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Fine Mapping of Prostate Cancer Aggressiveness Loci on Chromosome 7q22-35

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Abstract

Background—Deciphering the genetic basis of prostate cancer aggressiveness could provide valuable information for the screening and treatment of this common but complex disease. We previously detected linkage between a broad region on chromosome 7q22-35 and Gleason score—a strong predictor of prostate cancer aggressiveness. To further clarify this finding and focus on the potentially causative gene, we undertook a fine-mapping study across the 7q22-35 region.

Methods—Our study population encompassed 698 siblings diagnosed with prostate cancer. 3,072 single nucleotide polymorphisms (SNPs) spanning the chromosome 7q22-35 region were genotyped using the Illumina GoldenGate assay. The impact of SNPs on Gleason scores were evaluated using affected sibling pair linkage and family-based association tests.

Results—We confirmed the previous linkage signal and narrowed the 7q22-35 prostate cancer aggressiveness locus to a 370 kb region. Centered under the linkage peak is the gene *KLRG2* (killer cell lectin-like receptor subfamily G, member 2). Association tests indicated that the potentially functional non-synonymous SNP rs17160911 in *KLRG2* was significantly associated with Gleason score ($p = 0.0007$).

Conclusions—These findings suggest that genetic variants in the gene *KLRG2* may affect Gleason score at diagnosis and hence the aggressiveness of prostate cancer.

Keywords

prostate cancer; Gleason Score; siblings; SNP

INTRODUCTION

The aggressiveness of prostate cancer varies widely: some tumors progress to invasive, potentially life-threatening disease; whereas, others stay latent for an individual's remaining lifetime. Like other complex traits, both genetic and environmental factors are likely to affect this variability. Numerous genome-wide association studies (GWAS) [1-13] of prostate cancer have recently been undertaken. Only a few of these studies, however, have examined aggressive prostate cancer [1,9,12]. Furthermore, the majority of the SNPs identified from GWAS were not associated with Gleason score [14-18] or disease aggressiveness defined by both Gleason score and tumor stage [16-20]. A SNP at 17p12 has recently been associated with aggressive disease (i.e. Gleason score ≥ 7 or clinical stage T3/T4) in contrast to indolent disease [21]; nevertheless, the genetic basis of prostate cancer aggressiveness remains unclear.

Our research group has undertaken two genome-wide linkage scans for prostate cancer-aggressiveness loci using Gleason score as a quantitative trait. In the first study [22] we identified candidate regions at chromosomes 5q31-33, 7q32, and 19q12 linked to Gleason score using 326 affected sibling pairs. We expanded this study with an additional 108 new sibling pairs [23], and observed a stronger linkage signal at 7q32. This finding, and the chromosome 19q linkage, have been replicated by others [24,25].

To further narrow the 7q32 linkage region, we conducted an independent allelic imbalance (AI) study in prostate tumors at this locus [26]. The highest frequency of AI was observed for the neighboring markers D7S2531 (52%) and D7S1804 (36%), and interstitial AI involving one or both markers delineated a relatively narrow common region of AI spanning approximately 1Mb. However, the tumors also exhibited modest AI (20-30%) with markers spanning ~20 Mb. In addition, 7q31 has been commonly reported by others to have LOH in prostate cancer [27-31] and other tumor types [32-37]. Therefore, we sought to determine the genetic basis underlying the linkage and AI findings for prostate cancer aggressiveness by conducting a fine mapping study of the 7q22-35 linkage region, with a particular emphasis on the region 7q31-33 defined by markers D7S3061-D7S1824.

MATERIALS AND METHODS

Study Population

The prostate cancer families were recruited from medical institutions in St Louis, MO and Cleveland OH, as described in our previous linkage studies [22,23]. In the current fine mapping study, a total of 742 subjects were successfully genotyped by the Center for Inherited Disease Research (CIDR), including 18 blinded duplicates, 698 prostate cancer cases, and 26 non-affected sibling controls. Of the cases, there were 340 sibships with Gleason score information available (more than 75% with only two affected brothers). The Gleason Scores were determined by radical prostatectomy specimens when available, otherwise by biopsy specimens. Ninety-seven percent of the prostate cancer cases self-identified as being Caucasian. Institutional Review Board approval was obtained from the participating institutions, and all study participants gave informed consent. All samples were whole genome amplified (WGA) from 100ng genomic blood DNA (REPLI-g kit, Qiagen). The integrity of the WGA DNA was assessed by gel electrophoresis to ensure that the majority of product produced was $>10\text{kb}$.

SNP Selection and Genotyping

A total of 3,072 SNPs were genotyped by CIDR using the Illumina GoldenGate assay and were selected as follows. For the allelic imbalance (AI) region bounded by markers D7S3061 and D7S1824, we selected tagSNPs that were in high linkage disequilibrium (LD) with common SNPs (pairwise $r^2 \geq 0.8$) within the region (Tagger program, HapMap Rel 21). Then for the broader region under the original linkage peak (7q22-35: D7S1799 to D7S3058), we selected potentially functional SNPs that either result in nonsynonymous amino acid change or are located within human-mouse conserved regions and predicted to be functional variants (PupaSuite, <http://pupasuite.bioinfo.cipf.es/>), e.g., SNPs in putative transcription factor binding sites in the promoter region of a gene (up to 10kb upstream of the transcription start site), exonic splicing enhancers, exonic splicing silencers, microRNAs and their targets, and potential DNA triplexes. All the selected SNPs are common with minor allele frequency (MAF) $>5\%$ in Caucasians according to HapMap and dbSNP databases.

Once the 3,072 SNPs were genotyped, CIDR excluded 245 SNPs with poor performance (e.g., poor cluster definition, replicate errors, and/or all samples typing as heterozygous), leaving 2,827 SNPs. We further excluded 26 SNPs with atypical clustering patterns and one SNP that deviated from Hardy-Weinberg Equilibrium (HWE, $p < 10^{-4}$). The mean call rate of the remaining 2800 SNPs was $>99.8\%$, and the blind duplicate reproducibility rate was $>99.99\%$. 24 CEPH and 12 Yoruba controls were genotyped simultaneously. The genotype data of 12 unique CEPH samples showed high consistency with the HapMap genotype data ($>99.56\%$).

Statistical Analyses

We first tested for Hardy-Weinberg Equilibrium (HWE) using the programs Haploview (<http://www.broadinstitute.org/mpg/haploview>) and PLINK (<http://pngu.mgh.harvard.edu/~purcell/plink>). We then undertook nonparametric linkage analysis of Gleason score similarity among affected sibling pairs using a model-free regression method (implemented in the program MERLIN [38]). This extends the Haseman-Elston quantitative trait linkage analysis by using the trait (i.e., Gleason score) squared sums and differences among siblings to predict their identical-by-descent sharing [39]. We further excluded 21 SNPs with MAF $<2\%$ in the study population and 112 SNPs in complete LD ($r^2=1$) with other typed SNPs, leaving a total of 2,667 SNPs for the linkage analysis.

Next, we conducted a family-based association test of the relationship between SNPs and the Gleason score (FBAT, version 2.0.3) (<http://biosun1.harvard.edu/~fbat/fbat.htm>). In particular, the FBAT statistics were calculated using the empirical variance to test for association in the presence of linkage [40]. To minimize the variance of the test statistics [41], original Gleason scores were transformed into standardized scores (i.e. mean=0 and standard deviation =1). Two-locus haplotype analyses via a sliding window were also evaluated with the haplotype FBAT (HBAT) [42]. An additive genetic model was used throughout the study. For the association analyses we used all 2,800 SNPs, since FBAT excludes markers with less than 10 informative families.

We also undertook FBAT and HBAT analyses using measured and imputed SNPs. Imputation of unmeasured SNPs was undertaken for the Caucasians using the MACH program (version 1.0) CEU HapMap (rel 22) [43,44]. A total of 40,803 SNPs were initially imputed; 22,237 of these were removed from our analyses due to poor imputation quality ($rsq < 0.3$), deviation from HWE, or with MAF $<2\%$. This left a total of 18,566 imputed SNPs that, when combined with 2,800 typed SNPs, resulted in a total of 21,336 SNPs in the 679 Caucasians studied here for additional FBAT and HBAT analyses.

RESULTS

Linkage Analysis Fine Mapping

With fine mapping we narrowed down the previous Gleason score linkage peak to a 370 kb region (i.e., with LOD scores > 2) (Figure 1). There are only a few genes in this region. Centered under the linkage peak is killer cell lectin-like receptor subfamily G, member 2 (*KLRG2*). Other genes in the region include tetratricopeptide repeat domain 26 (*TTC26*), ubinuclein 2 (*UBN2*), chromosome 7 open reading frame 55 (*C7orf55*), Homo sapiens LUC7-like 2 (*S. cerevisiae*) (*LUC7L2*), Homo sapiens hypothetical LOC100129148 (*LOC100129148*), and C-type lectin domain family 2, member L (*CLEC2L*). Five SNPs were genotyped in *KLRG2*, including one missense SNP (rs17160911: Gly339Ala) and four intronic SNPs: rs10954649, rs12113878, rs7779375, and rs6945850. These results were not materially changed when using the residual of Gleason scores after adjusting for age or when excluding redundant SNPs with $r^2 > 0.8$ (not shown).

Association Analyses

Figure 2A shows the results from the FBAT single SNP analyses. Eighteen SNPs exhibited nominal association with Gleason score ($p < 0.01$). Table 1 lists the position, genes, MAF, and potential function for these SNPs, plus the four additional intronic SNPs genotyped in *KLRG2*. The smallest association p-value was for the missense SNP rs17160911 (Gly339Ala) in *KLRG2* (p value = 0.0007). This SNP was not in high LD with the other neighboring SNPs (i.e. maximum $r^2 = 0.31$ within 1MB). Two-locus haplotype analyses did not provide substantially more information (Figure 2B).

Figures 3A and 3B present the results for imputed and typed SNPs among Caucasians. In general, the associations were slightly stronger after excluding 17 African American and 2 Hispanic subjects, although the association between the missense SNP rs17160911 in *KLRG2* and Gleason score was slightly weaker (p value = 0.0014 vs. 0.0007). This may reflect lower power due to the reduced number of informative families, and suggests that the effect of rs17160911 may be relevant not only to Caucasians but also other ethnic groups. The smallest p-values ($= 0.0002$) from these analyses were observed in an imputed SNP (rs1851434) and a typed SNP (rs6947787). Neither of these SNPs is located in a specific gene nor have potential function. The strongest two-locus haplotype association with Gleason score ($p = 0.0001$) was observed for the three intergenic SNPs (rs2140138, rs12531207, rs11971770), that reside between antitiquitin-like 3 (*ATQL3*) and GTPase, IMAP family member 4 (*GIMAP4*).

DISCUSSION

In this fine mapping linkage and association study of prostate cancer aggressiveness, we first confirmed the previous findings of linkage to chromosome 7q22-35 using a panel of dense SNPs (i.e. 1 SNP per 7 kb in the AI region). We further narrowed the linkage peak with a LOD score > 2.0 to a 370 kb region. Within this region, a potentially functional SNP rs17160911 in the gene *KLRG2* was strongly associated with Gleason score and, therefore, potentially with prostate cancer aggressiveness.

Although the function of *KLRG2* (also known as *CLEC15B*) is not known, it contains a conserved C-type lectin/C-type lectin-like domain (CTL/CTLD) (GeneCards v3, www.genecards.org) which are believed to function in carbohydrate recognition and binding. CTL and CTLD receptors are expressed on many cell types of the immune system, and have been shown to be involved in cell adhesion and migration, cell-cell signaling, and pathogen recognition [45-47]. The CTLD of *KLRG2* shows most similarity to those of the natural killer cell receptors which are expressed on subsets of natural killer (NK) cells and T

cells and thus could influence the function of these immune cells. A recent study has demonstrated that *KLRG1* can inhibit NK and T cell functions by binding to E-cadherin [48,49]. The amino acid substitution from Gly to Ala at amino acid position 339 of *KLRG2* arising from the associated SNP rs17160911 is located in the C-type lectin-like domain and may lead to secondary structure change of this transmembrane protein (predicted by F-SNP database <http://compbio.cs.queensu.ca/F-SNP>). This could subsequently inhibit immune and inflammatory regulation, possibly impacting prostate cancer aggressiveness. Finally, this missense SNP (rs17160911) is located in an exonic splicing enhancer and thus may regulate splicing processes and subsequent mRNA stability.

We have previously undertaken association studies of two other genes in the chromosome 7q31-33 region: testis derived transcript (*TES*) [50] and podocalyxin-like (*PODXL*) [51]. For variants in *TES* we previously observed an association with prostate cancer aggressiveness, but this was restricted to African-American men. Therefore, the present study confirms our finding that genetic variants in *TES* do not appear to impact prostate cancer aggressiveness among Caucasians. With regard to *PODXL*, we previously detected an association between the missense SNP (rs3735035) and prostate cancer (340 G>A in ref [51]). However, we did not replicate this result in the current study of Gleason score. We also previously detected an association between an in-frame variable length deletion in coding exon 1 of *PODXL* and prostate cancer aggressiveness [51] that we were unable to evaluate in this SNP-based study. The different study designs might be detecting distinct genetic loci for prostate cancer in the region. Specifically, our previous *PODXL* results were from association studies of affected and unaffected individuals, and were detected for both disease risk and more aggressive disease. We subsequently showed that *PODXL* represented a biologically plausible prostate cancer candidate gene through *in vitro* studies [52]. In contrast, the current paper reports results from linkage / association studies of affected sibling pairs, looking at Gleason score as a quantitative trait. It is feasible that both *PODXL* and *KLRG2* represent independent markers of aggressive prostate cancer mapping to this region, or *KLRG2* may be a statistical artifact due to the multiple comparisons.

In summary, we confirmed and narrowed the previous linkage region for Gleason score on chromosome 7q22-35 and detected a promising association with a nonsynonymous SNP in *KLRG2*. We cannot eliminate the possibility that there are other genes in this region that impact prostate cancer aggressiveness in conjunction with, or independently, of *KLRG2*. This finding needs further confirmation by future studies of prostate cancer aggressiveness, progression, or mortality. If confirmed, functional studies may help distinguish the biological mechanisms underlying this result, and this knowledge may provide an avenue for improving prostate cancer screening and treatment.

Conclusions

Our findings suggest that genetic variants in the gene *KLRG2* may affect Gleason score at diagnosis and hence the aggressiveness of prostate cancer

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Figure 1.
Linkage Analysis of chromosome 7q22-35 SNPs and Gleason Score.

Figure 2.
Family-Based Association Tests (FBAT) in 698 prostate cancer cases: Gleason Scores & the typed SNPs on 7q22-35.

A. Single SNP Analyses

B. Two-Locus Haplotype Analysis by Sliding Window

Figure 3.

Results from Family-Based Association Tests (FBAT) in 679 Caucasian prostate cancer cases: Gleason Scores & the imputed and genotyped SNPs on 7q22-35.

A. Single SNP Analyses

B. Two-Locus Haplotype Analysis by Sliding Window

Table 1

Results from FBAT for Gleason Scores (Figure 2A): SNPs in gene *KLRG2* and SNPs with p-values ≤ 0.01 .

SNPs	Position (bp)	Genes	Function	Allele*	MAF**	P-value
rs11539696	111768243	<i>ZNF277</i>	missense	G/A	0.065	0.007
rs4357236	125392338			T/C	0.366	0.009
rs4731279	125410378			C/T	0.209	0.004
rs17149532	125718618			G/T	0.127	0.010
rs11563330	126847532			T/C	0.176	0.004
rs2347699	134041504			A/T	0.296	0.003
rs3800748	134200659	<i>CALD1</i>	intron	C/T	0.157	0.008
rs834789	135512847			C/T	0.292	0.004
rs17168743	135993029			T/A	0.146	0.009
rs1345934	136422815	<i>LOC100128744</i>	intron	A/G	0.236	0.001
rs322302	136582092	<i>PTN</i>	intron	A/G	0.112	0.005
rs322239	136607147	<i>PTN</i>	intron	C/A	0.119	0.001
rs10954649	138789091	<i>KLRG2</i>	intron	C/T	0.343	0.347
rs17160911	138789490	<i>KLRG2</i>	missense	C/G	0.149	0.0007
rs12113878	138793985	<i>KLRG2</i>	intron	C/G	0.232	0.479
rs7779375	138814003	<i>KLRG2</i>	intron	G/A	0.479	0.631
rs6945850	138814158	<i>KLRG2</i>	intron	C/T	0.364	0.589
rs6947787	139609865			A/G	0.096	0.002
rs269243	139948200	<i>DENND2A</i>	missense	G/T	0.054	0.003
rs3735081	149848181	<i>GIMAP7</i>	synonymous	T/C	0.372	0.006
rs6959171	149891749			C/G	0.222	0.006
rs759011	150070433	<i>GIMAP5</i>	synonymous	G/A	0.297	0.006

* The first allele showed positive FBAT test statistics

** Minor allele frequency in Caucasians.