Original Article

LHRH and LHR genotypes and prostate cancer incidence and survival

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Abstract: Despite their crucial role in initiating steroid-hormone synthesis, the hypothalamic and pituitary hormones (LH, LHRH) and their receptors have received scant attention in genetic studies of hormone-related diseases. This study included 1,170 men diagnosed with prostate cancer (PC) in Los Angeles County between 1999 and 2003. LHRH and LH receptor genotypes were examined for association with PC survival. Additionally, associations with PC incidence were examined by comparing PC cases to control men of similar age and race/ethnicity. The LHR 312 G allele was found to be associated with increased PC mortality (p=0.01). Ten years after diagnosis, 16% of men carrying two copies of the G allele (genotype GG) had died of PC, compared to 11% of those with genotype AG and 9% of those with AA. In a case-control comparison, this same allele was significantly associated with decreased PC risk: OR=0.68 (95% CI: 0.49, 0.93) for genotype GG vs. AA. These results suggest that androgens may play opposing roles in PC initiation and progression, and highlight the need to include these important but overlooked genes in future studies of PC etiology, prognosis, and treatment.

Keywords: Prostate cancer, LHRH, LHR, genetic polymorphism, survival, association study

Introduction

Prostate cancer (PC) (OMIM: 176807) affects almost 240,000 men in the United States each year and is the second leading cause of cancer related death in this population [1]. Androgens are essential survival factors for prostate epithelial cells, and have long been recognized as the primary drivers of cell proliferation in both normal and malignant prostate. Genetic variations in the androgen receptor and androgen metabolic pathway have been studied with respect to PC incidence (reviewed in [2]) and survival [3]. Despite their importance in initiating androgen synthesis, the hypothalamic and pituitary hormones, luteinizing hormone-releasing hormone (LHRH) and luteinizing hormone (LH), have received scant attention in genetic studies.

LHRH is secreted, under physiologic conditions, in a pulsatile manner from the hypothalamus. LHRH receptors in the pituitary, upon brief exposures to high levels of LHRH, trigger release of LH. LH plays a critical role in initiating testicular androgen synthesis. The binding of LH to its receptors on testicular Leydig cells induces cAMP production and subsequent StAR-mediated cholesterol transport, which is the first and rate-limiting step in testicular androgen biosynthesis. This pathway is central to the development of PC and remains the primary therapeutic target in advanced PC.

In this study, we selected all validated non-synonymous SNPs having a minor allele frequency of at least 5% in the following genes: LHB (encoding the LH beta subunit), LHCGR (encoding LH receptor), GNRH1 (encoding LHRH), and GNRHR (encoding the LHRH receptor). Only two SNPs fulfilled these criteria, rs2293275 in LHCGR and rs6185 in GNRH1, which we will subsequently refer to as LHR312 and LHRH16. These SNPs were genotyped in a cohort of 1,170 men diagnosed with PC in Los Angeles County from 1999-2003. Genotypes were examined for association with PC survival. Additionally, associations with PC incidence...
were examined by comparing PC cases to control men of similar age and race/ethnicity. Cases and controls were participants in the Los Angeles site of the California Collaborative Prostate Cancer Study.

Methods

Study population

Study subjects were Los Angeles County participants from the California Collaborative Prostate Cancer Study, a population-based case control study that has been previously described in detail [4-6]. Briefly, men diagnosed with PC between 1999 and 2003 were identified by the Los Angeles County Cancer Surveillance Program and from Los Angeles County Cancer Registry records. Cases were ascertained to enrich the study population for advanced stage disease [7]. Cases were linked to the California Cancer Registry records in March 2013 to obtain vital status and cause of death. Controls living in the same neighborhood as the cases were identified using a standard neighborhood walk algorithm and were frequency matched to cases on age (≥5 years) and race/ethnicity.

Written informed consent was obtained from all study participants. The study was approved by the Institutional Review Board of the University of Southern California.

This study included 1,170 prostate cancer cases and 538 controls. For the observed distributions of genotypes and survival probabilities shown in Table 1, minimal detectable hazard ratio for the log-rank test with a=0.05 and 80% power was 1.97 for LHR312 genotype GG vs. AA and 2.88 for LHRH16 genotype CC vs. GG. For case-control comparisons, minimal detectable effects correspond to odds ratios of 0.71 for LHR312 AG/GG vs. AA and 0.70 for LHRH16 GC/CC vs. GG.

Genotyping

SNPs were genotyped using DNA extracted from peripheral blood buffy coats. Genotypes were assayed using TaqMan allelic discrimination assays (Applied Biosystems) using the TaqMan Core Reagent Kit according to manufacturer’s recommendations. Fluorescent signals were measured using the ABI7900HT detection system. Each run included water blanks and 10% blind replicates. There were no discrepancies among replicates. Call rates were >98% for both assays.

Statistical analysis

Hazard ratios were estimated by fitting Cox proportional hazards models adjusted for race (black vs. non-black), age at diagnosis (≥70 vs. <70), tumor grade (high vs. low), and stage at diagnosis (regional/distant vs. local). Odds ratios were estimated from conditional logistic regression matching on race/ethnicity and...
Results

Survival

Table 1 describes the cohort of 1,170 cases included in this study. Age at diagnosis ranged from 42 to 94 years (median 66 years); 30% (349) were African American, 28% (327) were Hispanic, and 42% (494) were non-Hispanic white. Eight percent (89) were diagnosed with distant metastases, 41% (481) with regional disease, and 45% (528) with localized disease. Median follow-up was 10.0 years. At the time of linkage to the cancer registry, 36% had died, 11% from PC. PC-specific mortality appeared higher among men diagnosed at age 70 years or older, among those diagnosed with advanced or high-grade disease, and among those with LHR312 genotype GG.

Hazard ratios for PC-specific survival and genotype are shown in Table 2. The significant association between PC-specific survival and LHR312 genotype remained after adjusting for stage, grade, age at diagnosis, and race. There was no evidence that the association between genotype and survival varied by grade or stage of disease, age, or race/ethnicity, however power to detect heterogeneity was low. LHR312

Table 2. Hazard Ratios for PC-specific survival, by LHR and GnRHR genotype

<table>
<thead>
<tr>
<th></th>
<th>Hazard Ratio (95% CI)†</th>
<th>† Includes those cases with non-missing grade and stage (1000 for LHR 312 and 1010 for LHRH 16. ‡Cox Proportional Hazards models adjusted for race (Black vs. non-Black), age (≥70 vs. &lt;70), grade (high vs. low), and stage (regional/distant vs. local).</th>
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<tbody>
<tr>
<td><strong>LHR 312 genotype</strong></td>
<td></td>
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</tr>
<tr>
<td>AA</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>AG</td>
<td>1.28 (0.72, 2.27)</td>
<td>1.46 (0.80, 2.64)</td>
</tr>
<tr>
<td>GG</td>
<td>1.90 (1.07, 3.36)</td>
<td>2.25 (1.19, 4.26)</td>
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<tr>
<td>p trend = 0.01</td>
<td>0.01</td>
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<tr>
<td><strong>LHRH 16 genotype</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>1.00 (ref)</td>
<td>1.00 (ref)</td>
</tr>
<tr>
<td>GC</td>
<td>1.02 (0.65, 1.59)</td>
<td>0.91 (0.58, 1.44)</td>
</tr>
<tr>
<td>CC</td>
<td>1.38 (0.60, 3.17)</td>
<td>1.00 (0.43, 2.43)</td>
</tr>
<tr>
<td>p trend = 0.60</td>
<td>0.60</td>
<td>0.80</td>
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p=0.03). However, for approximately the first five years after diagnosis, survival was similar across genotypes. Poorer PC-specific survival among those with the GG genotype emerged only with long-term follow-up. At ten years follow-up, PC-specific survival was 84% among those with genotype GG, compared to 89% and 91% for those with genotypes AG and AA, respectively.

Figure 1. Kaplan-Meier Curves for PC-specific survival by genotype. A: Survival by LHR312 genotype; B: Survival by LHRH16 genotype. P-values are from the Log-Rank Test.
<table>
<thead>
<tr>
<th>Genotype</th>
<th>All Races</th>
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<tbody>
<tr>
<td></td>
<td>Controls</td>
<td>Cases</td>
<td>OR (95% CI)</td>
<td>Controls</td>
<td>Cases</td>
<td>OR (95% CI)</td>
<td>Controls</td>
<td>Cases</td>
</tr>
<tr>
<td>LHR321</td>
<td></td>
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</table>
| AA       | 115 (20%) | 283 (26%) | 1.00 (ref) | 45 (11%)  | 114 (15%) | 1.00 (ref) | 70 (45%)  | 169 (52%) | 1.00 (ref)  
| AG       | 272 (47%) | 466 (43%) | 0.70 (0.54, 0.93) | 204 (48%) | 339 (44%) | 0.65 (0.32, 0.97) | 68 (44%)  | 127 (39%) | 0.78 (0.51, 1.17) 
| GG       | 187 (33%) | 340 (31%) | 0.68 (0.49, 0.93) | 170 (41%) | 309 (41%) | 0.63 (0.32, 0.96) | 17 (11%)  | 31 (9%)  | 0.75 (0.38, 1.46) |
|          | 574 (100%)| 1089 (100%)|            | 419 (100%)| 762 (100%)|            | 155 (100%)| 327 (100%)|            |
| LHRH16   |           |          |            |           |            |            |           |          |            |
| GG       | 360 (66%) | 757 (69%) | 1.00 (ref) | 241 (61%) | 471 (62%) | 1.00 (ref) | 119 (80%) | 286 (85%) | 1.00 (ref)  
| GC       | 159 (30%) | 295 (27%) | 0.93 (0.73, 1.19) | 130 (35%) | 248 (32%) | 0.97 (0.73, 1.28) | 29 (19%)  | 47 (14%) | 0.78 (0.46, 1.32) 
| CC       | 19 (4%)   | 46 (4%)   | 1.21 (0.68, 2.16) | 18 (5%)   | 44 (6%)   | 1.26 (0.69, 2.29) | 1 (1%)    | 2 (1%)   | 0.47 (0.04, 5.32) |
|          | 538 (100%)| 1098 (100%)|            | 389 (100%)| 763 (100%)|            | 149 (100%)| 335 (100%)|            |

Odds ratios calculated from conditional logistic regression, adjusting for age, 1st degree family history of PC, race/ethnicity, and SES.
LHRH & LHR genotypes and PC

genotype was not associated with all-cause mortality (p trend = 0.82; data not shown).

Genotype at the LHRH16 locus was not significantly associated with PC survival (Figure 1B, Log rank test p=0.66). There appeared to be a modest increase in hazard for those with genotype CC vs. GG; however, power to detect such a difference was limited by the small number of men with genotype CC, and the apparent association disappeared after adjusting for stage, grade, age at diagnosis and race (Table 1). LHRH 16 genotype was not associated with all-cause mortality (Table 1, P trend = 0.57).

Incidence

Table 3 compares genotype frequencies among PC cases to those among controls. Odds of PC were approximately 30% lower for men who carried at least one LHR312 G allele, compared to men with the AA genotype. This association between PC and LHR genotype was observed in both racial groups, though the association was not statistically significant among African Americans. The frequency of the G allele was substantially lower among African Americans (33%) than among whites (65%). Genotype frequencies did not depart from Hardy-Weinberg equilibrium among controls in either racial group (p>0.05). There was no evidence of heterogeneity by stage or grade of disease. Odds ratios (and 95% confidence intervals) for genotype GG vs. AA were 0.65 (0.45, 0.95) and 0.72 (0.49, 1.07) for advanced and localized stage disease and 0.62 (0.41, 0.95) and 0.67 (0.47, 0.95) for high- and low-grade disease, respectively (data not shown).

LHRH16 genotype was not associated with PC incidence, either overall or when stratified by race, stage, or grade of disease. The C allele was less frequent among African Americans than among whites (10% vs. 22%). There were no departures from Hardy-Weinberg equilibrium among either racial group (p>0.05).

Discussion

In this cohort of 1,170 prostate cancer cases, diagnosed more than a decade ago, we found that the LHR312 G allele is associated with increased PC mortality (p=0.01). Ten years after diagnosis, 16% of men carrying two copies of the G allele (genotype GG) had died of PC, compared to 11% of those with genotype AG and 9% of those with AA. Yet, in a case-control comparison, this same allele was significantly associated with decreased PC risk.

The LHR312 G and A alleles respectively encode serine and asparagine at position 312 in exon 10, an exon that is critical for LH receptor activation [8]. The SNP lies adjacent to a potential N-linked glycosylation site: 313N-314K-315T, however it has not been found to affect glycosylation at 313Asn [9].

Although in vitro evidence for functionality is lacking, association studies provide evidence that the SNP may have subtle functional effects. The few association studies that have examined this polymorphism consistently link the G allele to decreased LH receptor signaling. The A allele was over-represented in two independent samples of patients with breast cancer [9], a disease associated with increased steroid hormone exposure. Conversely, the G allele has been overrepresented in conditions associated with decreased testosterone production, such as testicular maldescent and male infertility without maldescent [10], testicular cancer [11], and male genital under-masculination [12].

In light of these reports, our results suggest that modestly impaired LH signaling (marked by the LHR321 G allele) may be associated with decreased PC risk but worse PC prognosis. This first observation (association of the G allele with decreased risk) is in agreement with the androgen hypothesis of PC causation and progression, which has been universally accepted since the seminal studies of Huggins [13] more than seven decades ago. Within the last decade, however, a more nuanced appreciation of the relationship between androgens and PC has emerged. A number of studies from around the world have reported that testosterone levels around the time of PC diagnosis are not positively associated with prognosis. One recent study reported a U-shaped association, with both low and high serum testosterone levels being associated with high risk PC [14]. Most studies have found that low testosterone levels are associated with higher Gleason scores, higher PSA levels, more poorly differentiated disease, and increased risk of biochemical recurrence [15-18]. Consistent with these studies, we found that high-grade disease was
more common among men carrying a G (low activity) allele (39%) compared to those with genotype AA (31%) (p=0.02, data not shown).

Strengths of this study include the large sample size of more than 1,000 PC cases ascertained via cancer registry from a multi-ethnic population, with more than 10 years of follow-up. The main study limitations are related to the incomplete assessment of genetic variation in the LH gene pathway and to the unavailability of clinical data on PC treatment history for cancer registry-identified cases. Our results highlight the need to include these important overlooked genes in future studies of PC etiology, prognosis, and treatment.

Acknowledgements

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Disclosure of conflict of interest

None.

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