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Report

Confirmation of Linkage of Prostate Cancer Aggressiveness with Chromosome 19q

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Regions on chromosomes 7 and 19 were recently reported to contain susceptibility loci that regulate tumor aggressiveness of prostate cancer. To confirm these findings, we analyzed genome scan data from 161 pedigrees affected with prostate cancer. Using the Gleason score as a quantitative measure of tumor aggressiveness, we regressed the squared trait difference, as well as the mean-corrected cross product, on the estimated proportion of alleles shared identical-by-descent at each marker position. Our results confirm the previous linkage results for chromosome 19q (D19S902, $P < .00001$). In addition, we report suggestive evidence for linkage on chromosome 4 (D4S403, $P = .00012$). The results of previous findings, together with our results, provide strong evidence that chromosome 19 harbors a gene for tumor aggressiveness.

Prostate cancer (MIM 176807) is the most common type of cancer and the second leading cause of cancer death among men in the United States. Substantial evidence has shown that it clusters in families (Steinberg et al. 1990; Goldgar et al. 1994; Whittemore et al. 1995), with a two- to fourfold increased risk of disease for first-degree relatives of an affected man. Furthermore, this clustering can partially be attributed to genetic causes (Ahlbom et al. 1997; Schaid et al. 1998). As a result, research efforts have focused on mapping the susceptibility genes(s) using linkage analysis methods (Smith et al. 1996; Berthon et al. 1998; Xu et al. 1998, 2001; Gibbs et al. 1999, 2000; Berry et al. 2000; Tavtigian et al. 2001). Recently, an analysis was conducted by Witte et al. (2000), in which they used the total Gleason score as a quantitative trait. The Gleason score is a cancer grading system that is based on the architectural pattern of biopsy or prostatectomy specimens. It is obtained by adding two histological pattern grades, each of which is individually scored from 1 to 5. The total Gleason score thus ranges from 2 to 10, with a high score generally indicating higher-grade disease associated with poor prognosis (Epstein et al. 1993; Lerner et al. 1996).

Witte et al. (2000) used the Gleason score as a quantitative trait to search for genes related to tumor aggressiveness. They conducted a genomewide scan using a total of 236 sib pairs. Strong evidence for linkage was found on 5q31 ($P = .0002$), 7q32-q34 ($P = .0007$), and 19q12 ($P = .0004$). However, in follow-up analyses, only the findings on chromosomes 7 and 19 were maintained (Neville et al. 2002).

To confirm the findings of Witte et al. (2000), we analyzed our genome scan data of families with prostate cancer, using the Gleason score as a quantitative trait. These families with prostate cancer were identified from surveys of men treated by radical prostatectomy or radiation therapy at the Mayo Clinic between 1967 and 1997. A pedigree was ascertained if it had a minimum of three closely related men affected with prostate cancer and if at least two of the affected men were willing to give blood. All affected men who contributed a blood specimen had their cancers verified by review of medical records and pathologic confirmation (see Schaid et al. [1998] and Berry et al. [2000]). A total of 161 families were ascertained. These families consisted of 448 men affected with prostate cancer; Gleason scores were available on 364 of the men. The scores were obtained from either radical prostatectomy specimens, or, if no specimens were available, the score was obtained from six

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core needle biopsies. Collection of blood and family history data was approved by the Mayo Clinic Internal Review Board.

For the genomewide screen, 400 markers from the ABI Prism Linkage Mapping Set 10cM were used. Forward primers were labeled with phosphoramidite dyes. Each 15μl reaction contained 25ng of genomic DNA, 200 μM dNTPs, 0.33 μM each primer, 0.5 U Ampli*Taq* Gold (PE Biosystems), and 1.5–2.5 mM $MgCl_2$. Reactions were cycled in either a Perkin Elmer 9600 GeneAmp PCR System or a MJR Tetrad Cycler, as follows: 10 min at 95°C; 35 cycles of 30 s at 95°C, 30 s at 58°C or 55°C, and 30 s at 72°C; and then a final 10-min extension at 72°C. Reactions were held at 5°C until analysis. PCR products were resolved on 5% denaturing polyacrylamide gels using an ABI 377 DNA sequencer. Genotypes were analyzed using ABI Genescan 2.1 and ABI Genotypes 2.0 and 2.5 software packages. Through the use of SAS and Splus programs, we checked for Mendelian errors, mislabeling of marker names, out-of-range alleles, and Hardy-Weinberg equilibrium. We also used the program RELPAIR (Boehnke and Cox 1997) to confirm familial relationships.

Similar to Witte et al. (2000), we used the Haseman-Elston (HE) method of quantitative-trait linkage analysis (Elston et al. 2000; S.A.G.E. 2002). This method regresses either the trait-difference-squared (original HE) or the trait-mean-corrected-product (revised HE) on the estimated proportion of alleles shared identical-by-descent (IBD) at each marker locus. The sample mean Gleason score was used for the revised HE function. For multiple brother-pairs from the same family, their nonindependence is corrected in the analysis through the use of weighted least squares (Elston et al. 2000). We used multipoint estimates of IBD sharing to achieve the most accurate IBD probability estimates, and we regressed the trait on each marker position (as opposed to simultaneously modeling multiple markers). For the chromosome X analysis, we used ordinary least squares, assuming all brother-pairs are independent, to model either the original HE or the revised HE on the IBD-sharing probabilities. The IBD information was generated in MERLIN (Abecasis et al. 2002).

A total of 197 affected brother pairs were available for genetic analysis. The mean Gleason score is 5.68 (range 2–10). The distribution of scores are: Gleason 2 (4.9%), Gleason 3 (2.5%), Gleason 4 (6.3%), Gleason 5 (33.8%), Gleason 6 (22.8%), Gleason 7 (21.7%), Gleason 8 (4.7%), Gleason 9 (3.0%), and Gleason 10 (0.3%).

Singlepoint linkage results from the genome screen are presented in figure 1. The vertical axis of each plot is $-\log(P)$, where P is the P value from each of the two HE analyses. Following the criteria for declaring significance (Lander and Kruglyak 1995), chromosome 19q13 shows significant evidence for linkage on the basis of the original HE analysis. The highest peak occurs at marker D19S902 ($P < .0001$). The span of this region within which $P < .001$ is ~6.5 cM in length. Of great importance, this region is near the region showing evidence for linkage by Witte et al. (2000). With the use of the revised HE approach, the authors report evidence for linkage ($P = .0004$) at marker D19S433. The distance between the markers D19S433 and D19S902 is ~20 cM, on the basis of the Marshfield map. To our knowledge, our finding is the first replication, using independent data, of the positive linkage findings of Witte et al. (2000). This is an extraordinary finding, because replication of results from gene-mapping studies of complex diseases is difficult (Vieland 2001), particularly for prostate cancer (Ostrander and Stanford 2000).

We also observed suggestive evidence for linkage on chromosomes 4 and 15q (fig. 1), on the basis of the original HE approach. The peak on chromosome 4 occurs at marker D4S403 ($P = .00012$), a region not identified by Witte et al. (2000). However, two other genomewide screens have reported linkage between this marker and prostate cancer. Smith et al. (1996) reported a two-point LOD score of ~1.6, and Goddard et al. (2001) reported a LOD score of 1.80, after adjusting for age at onset in the analysis. The region on chromosome 15q23 was located at marker D15S131 ($P = .0014$). This region was not identified by Witte et al. (2000) nor by other groups. For the other linked regions on chromosomes 5 and 7 that were identified by Witte et al. (2000), we observed an elevated peak on only chromosome 5q31, located in the same region but not as significant ($P = .005$) as that observed by Witte et al. (2000). However, the results for this region are still unclear, since, in a follow-up analysis, members of the Witte et al. (2000) group reported that the LOD scores diminished on 5q31 (Neville et al. 2002).

Our findings tended to show the squared-trait-difference to be more significant than the cross product (the opposite of the results of Witte et al. [2000]). There are four possible explanations for this. First, theoretical calculations for large sample sizes indicate that the squared-trait-difference has greater power than the cross product when the sibling correlation is >0.27 (Visscher and Hopper 2001). In our data, the sibling correlation of Gleason score was 0.22. It may be that, in our data, the correlation is bordering the threshold for which the squared-trait-difference achieves greater power. Second, ascertainment may also have a role here. Under strong ascertainment, the trait difference has been shown, via simulations, to have greater power than the cross product (Palmer et al. 2000). Our pedigrees were ascertained on the basis of a list of index cases who were treated at the Mayo Clinic. These individuals may have a more severe form of prostate cancer, and, as a result, they may have shifted the distribution of Gleason scores toward higher scores. However, the distribution of Gleason

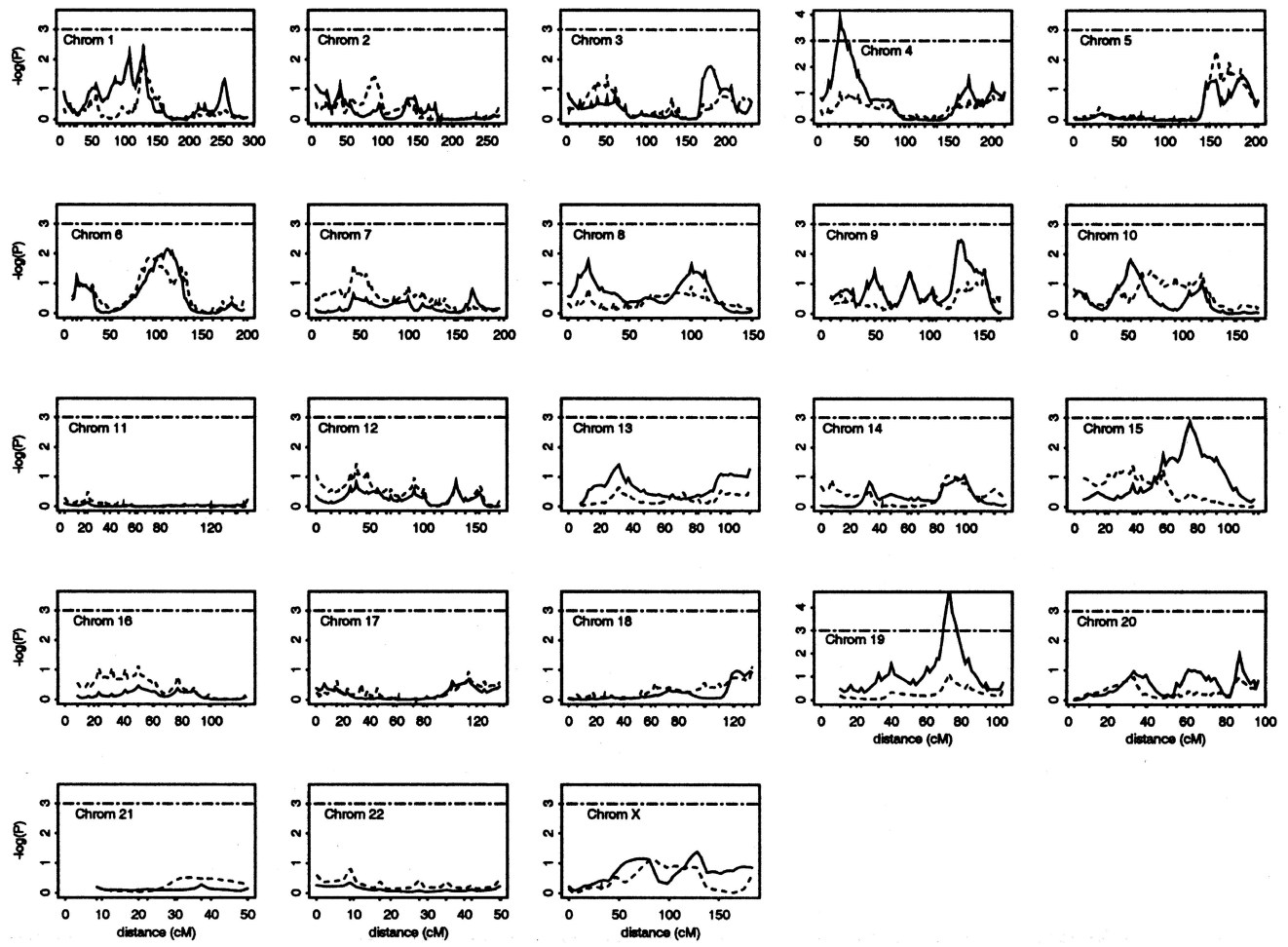

Figure 1 Linkage scan for loci segregating with prostate tumor aggressiveness. The solid line denotes the original HE analysis (i.e., the trait-difference-squared); the broken dotted/dashed line denotes the revised HE analysis (i.e., trait-mean-corrected product). The horizontal dotted/dashed line in each subfigure indicates where $P = .001$ ($-\log[P] = 3$). Points above this line denote $P < .001$. Tick marks on the *X*-axis denote marker locations.

scores for Mayo prostate cancer cases is comparable to that of Witte et al. (2000). Moreover, owing to PSA screening, the Gleason scores might be attenuated because prostate cancer is detected earlier. Thus, the effect of ascertainment is not likely to be a major influence. Third, the power of the cross product is reduced when the mean used in the calculations deviates from the true population mean (Palmer et al. 2000). We used the sample mean in our analysis of the cross product; however, we tried other prespecified values to explore the sensitivity of our results on chromosome 19. The significance level of the cross product varied across the values of the mean, but none reached the significance level of the difference-squared (results not shown). Finally, random chance might explain the greater significance with the squared-trait difference.

Nineteen percent of our cases (or 84 individuals) were missing Gleason scores. This missing data will have very little effect on our results, because 78 of these cases were affected fathers who had no affected brothers and, hence, would not have entered into the analysis.

In summary, we report strong evidence for linkage on chromosome 19q13 and suggestive evidence on chromosome 4q. Our finding on chromosome 19, together with previous findings of Witte et al. (2000), strongly suggests that susceptibility loci exist for tumor aggressiveness, and, since we found this locus with the trait-difference-squared outcome, which is a similarity score, this finding suggests that the putative locus may have alleles that not only promote more aggressive tumors, but also lead to less aggressive disease.

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Electronic-Database Information

Accession number and URL for data presented herein are as follows:

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

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