were suggestive of linkage to the *TMPRSS2-ERG* fusion-positive prostate cancer phenotype with linkage-of-disease scores up to 2.16 and nonparametric linkage scores up to 2.77. This suggests the presence of an inherited susceptibility to developing the *TMPRSS2-ERG* fusion. Given the association of *TMPRSS2-ERG* fusion and aggressive prostate cancer, close surveillance of relatives of patients with established fusion-positive prostate cancer or a family history of prostate cancer in general would be warranted. [Cancer Res 2009;69(2):640–6]

Introduction

Prostate cancer remains one of the predominant causes of cancer-related deaths for males in the United States and worldwide. Risk factors for prostate cancer include age, race, and a positive family history, and studies analyzing familial occurrence of prostate cancer report an at least 2-fold relative risk if relatives are affected. This risk is associated with the number of affected relatives and is further increased if the relatives were diagnosed at an earlier age (1). More recent work suggests that specific single nucleotide polymorphisms on chromosomes 8q24 and 17 are also associated with the risk of developing prostate cancer (2, 3), and previously, putative chromosomal regions linked to prostate cancer were validated in other studies (e.g., regions on chromosome #17; refs. 4, 5).

Recent work has identified a common recurrent gene fusion, *TMPRSS2-ERG*, in the majority of PSA-screened prostate cancers (6). This fusion involves the hormone-regulated gene *TMPRSS2* and the oncogene *ERG*, a member of the *ETS* family of transcription factors, both of which are located on chromosome 21. More than 60% of the fusion occur through an interstitial deletion between *TMPRSS2* and *ERG*, whereas in the remaining cases, the fusion occurs through an insertion (7). Recent data also suggest that *TMPRSS2-ERG* fusion is present in 15% to 20% of high-grade PIN (8–10), although the prevalence in early prostate cancer still needs to be determined and seems to be present in ∼50% of PSA-screened primary prostate cancers. Several studies have analyzed the association of *TMRSS2-ERG* with patient outcome. The association of positive fusion status with PSA recurrence (or other surrogate parameter of prognosis such as stage or Gleason score) remains controversial, showing favorable (11), worse (12, 13), or no change (14) in prognosis of patients carrying the *TMPRSS2-ERG* fusion. Similarly, studies focusing on death of disease found it either to be associated with prostate cancer–specific death (15, 16) or to not influence patient survival (17). However, all these studies are based on patient cohorts that differ greatly in both composition and treatment. The one study that implemented a watchful waiting cohort following the natural history of prostate cancer without initial treatment did find a significant association of *TMPRSS2-ERG* status with prostate cancer–specific death (16). To date, only sporadically detected prostate cancers have been evaluated. However, the widespread prevalence of the *TMPRSS2-ERG* fusion, the presence in a subset of precursor lesions, and the association with aggressive disease raise the question of whether *TMPRSS2-ERG* fusion is further enriched in hereditary prostate cancer.

In the current study, we analyzed the frequency of *TMPRSS2-ERG* fusion status (also subdivided in fusion through deletion and fusion through insertion) in our cohort of familial prostate cancer cases. We then expanded this study and performed a genome-wide linkage search for susceptibility loci to explore a potential genetic component of this molecular phenotype. We specifically focused our analysis on whether the fusion is linked to chromosome #21 and whether other loci are involved in a predisposition.

Note: Supplementary data for this article are available at Cancer Research Online (http://cancerres.aacrjournals.org/).

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Table 1. Individual persons and families included in the analyses

Analysis subgroup—inclusion criterion	Patients/families
TMPRSS2-ERG assay on TMA, total of successful hybridizations	86/47
Familial clustering, frequency—at least two probands/family with TMA data	75/36
λ_s calculation—restricted to relationship of brothers	66/33*
Linkage analysis, total of genotypes	309/139
At least two probands per family with TMA data and genotypes	48/23
At least two probands per family with fusion-positive tumor and genotypes	21/10

Abbreviation: TMA, tissue microarray.
*For calculation of λ_s, of all 36 families with at least 2 members showing successful hybridization, only the 33 families represented by brother-brother pairing were included.

Materials and Methods

Study cohort. Prostate cancer families, which were derived from all over Germany, were collected at the University of Ulm. Familial prostate cancer was defined by the occurrence in at least two first-degree relatives, or occasionally two second-degree relatives affected with prostate cancer. Hereditary prostate cancer, as defined by the Johns Hopkins Criteria (18), was present in ~36% of the collected families. Initially, patients from 47 prostate cancer families (including 17 with hereditary prostate cancer) were analyzed for *TMPRSS2-ERG* fusions in tumor samples, and some of these families were represented in the collection of 139 prostate cancer pedigrees (including 50 with hereditary prostate cancer) that underwent a genome-wide linkage scan previously (19). The overlapping fractions, as well as the subgroups used for different considerations of this study, are described in Table 1.

Tissue blocks were available from a total of 281 patients that underwent radical prostatectomy as monotherapy for biopsy-proven prostate cancer in Germany between 1989 and 2001. Tissue microarrays were constructed from these samples as described elsewhere (20). Each prostate cancer sample was arrayed in triplicate. Tissue blocks and genomic DNA samples from peripheral blood were obtained under Institutional Review Board approval from the University of Ulm, Germany, and the Brigham and Women's Hospital. Sufficient cancer tissue and fluorescence *in situ* hybridization (FISH) hybridization was present for a subset of our 281 patients and an indisputable diagnosis of either presence or absence of *TMPRSS2-ERG* fusion could be made for 86 patients. Of those 86 patients from 47 separate families, we identified 75 patients who had at least one other family member with a fusion diagnosis. As described in Table 1, these patients belonged to 36 families and consisted mostly of brother pairs (n = 33 families), but in three families they were father and son. All 36 families were included in the subsequent analysis, except for the calculation of the sibling recurrence ratio, which was based solely on the 33 families, which were represented as brother pairs.

Assessment of TMPRSS2-ERG fusion status by dual-color interphase FISH. To interrogate our cohort for the *TMPRSS2-ERG* fusion status, we applied a dual-color break-apart interphase FISH assay as described elsewhere in detail (9). Because *TMPRSS2* and *ERG* are located too close together on 21q (distance of ~2.8 Mb) for a direct evaluation, we developed a break-apart assay to indirectly assess for *TMPRSS2-ERG* fusion. In brief, two differentially labeled probes were designed to span the telomeric and centromeric neighboring regions of the *ERG* locus. This break-apart probe system allows differentiation between *TMPRSS2-ERG* fusion through insertion, *TMPRSS2-ERG* fusion through an intronic deletion of DNA between *TMPRSS2* and *ERG*, and no gene rearrangement. A nucleus without *ERG* rearrangement shows two pairs of juxtaposed red and green signals (mostly forming two yellow signals; Fig. 1*C*). A nucleus with an *ERG* break-apart (reflecting a *TMPRSS2-ERG* fusion through insertion) shows split apart of one red-green (yellow) signal pair, resulting in a single red and green signal for the rearranged *ERG* allele, and a still combined (yellow) signal for the non-rearranged *ERG* allele in each nucleus (Fig. 1*B*). Finally, a nucleus with deletion of the telomeric (green) *ERG* break-apart probe (reflecting a *TMPRSS2-ERG* fusion through deletion) shows one juxtaposed red-green signal pair (yellow) for the non-rearranged allele and a single red signal for the rearranged (fusion through deletion) allele (Fig. 1*A*).

Determination of sibling recurrence risk ratio. The sibling recurrence risk ratio, λ_s, is defined as the ratio of risk of disease manifestation, given that one's sibling is affected, as compared with the disease prevalence in the general population. Estimation of this measure for familial recurrence of *TMPRSS2-ERG* fusions was carried out in a sample of 33 families where tumor tissues of at least two brothers with prostate cancer had been probed (32 pairs and 1 triplet of brothers as shown in Table 1; in the triplet of brothers, two of them were chosen randomly.). Precisely, λ_s was calculated as the relative frequency of families with each of the two brothers affected by the fusion, divided by a squared prevalence estimate in general prostate cancer patients, according to the formula in ref. 7. The prevalence of the fusion depends on ascertainment and ranges between 15% in cases from population-based screening (19) and up to 78% in other studies with less defined patient cohorts (21). For our calculations, we therefore assumed a prevalence between 50% and 15%, emphasizing the findings of larger, population-based studies.

Family sample for linkage analysis. Recently, a genome-wide linkage scan for prostate cancer susceptibility loci was done in 139 families from Ulm, Germany (19). Genotyping was done by in-house techniques at deCODE genetics in a panel of 500 marker loci. The methodologic details as well as the results of this scan are previously described in detail (19).

For the present study, tumor tissue was screened in 23 of these families (see Table 1) based on availability of tissue. Ten of the families showed consistent *TMPRSS2-ERG* fusions (i.e., at least two family members showed this molecular phenotype). These families were selected as a subset for reanalysis of linkage using the methods already specified in ref. 10. This means that, altogether, 36 genotyped individuals (average of 3.6 per family; range, 2–9) were incorporated in the present linkage scan. Among these were 21 men affected by prostate cancer (9 sibpairs and 3 brothers in one family).

Linkage scores. To assess the degree of linkage of genetic marker loci to the phenotype "fusion-positive prostate cancer" (null hypothesis: no linkage), model-based linkage-of-disease (LOD) scores and nonparametric linkage (NPL) scores were calculated. Other than in the previous linkage analysis of the whole family sample, the *TMPRSS2-ERG* fusion was considered the phenotype. Note that in the subset, each man (but one) affected by prostate cancer is also characterized by a *TMPRSS2-ERG* fusion, so these two phenotypes (prostate cancer and fusion-positive prostate cancer) were almost congruent.

Linkage results were mainly based on multipoint calculations implemented in the GENEHUNTER-PLUS software (22), with a resolution of five intermediate steps between two adjacent marker loci on each chromosome. LOD scores were calculated under a dominant model of inheritance,

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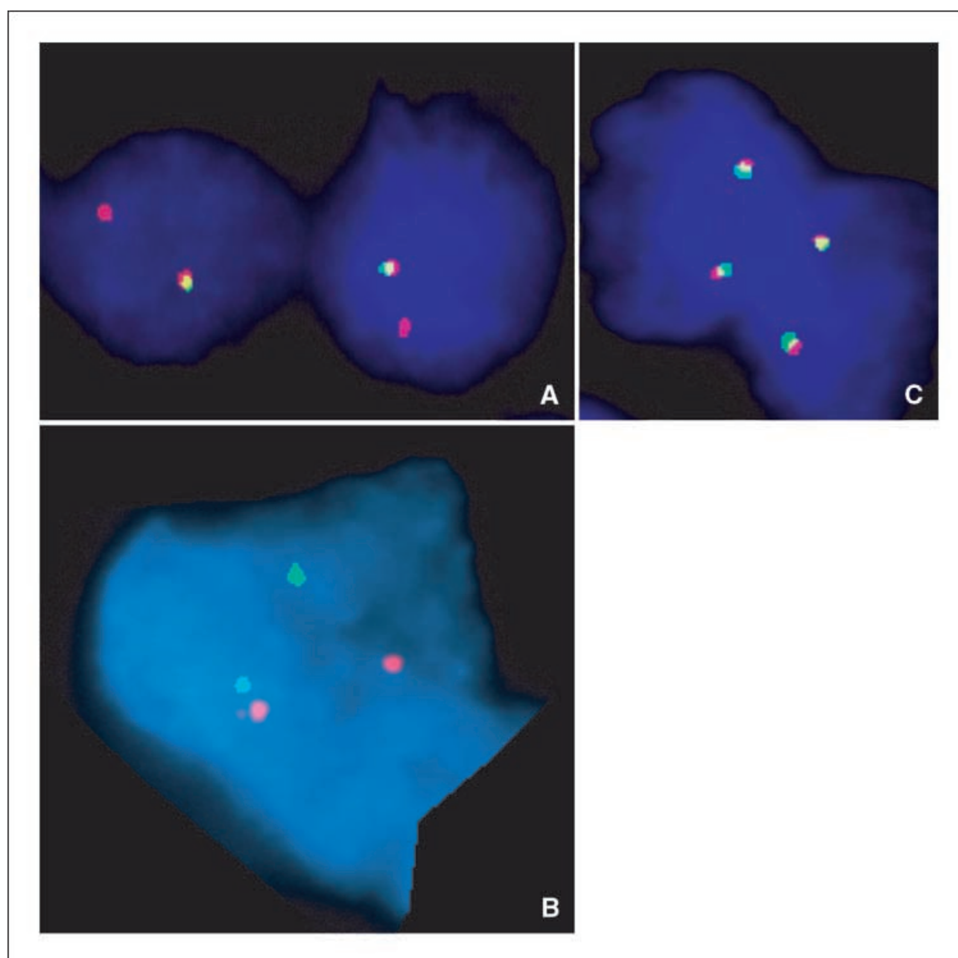

Figure 1. FISH assay for *TMPRSS2-ERG*. In case of fusion through deletion, only one red signal is present in addition to the green/red (*yellow*) signal of the intact chromosome (*A*). In case of a fusion without deletion (*B*), the red (*TMPRSS2*) and the green (*ERG*) probe signals are distinctively separated in addition to the red/green signal of intact chromosome. Two yellow signals are present if no fusion of *TMPRSS2-ERG* is present (*C*).

specifically, frequency of susceptibility allele = 0.003, penetrance in carriers of this allele = 1.0, and penetrance in noncarriers = 0.001 (ref. 23). This is the model of the original evaluation of the scan (19) and has been widely used for prostate cancer linkage studies. Marker allele frequencies were estimated by counting alleles across the entire sample of 139 families, without consideration of genetic relationships, as was done in ref. 19. However, the conclusions of our study are mainly based on the model-free NPL scores. In addition, single-point linkage scores were obtained at those marker loci with noticeable multipoint scores.

Significance of results. Genomic regions with at least one multipoint LOD score ≥1.86 or multipoint NPL score ≥2.93 are reported; additionally, single-point LOD scores are presented. The limits correspond to *P* values of 0.0017 based on the underlying asymptotic null distributions (24). They are supposed to be "suggestive" for linkage, representing "statistical evidence that would be expected to occur one time at random in a genome scan." The limits for genome-wide significance of a linkage result (3.3 and 3.9, respectively) were not exceeded in this study. Suspecting that asymptotic *P* values potentially are not valid in our small family sample, we rechecked all *P* values empirically in a simulation study. For this purpose, at any locus, empirical *P* values were calculated with respect to the null hypothesis of no linkage of the respective marker to the disease. Note that asymptotic *P* values potentially do not hold in our small family sample. Using the SIMULATE software (25), we generated a thousand copies of each family, but with random marker alleles at each locus and, thus, without linkage to the phenotype. Allele drawing was carried out under the observed allele frequencies in the underlying families. At any marker locus, the empirical unadjusted *P* value is the relative frequency of events among the 1,000 simulations, with a simulated score equal to or above the corresponding observed score in the actual data. Genome-wide adjustment of *P* values was done using the minP method (26) under resampling from the pool of family copies that were created by runs of SIMULATE. The latter calculations were conducted to check whether the asymptotic criteria given by Lander and colleagues (24) are reliable in the underlying family sample.

Empirical *P* values were also obtained by randomly drawing a set of 10 families from all 139 families that had been evaluated in the previous genome-wide scan for prostate cancer loci. This approach is based on the assumption that, for the loci of interest, the null hypothesis of no linkage is true in the whole sample. Although family structures and observed linkage scores varied considerably among the 139 pedigrees, the resulting *P* values were quite close to those generated with SIMULATE. Therefore, only *P* values computed under simulated marker distributions are quoted. In the genome-wide scan, we quote both unadjusted and adjusted (genome-wide) empirical *P* values. For further tests, like the χ^2 test of association between fusion status and Gleason scores, we do not adjust for multiple testing. We call a result statistically significant if $P < 0.05$ and distinguish between unadjusted and genome-wide *P* values.

Results

TMPRSS2-ERG fusion status was assessable in prostate cancer samples from 86 patients. All tissue microarray cores from the same patient showed either concordant presence or absence of *ERG* rearrangement. Seventy-five patients from 36 separate families affected by prostate cancer could be subsequently included in our analyses. Patient characteristics of our study cohort are presented in Table 2. Of note, about half of the patients (41 of 75)

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Table 2. Patient characteristics

		n (%)
T stage		
1		4 (5)
2		37 (49)
3		29 (39)
4		5 (7)
N stage		
0		56 (85)
1		10 (15)
Missing		9
M stage		
0		74 (99)
1		1 (1)
Gleason score		
4–6		29 (41)
7		34 (48)
8–10		8 (11)
Missing		4
PSA recurrence		
No		45 (70)
Yes		19 (300)
Missing		11
	n	**Range (mean)**
Pre-op PSA (ng/mL)	63	0.5–210 (14.0)
Age (y)	75	47–78 (62.5)

had organ-confined disease (T1 and T2) and half of them (34 of 75) had extraprostatic extension in the periprostatic soft tissue or adjacent organs (T3-T4). Fifteen percent (10 of 66) were lymph node positive (N1). Gleason scores ranged from 4 to 10, with 89% (63 of 71) having a Gleason score ≤7. PSA failure has been detected in 30% (19 of 64) of the cases with a mean follow-up time of 67.1 months (range, 8–156 months). We found a significant association between *TMPRSS2-ERG* fusion-positive prostate cancers and higher Gleason scores [7 and 8–10 versus 4–6, $P = 0.027$ (χ^2 test)]. In detail, *TMPRSS2-ERG* fusion was present in 14 of 29 (48%) patients with a Gleason score of 4 to 6, in 26 of 34 (76%) patients with a Gleason score of 7, and in 3 of 8 (38%) patients with a Gleason score of 8 to 10. Other clinical parameter such as age, stage, and PSA biochemical failure showed no significant association with the fusion status in this limited patient cohort.

TMPRSS2-ERG fusion was detected in 44 of 75 patients (59%). Fusion through deletion occurred in 25 of 44 (57%) fusion-positive tumors. Representative images from our FISH analysis are presented in Fig. 1. We defined a positive family history of carrying the fusion when it was concordantly present in two relatives. Table 3 presents the observed and the expected (under a normal distribution) frequencies of families that showed concordance and discordance of the fusion phenotype (presence or absence of *TMPRSS2-ERG* fusion) of prostate cancer among their members. We observed fewer families with discordant *TMPRSS2-ERG* status as expected under independence of trait between brothers, suggesting that the fusion (or a susceptibility to it) exists as a trait in certain families ($P = 0.07$, χ^2 goodness-of-fit test). Restricting the *TMPRSS2-ERG* fusion status to the subtype of fusion through deletion, a similar but less obvious deviation from the expectation was observed (Table 3). Of note, the average time between cancer diagnosis for brothers concordant in the fusion status was 21.4 months, compared with 47 months in brothers discordant for the fusion status. This difference was not significant ($P = 0.11$).

According to the formula in ref. 27, a rough estimate of the sibling recurrence risk ratio (λ_s), the probability of somatic *TMPRSS2-ERG* fusions in tumor tissue in two brothers affected by prostate cancer, was estimated as 14 of 33 (42%). The prevalence in the general population is reported to be between 15% and 78%, depending on the method of detection [reverse transcription-PCR (RT-PCR) versus FISH] and patient cohort used. In general, studies using RT-PCR to detect *TMPRSS2-ERG* fusion tend to show an increased frequency, which is confounded by the use of smaller, less well-defined patient cohorts and/or by a selection bias toward highly aggressive or metastatic disease (21, 28–30). On the other hand, larger and well-defined patient cohorts analyzed by FISH show frequency rates on the lower edge of the spectrum (7, 16, 31). In a recent study by Perner and colleagues (7), both detection method (FISH) and patient cohort (German prostate cancer patients with incomplete PSA screening history) are comparable with our current collective, although the frequency of high-risk patients in this cohort is increased (32). We therefore assumed for our calculations a prevalence rate between 50% and 15%. This translates to a sibling recurrence risk ratio (λ_s) for *TMPRSS2-ERG* fusions to be in the interval from 1.7 to 18.9. This broad range reflects different schemes of ascertainment possibly with inherent

Table 3. Classification of families based on recurrence of *TMPRSS2-ERG* status

Family classification	Observed (n)	Expected (n)
Fusion positive (≥2 members positive)	16/36	13/36*
Discordant (one single positive proband)	12/36	17/36*
Fusion negative (concordantly negative probands)	8/36	6/36*
Fusion-with-deletion positive (≥2 members positive)	6/36	4/36 †
Discordant (one single positive proband)	13/36	16/36 †
Fusion-with-deletion negative (concordantly negative)	17/36	16/36 †

*Assumed frequency of fusion is 0.59 (44/75) among all probands.
†Assumed frequency of fusion-with-deletion is 0.33 (25/75) among all probands.

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Table 4. Potential susceptibility loci for *TMPRSS2-ERG* fusions from genome-wide scan in 10 prostate cancer families

Marker (chromosomal position)	DeCODE position (cM)	Multipoint NPL (*P*)	Multipoint LOD (*P*)	Single-point LOD
D9S175 (9q21)	73 (MapViewer: 71)	1.86 (0.029)	1.87 (0.003)	1.16
D18S478 (18q11)	52 (MapViewer: 51)	2.77 (0.001)	0.64 (0.049)	−0.7
D18S68 (18q22)	90 (MapViewer: 89)	2.17 (0.011)	1.90 (0.002)	1.21
DXS8013 (Xq27)	149 (MapViewer: 142)	2.21 (0.002)	2.16 (0.002)	1.45
D10S217 (10q24)	154 (MapViewer: 160)	2.17 (0.009)	1.73 (0.001)	1.57
D11S968 (11q25)	156 (MapViewer: 152)	2.30 (0.001)	1.81 (0.002)	1.47
D12S326 (12q21)	93 (MapViewer: 91)	2.50 (0.003)	1.79 (0.004)	1.56

NOTE: Multipoint LOD and NPL scores as well as single-point LOD scores are shown. The *P* values are empirical and not adjusted for multiple testing. They have been obtained in a simulation study. The deCODE positions of the markers are given (*a*) as used in the evaluation of the original scan and (*b*) as retrieved from MapViewer.

biases; however, it suggests a genetic component of this molecular phenotype. To avoid external estimates of the prevalence, we compared the recurrence risk in fusion-positive "index cases" with the risk of a negative index case having a brother that is positive for the *TMPRSS2-ERG* fusion. Based on the prostate cancer index cases, this risk ratio is 1.75 in our cohort, emphasizing a genetic background of the *TMPRSS2-ERG* fusion phenotype.

Because we identified an accumulation of *TMPRSS2-ERG* fusion events in certain families, we reanalyzed a genome-wide linkage scan based only on these families, using the *TMPRSS2-ERG* fusion as the phenotype under investigation. Loci that reach suggestive linkage to the phenotype could be detected on chromosomes #9, #18, and X. Table 4 shows the degree of evidence at the significant marker loci next to the peaks of the NPL and LOD curves.

Chromosome #9 shows linkage signals on 9q13–9q21 from about 65 to 80 cM (unadjusted $P < 0.05$). However, simulation yielded nonsignificant genome-wide *P* values for both the multipoint LOD and NPL scores (all genome-wide $P > 0.4$). The peak is isolated and the chromosome does not exhibit further notable regions.

In contrast, the linkage on chromosome #18 encompasses a vast area from 18p11 to 18q22 with scores that are suggestive or nearly suggestive (Fig. 2) of linkage. After adjustment for genome-wide testing, these loci had *P* values of >0.38 (multipoint LOD) and >0.13 (NPL). For a list of genes located in this area on chromosome #18, which are potentially associated with prostate cancer susceptibility via *TMPRSS2-ERG* fusion, please refer to Supplementary Table S1.

Further, on the X chromosome, a rather isolated peak LOD of 2.16 was observed in the region Xq25–Xq27 (genome-wide $P = 0.14$). Table 4 shows an extended list of loci potentially linked to the phenotype of *TMPRSS2-ERG* fusion.

Chromosome #21, which harbors the *TMPRSS2-ERG* fusion, did not show any evidence for linkage to this phenotype. At none of the nine genetic markers spread along this chromosome were LOD scores >0 or NPL scores >0.5 observed.

Discussion

The *TMPRSS2* fusion with members of the *ETS* oncogene family is prevalent in a remarkable portion of prostate cancer cases, ranging from 15% [among patients incidentally diagnosed by TURP because of benign prostatic hyperplasia–related voiding problems] to >50% (in PSA-screened cohorts). The fusion of *TMPRSS2* with *ERG* constitutes the most common subtype (~90% of fusion-positive tumors) and was the focus of the current study. To date, investigations have largely been restricted to sporadic cases, and here we expanded our previous research to cases of familial prostate cancer. Specifically, we focused on the concordance of *TMPRSS2-ERG* fusion in brothers of families with an established family history of prostate cancer. We observed an overall prevalence of 59% among affected men. Given that the vast majority of cases were detected clinically in our cohort, this constitutes a considerable increase in the fraction of *TMPRSS2-ERG*–positive prostate cancer cases compared with PSA-screened

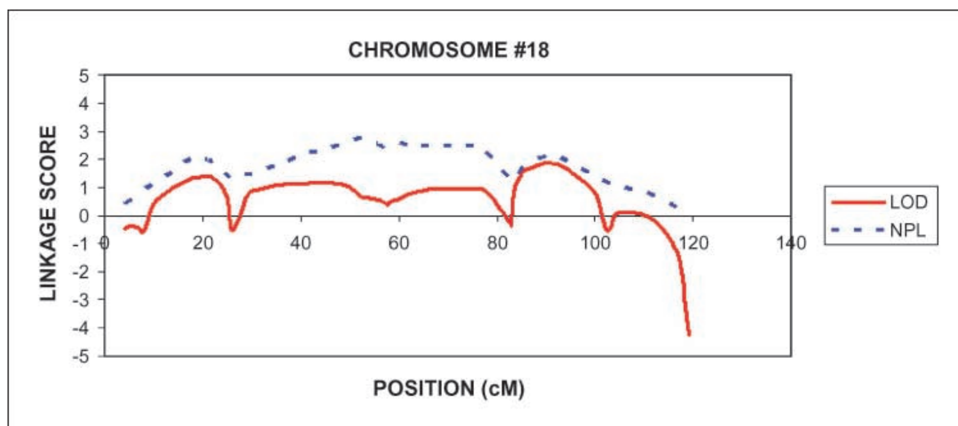

Figure 2. LOD scores and NPL scores for linkage with the TMPRSS2-ERG fusion phenotype on chromosome #18. Peaks were observed at 52 and 90 cM corresponding to 18q11 and 18q22, respectively.

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patient cohorts in which the prevalence of *TMPRSS2-ERG* fusion seems to be higher. In addition, 73% of cases accumulated within the same individual families. *TMPRSS2-ERG* fusion through deletion was found in 57% of fusion-positive cases, but most cases positive for *TMPRSS2-ERG* fusion through deletion were scattered among families showing no accumulation within families. This suggests that it is rather the rearrangement and not its mechanism for which susceptibility may be inherited. In our cohort, the *TMPRSS2-ERG* fusion was significantly associated with higher Gleason scores. The fusion was present in only less than half of patients with a Gleason score ≤6 but in more than three quarters of patients with a Gleason score of 7. However, the association of *TMPRSS2-ERG* fusion with higher Gleason scores has not consistently been found either by our group or by others (29, 31, 33–36), and although we attribute this to the low number of patients in this category (n = 8), the fusion frequency in patients with a Gleason score of 8 to 10 was lower than in any other category (38%). Based on the vast difference in fusion frequency among patients in different Gleason categories, however, we believe that, at least among hereditary prostate cancers, there may be an association of fusion-positive status and more aggressive disease.

We found an elevated sibling recurrence risk ratio (λ_s) of fusion-positive carcinomas among brothers, which ranged from 1.7 to 18.9. Although limited by the number of samples and varying prevalence estimates in the general prostate cancer population, this estimate of λ_s is suggestive of the presence of a genetic component predisposing for the *TMPRSS2-ERG* fusion. Our cases included in the cohort were collected from all over Germany; therefore, we have only limited information about the exact clinical presentation of each patient or treatment modality he chose. Given the standard treatment approaches between 1989 and 2001, it is possible that additional siblings may have opted out of a surgical treatment of biopsy-proven prostate cancer. Based on the fact that the majority of brothers in the current study were concordantly positive with regard to their *TMPRSS2-ERG* fusion status, we would assume that the actual sibling recurrence risk ratio would have been even higher, further supporting a hereditary component in developing *TMPRSS2-ERG*–positive prostate cancer.

A search for this genetic component by a genome-wide linkage scan revealed linkage signals on chromosomes #9, X, and #18. A comparatively broad region with potential linkage to prostate cancer on chromosome #18 had also been described by the ACTANE Consortium (37). Using the same genetic model as in our study, a heterogeneity LOD score of 1.40 (P = 0.011) at position 102 cM and a second peak with a heterogeneity LOD of 1.34 (P = 0.014) at 58 cM had been identified. That peak shows perfect concordance with our linkage scan results (see Fig. 2). This region contains a number of coding genes with a wide functional spectrum (please refer to Supplementary Table S1). Many of these genes, however, have not yet been characterized. Of note, our study was limited by the number of markers previously genotyped; thus, additional loci linked to *TMPRSS2-ERG* fusion phenotype were not identified.

The linkage signal on chromosome X is a match to the long known hereditary prostate cancer locus at Xq27 (HPCX, OMIM #300147), where the predisposing gene still remains to be identified. This may suggest that families with occurrence of *TMPRSS2-ERG* fusions may be more homogeneously linked to HPCX than unselected samples for this molecular phenotype. Based on our findings, we suspect that any of the loci on chromosomes #9, #18, and X harbors a gene involved in DNA repair. Based on the strength of the signals and the findings of the ACTANE Consortium (37), we specifically suspect chromosome #18 to harbor a candidate gene involved in conveying susceptibility to obtaining the *TMPRSS2-ERG* fusion or fusions of *TMPRSS2* with other members of ETS transcription factors.

It is noteworthy that the potential susceptibility loci found in our study show no overlap with risk regions such as those located on chromosome 8q24 recently identified in genome-wide association approaches for prostate cancer (20–22). We would not expect such a coincidence because common risk variants that arise from case control studies usually confer minor disease effects that cannot be detected by linkage analyses. However, the frequency of the now known risk single-nucleotide polymorphisms has not yet been assessed in the sample of fusion-positive families and, thus, a low-penetrance contribution to that special subgroup of prostate cancer cases remains to be determined.

The linkage scan further revealed that the occurrence of *TMPRSS2-ERG* fusion is not linked to chromosome #21. It is reasonable that the short distance between the two genes at 21q, as well as the fact that *ERG* resides on the same transcriptional direction downstream of *TMPRSS2*, may predispose to the fusion gene via simple genomic deletion. Homologous sequences within the interval could render this locus even more prone to intrachromosomal rearrangements [e.g., similar to intrachromosomal deletions on chromosome #17 in neurofibromatosis type 1; ref. 23]. However, our results suggest that there are no elements at 21q that could be present in a specific risk group (e.g., familial prostate cancer), whereas lower-risk individuals would not carry such fusion-prone sequences. In other words, lack of linkage at 21q excludes any genetic variability within the deleted region that would give rise to familial clustering of fusion-positive prostate cancer.

This interpretation fits the observation that fusion breakpoints are highly variable and that different breakpoints may occur in the same patient (8). Familial aggregation of the fusion could also be due to a genetically determined chromosomal instability such as being conveyed by variations in DNA repair enzymes. For example, variants of DNA repair genes have been shown to confer breast cancer susceptibility via genomic instability (e.g., RAD50 and NBS1; ref. 38).

The main limitation of this study is its relatively small sample size. For that reason, some results (e.g., estimates of the recurrence ratio) are subject to larger statistical variation. Possibly some linkage peak may shift in further investigations, and due to limited power, moderately linked loci may have been missed. However, although not every detail may be confirmed in future studies, we believe that our findings are on firm ground. Our results suggest that the susceptibility to acquiring prostate cancer in conjunction with the *TMPRSS2-ERG* fusion may be an inherited phenotype. We believe that the reported association of *TMPRSS2-ERG* fusion with an aggressive subtype of prostate cancer, as reported before (7, 16) and as suggested by its potential association with higher Gleason scores in the current analysis, warrants close surveillance of brothers of fusion-positive prostate cancer patients. We further believe that the presence or absence of *TMPRSS2-ERG* fusions should constitute strata in future genetic epidemiologic linkage studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Kalish LA, McDougal WS, McKinlay JB. Family history and the risk of prostate cancer. Urology 2000;56:803–6.
2. Gudmundsson J, Sulem P, Manolescu A, et al. Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24. Nat Genet 2007;39:631–7.
3. Wang L, McDonnell SK, Slusser JP, et al. Two common chromosome 8q24 variants are associated with increased risk for prostate cancer. Cancer Res 2007;67:2944–50.
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. 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.
6. Tomlins SA, Rhodes DR, Perner S, et al. Recurrent fusion of TMPRSS2 and ETS transcription factor genes in prostate cancer. Science 2005;310:644–8.
7. Perner S, Demichelis F, Beroukhim R, et al. TMPRSS2: ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. Cancer Res 2006;66:8337–41.
8. Clark J, Merson S, Jhavar S, et al. Diversity of TMPRSS2-ERG fusion transcripts in the human prostate. Oncogene 2007;26:2667–73.
9. Perner S, Mosquera JM, Demichelis F, et al. TMPRSS2-ERG fusion prostate cancer: an early molecular event associated with invasion. Am J Surg Pathol 2007;31:882–8.
10. Tomlins SA, Laxman B, Varambally S, et al. Role of the TMPRSS2-ERG gene fusion in prostate cancer. Neoplasia 2008;10:177–88.
11. Winnes M, Lissbrant E, Damber JE, Stenman G. Molecular genetic analyses of the TMPRSS2-ERG and TMPRSS2–1 gene fusions in 50 cases of prostate cancer. Oncol Rep 2007;17:1033–6.
12. Nam RK, Sugar L, Yang W, et al. Expression of the TMPRSS2: ERG fusion gene predicts cancer recurrence after surgery for localised prostate cancer. Br J Cancer 2007;97:1690–5.
13. Wang J, Cai Y, Ren C, Ittmann M. Expression of variant TMPRSS2/ERG fusion messenger RNAs is associated with aggressive prostate cancer. Cancer Res 2006;66:8347–51.
14. Yoshimoto M, Joshua AM, Cunha IW, et al. Absence of TMPRSS2: ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. Mod Pathol 2008;21:1451–60.
15. Attard G, Clark J, Ambroisine L, et al. Duplication of the fusion of TMPRSS2 to ERG sequences identifies fatal human prostate cancer. Oncogene 2008;27:253–63.
16. Demichelis F, Fall K, Perner S, et al. TMPRSS2: ERG gene fusion associated with lethal prostate cancer in a watchful waiting cohort. Oncogene 2007;26:4596–9.
17. FitzGerald LM, Agalliu I, Johnson K, et al. Association of TMPRSS2-ERG gene fusion with clinical characteristics and outcomes: results from a population-based study of prostate cancer. BMC Cancer 2008;8:230.
18. Carter BS, Bova GS, Beaty TH, et al. Hereditary prostate cancer: epidemiologic and clinical features. J Urol 1993;150:797–802.
19. Maier C, Herkommer K, Hoegel J, Vogel W, Paiss T. A genomewide linkage analysis for prostate cancer susceptibility genes in families from Germany. Eur J Hum Genet 2005;13:352–60.
20. Rubin MA, Dunn R, Strawderman M, Pienta KJ. Tissue microarray sampling strategy for prostate cancer biomarker analysis. Am J Surg Pathol 2002;26:312–9.
21. Soller MJ, Isaksson M, Elfving P, Soller W, Lundgren R, Panagopoulos I. Confirmation of the high frequency of the TMPRSS2/ERG fusion gene in prostate cancer. Genes Chromosomes Cancer 2006;45:717–9.
22. Kruglyak L, Daly MJ, Reeve-Daly MP, Lander ES. Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 1996;58:1347–63.
23. 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.
24. Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. Nat Genet 1995;11:241–7.
25. Terwilliger JD, Speer M, Ott J. Chromosome-based method for rapid computer simulation in human genetic linkage analysis. Genet Epidemiol 1993;10:217–24.
26. Westfall PH, Zaykin DV, Young SS. Multiple tests for genetic effects in association studies. Methods Mol Biol 2002;184:143–68.
27. Risch N. Linkage strategies for genetically complex traits. I. Multilocus models. Am J Hum Genet 1990;46:222–8.
28. Furusato B, Gao CL, Ravindranath L, et al. Mapping of TMPRSS2-ERG fusions in the context of multi-focal prostate cancer. Mod Pathol 2008;21:67–75.
29. Lapointe J, Kim YH, Miller MA, et al. A variant TMPRSS2 isoform and ERG fusion product in prostate cancer with implications for molecular diagnosis. Mod Pathol 2007;20:467–73.
30. Rouzier C, Haudebourg J, Carpentier X, et al. Detection of the TMPRSS2-ETS fusion gene in prostate carcinomas: retrospective analysis of 55 formalin-fixed and paraffin-embedded samples with clinical data. Cancer Genet Cytogenet 2008;183:21–7.
31. Tu JJ, Rohan S, Kao J, Kitabayashi N, Mathew S, Chen YT. Gene fusions between TMPRSS2 and ETS family genes in prostate cancer: frequency and transcript variant analysis by RT-PCR and FISH on paraffin-embedded tissues. Mod Pathol 2007;20:921–8.
32. Hofer MD, Kuefer R, Huang W, et al. Prognostic factors in lymph node-positive prostate cancer. Urology 2006;67:1016–21.
33. Barry M, Perner S, Demichelis F, Rubin MA. TMPRSS2-ERG fusion heterogeneity in multifocal prostate cancer: clinical and biologic implications. Urology 2007;70:630–3.
34. Mosquera JM, Perner S, Demichelis F, et al. Morphological features of TMPRSS2-ERG gene fusion prostate cancer. J Pathol 2007;212:91–101.
35. Rajput AB, Miller MA, De Luca A, et al. Frequency of the TMPRSS2: ERG gene fusion is increased in moderate to poorly differentiated prostate cancers. J Clin Pathol 2007;60:1238–43.
36. Saramaki OR, Harjula AE, Martikainen PM, Vessella RL, Tammela TL, Visakorpi T. TMPRSS2: ERG fusion identifies a subgroup of prostate cancers with a favorable prognosis. Clin Cancer Res 2008;14:3395–400.
37. Edwards S, Meitz J, Eles R, et al. Results of a genome-wide linkage analysis in prostate cancer families ascertained through the ACTANE consortium. Prostate 2003;57:270–9.
38. Heikkinen K, Rapakko K, Karppinen SM, et al. RAD50 and NBS1 are breast cancer susceptibility genes associated with genomic instability. Carcinogenesis 2006;27:1593–9.

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