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Pathologic Aggressiveness of Prostatic Carcinomas Related to *RNASEL* R462Q Allelic Variants

Benjamin T. Larson¹, Cristina Magi-Galluzzi², Graham Casey³, Sarah J. Plummer³, Robert Silverman³, and Eric A. Klein⁴

¹Cleveland Clinic Lerner College of Medicine of Case Western Reserve University, Cleveland Clinic, Cleveland, OH

²Department of Anatomic Pathology, Cleveland Clinic, Cleveland, OH

³Department of Cancer Biology, Lerner Research Institute, Cleveland Clinic, Cleveland, OH

⁴Glickman Urological Institute, Cleveland Clinic, Cleveland, OH

Abstract

Purpose—Allelic variations in the *HPC1/RNASEL* gene, especially the R462Q single nucleotide polymorphism, have been associated with increased susceptibility to prostate cancer. Prior studies have suggested that *HPC1*- or R462Q-associated tumors present with more aggressive clinical features. We assessed a series of men undergoing radical prostatectomy for clinical and pathological measures of tumor aggressiveness according to the *RNASEL* R462Q genotype.

Methods—A prospective analysis of 232 men treated for prostate cancer by radical prostatectomy was performed. Disease aggressiveness at time of diagnosis was assessed by age of disease onset, biopsy Gleason score, clinical T stage, and pretreatment PSA level. Tumor aggressiveness was assessed pathologically by tumor volume, extraprostatic extension, seminal vesicle involvement, and lymph node metastases. Clinical and pathological characteristics were then correlated with *RNASEL* genotype.

Results—Of the 232 men studied, 104 (45%) were homozygous wild-type (RR), 101 (43%) were heterozygous (RQ), and 27 (12%) were homozygous for the R462Q variant (QQ), mirroring the distribution seen in the general population. No significant differences were seen between genotypes in age of disease onset, pretreatment characteristics or in pathologic features as assessed by surgical grade and pathological stage. QQ tumors were of smaller volume than other genotypes ($p=0.02$).

Conclusions—This prospective study suggests that prostate cancer in patients with the R462Q allelic variant of the *HPC1/RNASEL* gene are not associated with more aggressive clinical or pathological features in radical prostatectomy specimens.

Keywords

prostate cancer; *RNASEL*; *HPC1*; genetic susceptibility; aggressiveness

Introduction

In 1996, Smith et al. mapped the first gene linked to prostate cancer susceptibility to chromosome 1q24-25, and named it hereditary prostate cancer gene 1 (*HPC1*).¹ In 2002, the gene encoding the antiviral and proapoptotic enzyme RNase L was putatively identified as *HPC1*.² Subsequently, several studies have linked allelic variants in *RNASEL* to increased susceptibility to prostate cancer.^{2,3,4,5,6} Of these variants, the single nucleotide polymorphism (SNP) at nucleotide position 1385 (G1385A), which results in a glutamine instead of arginine at amino acid position 462 (R462Q), has demonstrated increased risk of prostate cancer in both heterozygotes (1.5 fold increase) and homozygotes (2.0 fold increase), and may account for up to 13% of prostate cancer cases in the US.³

Prior case-control and cohort studies have suggested that linkage of *HPC1* or presence of the 462Q variant in prostate tumors is associated with early age of onset^{7,8} and some have suggested that these tumors present with more aggressive clinical features as measured by tumor grade or clinical stage.^{9,10,11} No prior studies have assessed disease severity associated with the R462Q variant in radical prostatectomy specimens. Since the identification of the association between the R462Q variant and prostate cancer susceptibility, we have prospectively collected clinical, pathological, and genotypic information on men undergoing radical prostatectomy. In this study we investigated clinical and pathological features of tumor aggressiveness in men with localized and locally advanced disease and correlate them with *RNASEL* genotype, hypothesizing that more aggressive features would be observed in men homozygous for R462Q.

Methods

Study Population

We investigated 232 consecutive patients who underwent radical prostatectomy without neoadjuvant therapy at our institution for the treatment of prostate cancer between May 2003 and October 2005. Clinical characteristics including age, clinical T stage, PSA, and biopsy Gleason grade were recorded at the time of presentation. All patients were genotyped for the R462Q variant in the *RNASEL* gene. The study was IRB-approved and all patients gave written informed consent for participation.

Laboratory Methods

Genotyping—The 1385G→A (R462Q, rs486907) variant in *RNASEL* was identified using the 5' nuclease Taqman allelic discrimination assay. Reactions were performed using a predesigned primer/probe set (C_935391_1, Applied Biosystems, Foster City, CA), RealMasterMix Probe ROX (Eppendorf, Westbury, NY) and 2.5ng of genomic DNA extracted from white blood cells (Biorobot EZ1, Qiagen, Valencia, CA). The fluorescent intensities of the products were read on an AB7900HT sequence detection system and genotypes were called by the instrument software (SDS2.2, Applied Biosystems).

Pathological Analysis of Radical Prostatectomy Specimens—Radical prostatectomy specimens were inked and fixed in formalin solution for 24 to 48 hours before sectioning. Specimens were sectioned transversely at 3-mm intervals and mounted as half or

quarter sections for microscopic review. If no gross lesions were identified, standard sections of the prostate, including the apical shave margin, the bladder-neck shave margin, the entire peripheral zone and alternative sections of the anterior zone, and the base of the seminal vesicles were submitted for microscopic examination. In addition, any gross lesion suspicious for cancer was completely blocked, sectioned and submitted in its entirety.

The specimens were evaluated for Gleason score, tumor volume, extraprostatic extension (EPE), seminal vesicle (SV) and lymph node (LN) involvement, and margin status. Tumor volume was categorized as either Low (< 0.5 cc), Medium (0.5- 2 cc) or Extensive (> 2 cc) depending on the sum of the areas of the two largest cancer foci. This method of visual estimation has been previously described and shown to have excellent correlation with actual tumor volumes as measured by computer-assisted image analysis.¹²

Statistical Analysis—Prostate tumor aggressiveness was assessed by three methods: age of disease onset, tumor characteristics at diagnosis, and pathologic analysis of the RP specimen. Age of disease onset was defined as the time of tumor diagnosis. Tumor characteristics at diagnosis included biopsy Gleason score, 1997 AJCC clinical T stage, and PSA level. Tumor aggressiveness at presentation was also analyzed by categorizing patients into low, intermediate and high risk groups, based on biopsy Gleason score, PSA and clinical T stage. Low risk was defined as Gleason score ≤ 6 , PSA ≤ 10 ng/mL, and clinical stage T1c or T2a; intermediate risk was defined as any Gleason score 7, PSA 10.1- 20.0 ng/ml, or clinical stage T2b or c; and high risk was defined as Gleason score ≥ 8 , PSA > 20.0 ng/ml, or clinical stage T3. Pathological characteristics included surgical Gleason score, tumor volume, extraprostatic extension (EPE), seminal vesicle invasion and lymph node status. Tumor confinement was compared and defined as non-organ confined if there was any EPE, seminal vesicle invasion, or lymph node metastasis. *RNASEL* genotype was characterized as homozygous wild type (RR), heterozygous (RQ), or homozygous for the R462Q variant (QQ).

Differences in age of onset between genotypes were analyzed by use of the Kaplan-Meier method using the log-rank statistic. Odds ratios and 95% confidence intervals were calculated for all nominal variables using RR as the reference group; standard deviations were calculated for all continuous variables. Differences in tumor aggressiveness were analyzed by chi-squared tests between the genotype groups. Age was controlled for at a set point of 60 years of age at disease onset and also as a continuous variable using linear regression models. Genotype distribution was compared to Hardy-Weinberg equilibrium. P-values < 0.05 were considered significant. This study has a power of 93% to identify a 25% difference in pathologic characteristics. All statistical analyses were performed using JMP 5.1 software package.

Results

Of the 232 men studied, 104 (45%) were homozygous wild-type (RR), 101 (43%) were heterozygous (RQ), and 27 (12%) were homozygous for the R462Q variant (QQ) (Table 1), mirroring the distribution seen in the general population and in Hardy-Weinberg equilibrium ($p=0.95$).³ The distribution of ethnicities was not equal to that of the general population

(Table 1), likely reflecting referral biases in this surgical population. Median age of disease onset for the entire cohort was 58 years and 11 months (range: 39y7m – 73y2m). A positive family history was reported by 53 men (22.3%), evenly distributed across genotypes (Table 1). Although younger age of onset predominated in the QQ genotype, there were no statistically significant differences observed between groups (log-rank $p=0.6$, Table 1).

Comparisons of pretreatment measures of tumor aggressiveness revealed no differences between genotypes. Median biopsy Gleason score was the same for all genotypes (6, $p=0.7$); the majority of patients with each genotype presented with clinical stage T1c (81.5% for QQ, 84.2% for RQ, 82.7% for RR, $p=0.6$); and mean PSA levels at diagnosis were 7.30 (range 0.68-31.0 ng/ml) for QQ, 5.79 (0.85-20.0 ng/ml) for RQ, and 6.72 (0.82-24.9 ng/ml) for RR ($p=0.1$). When categorized as low, intermediate, and high risk groups, there were no statistically significant differences between genotypes ($p=0.9$, Table 2). This result also held when low vs. intermediate plus high risk was examined ($p=0.7$).

Comparison of tumor aggressiveness determined by pathological characteristics of radical prostatectomy specimens demonstrated some differences among genotypes, but did not indicate a pattern of tumor aggressiveness associated with a specific genotype. Gleason score based on surgical specimens was similar for all genotypes (median = 7, $p=0.8$). Comparison of tumor volumes by genotype were significantly different because of a slight preponderance of low volume tumors for QQ patients ($p=0.02$, Table 2); this difference disappeared when QQ was compared to RQ + RR ($p=0.8$). Seminal vesicle involvement and lymph node metastases demonstrated no significant differences among genotype groups ($p=0.1$ and $p=0.7$, respectively). Rates of organ-confined disease were not significantly different among genotypes ($p=0.6$, Table 2).

When patients were stratified into two age groups (≤ 60 years old at age of disease onset and >60) and all analyses were repeated, no significant differences ($p>0.05$) were noted between genotypes. When age was controlled as a continuous variable, using linear regression modeling, no significant differences ($p>0.05$) were noted between genotypes in any of the clinical or pathologic parameters.

Discussion

Several prior studies have suggested that *HPCI*-associated prostate cancers present at an earlier age^{7,8} and with more aggressive clinical features.^{9,10,11} In a retrospective case series, Gronberg et al. reported higher grade tumors and more advanced stage at the time of diagnosis in 33 *HPCI*-linked families compared to 44 unlinked families with hereditary prostate cancer defined by early age of onset, multiple affected family members, or affected men in 3 generations.⁹ In a retrospective study, Goode et al reported higher rates of aggressive disease defined as either Gleason score ≥ 7 or advanced clinical stage (Whitmore-Jewett Stage C or D) in a subset of 505 affected men linked to *HPCI*.¹⁰ In a case-control study, Rennert et al. reported a mixed association of disease severity and *HPCI* linkage by family history and race, suggesting that advanced clinical stage was linked to the R462Q variant in African Americans with a positive family history. None of these studies

assessed disease severity by commonly used clinical groupings and none included detailed pathological analysis of radical prostatectomy specimens.

In the present study we assessed the aggressiveness of prostate tumors among all genotypes of the R462Q variant of *RNASEL* in a prospective series of unselected men undergoing radical prostatectomy. Unlike prior studies, we observed that clinical characteristics at the time of diagnosis including tumor grade, PSA, T stage and clinical risk groupings did not significantly differ among genotypes. Younger age of onset predominated in the QQ phenotype (Table 1), but this difference was not statistically significant, perhaps owing to a small sample size. Our hypothesis that more aggressive pathological features would be observed in QQ homozygotes was not supported by measures of tumor grade, volume, and pathological stage, even when age of disease onset was controlled. These observations may be influenced by the fact that our study was performed later in the PSA era, where the effects of repeated screening are to identify disease at an earlier clinical and pathological stage than might have occurred in prior studies.¹³ It is possible that the QQ men in this study were screened more frequently or at an earlier age (as suggested by the observations on age of onset), resulting in tumors detected at an earlier stage and abrogating the effect of genotype. A propensity for those with a positive family history of prostate cancer to be screened more frequently¹⁴ is unlikely to account for our findings because the frequency of this attribute was equally distributed across genotypes. Another potential explanation, and a weakness of this study, is that we did not routinely genotype men presenting with more advanced disease or those who chose to be treated by other means, and it is possible that *HPC1* allelic variants that predispose to prostate cancer are overrepresented in that group. In addition we did not investigate the association between R462Q and clinical course; this work is ongoing and will require longer follow-up before mature results are obtained.

Although the QQ genotype leads to impaired RNase L function, the pathophysiology of *HPC1*-associated cancers is not fully understood.¹⁵ RNase L is the terminal enzyme of the 2-5A system, an RNA degradation pathway that plays an important role in mediating the biologic effects of interferons (IFNs) in response to viral infection. Type I IFNs induce a family of 2-5A synthetases that are activated by dsRNA resulting in the conversion of ATP to a series of short 2'5' linked oligoadenylates (2-5A). These bind with high affinity to RNase L, converting it from its inactive form as a monomer to a potent dimer which degrades single stranded RNA, preventing viral replication, interfering with protein synthesis, and causing caspase-mediated apoptosis.¹⁵ Decreased RNase L activity due to the R462Q variant has been postulated to allow tumor initiation because of deficient apoptosis of mutated cells.^{3,6,15} Another potential pathophysiologic mechanism linked to this variant is impaired antiviral defense, a hypothesis that led to the discovery of the novel retrovirus Xenotropic Murine Leukemia-related Virus (XMRV) in patients homozygous for R462Q.^{16,17} XMRV may be one of a variety of infectious agents that result in chronic inflammation that has been postulated to underlie the development of prostate cancer.¹⁸ While we have not yet completed screening for XMRV of all of the tumors included in this study, of the 11 XMRV-positive cases included 9 were associated with the QQ phenotype (and 1 each with RR and RQ), and all but one was organ-confined on histologic analysis of

the radical prostatectomy specimen. What role, if any, that XMRV plays in tumor initiation, promotion, or maintenance remains to be elucidated.

Conclusions

In conclusion, the R462Q allelic variant of *HPC1/RNASEL* is not associated with tumor aggressiveness at disease presentation as assessed by an unselected series of men undergoing radical prostatectomy for prostate cancer. Additional work is needed to understand the relationship of this genotype to the pathogenesis of prostate cancer.

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Table 1

Pre-treatment Characteristics by Genotype

Genotype	RR		RQ		QQ	
	N	(%)	N	(%)	N	(%)
	104	(44.8)	101	(43.5)	27	(11.6)
Race						
Caucasian	86	(82.7)	97	(96.1)	27	(100)
African American	12	(11.5)	0	(0)	0	(0)
Other	6	(5.8)	4	(.9)	0	(0)
*OR (95% CI)	1.0 (ref. group)		5.08 (1.65-15.58)		N/A	
Positive Family History**						
	25	(24.0)	22	(21.8)	6	(22.2)
OR (95% CI)	1.0 (ref. group)		0.88 (0.46-1.69)		0.90 (0.33-2.49)	
Age at disease onset†						
Median	58 years 11 months		59 years 4 months		58 years 0 months	
Range	(43y6m-73y2m)		(43y7m-73y1m)		(38y7m-69y4m)	

* OR = likelihood Caucasian

** p = 0.9, chi-square

† p = 0.6, log rank

Table 2
Measures of Disease Aggressiveness by Genotype

	Genotype			P Value
	RR (n=104)	RQ (n=101)	QQ (n=27)	
Clinical Characteristics				
Biopsy Gleason score Median	6	6	6	0.7
OR (95% CI)	1.0 (ref. group)	1.04 (0.59-1.86)	1.26 (0.50-3.15)	
Tumor Stage Median	T1c	T1c	T1c	0.6
OR (95% CI)	1.0 (ref.group)	1.11 (0.53-2.32)	0.92 (0.31-2.76)	
PSA level at diagnosis Mean (SD)	6.72 (4.10)	5.79 (3.00)	7.30 (6.91)	0.1
Risk Group				0.9
% Low	58	61	67	
OR (95% CI)	1.0 (ref. group)	1.17 (0.67-2.04)	1.47 (0.60-3.57)	
% Intermediate	36	29	30	
OR (95% CI)	1.0 (ref. group)	0.73 (0.40-1.31)	0.76 (0.30-1.91)	
% High	7	6	4	
OR (95% CI)	1.0 (ref. group)	0.88 (0.28-2.70)	0.53 (0.06-4.53)	
Pathologic Characteristics				
Surgical Gleason Score Median	7	7	7	0.8
OR (95% CI)	1.0 (ref. group)	0.75 (0.42-1.35)	0.92 (0.35-2.40)	
Tumor volume				0.02
Low	19	19	8	
OR (95% CI)	1.0 (ref. group)	1.04 (0.51-2.10)	1.88 (0.72-4.94)	
Medium	43	64	10	
OR (95% CI)	1.0 (ref. group)	2.45 (1.40-4.31)	0.83 (0.35-2.00)	
Extensive	41	18	9	
OR (95% CI)	1.0 (ref. group)	0.33 (0.18-0.63)	0.77 (0.32-1.87)	
Disease Confinement				0.6
Organ Confined	55	56	19	
OR (95% CI)	1.0 (ref. group)	1.11 (0.64-1.92)	2.12 (0.85-5.26)	
Non-organ confined	49	45	8	
OR (95% CI)	1.0 (ref. group)	0.90 (0.52-1.56)	0.47 (0.19-1.18)	