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Analysis of the *RNASEL/HPC1*, and *Macrophage Scavenger Receptor 1* in Asian-Indian Advanced Prostate Cancer

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Abstract

Objectives—Prostate cancer (PC) varies widely by geographic location and ethnicity. American men have a high PC risk but most have localized disease. In contrast, Asian Indians have a low PC risk but most are diagnosed with metastatic disease. Epidemiological and genetic data suggest an important role of genetic susceptibility in PC. Most studies were performed in whites. Substantially less is known about gene variation–associated PC in low-risk populations. The objective of this study was to investigate the role of *RNASEL* and *MSR1* in Asian-Indian men with advanced PC.

Methods—We genotyped DNA samples obtained from 113 cases and 245 age-matched controls (Northern India).

Results—For *RNASEL*, we identified 8 variants (7 novel and 1 previously published, D541E), including 4 exonic, 3 intronic, and 1 change in the 3′-noncoding region. Of these, we detected a novel 4-bp truncation mutation (Val51ArgfsX2) in 2 controls. For *MSR1*, we identified 4 novel variants (2 intronic and 2 exonic) and 2 previously reported variants (P275A and promoter −4,637 A>G). We also genotyped 3 common *MSR1* variations (promoter −14,742 A>G, IVS5-59 C>A, and IVS7 delinsTTA). We found no associations among any of the sequence variations and PC. Three major haplotypes account for most of all *MSR1* haplotypes in Asian Indians. Haplotype frequencies were not significantly different between cases and controls.

Conclusions—Our results do not support a role for *RNASEL*, or *MSR1* mutations in advanced Asian-Indian PC. This study warrants additional investigations of these genes in etiology particularly among individuals from diverse ethnic and geographic groups.

Prostate cancer is the second leading cause of cancer deaths in Americans.[1] In 2007, an estimated 218,890 men will be newly diagnosed and 27,050 American men will die from prostate cancer.[2] Most of deaths from prostate cancer are related to hormone-refractory disease which occurs when hormone therapy fails to stop the growth and spread of prostate cancer.[3] Prostate cancer varies widely by geographic location and ethnicity, and is more common in black men (age-adjusted incidence of 137 per 100,000) in whom it tends to be more aggressive.[4,5] In contrast, this rate is strikingly low in native Asians (2.3 to 9.0 per 100,000).[1]

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Prostate cancer is a complex and multifactorial disease likely to be caused by combination of environmental and genetic factors.[6] Family-based studies have yielded numerous prostate cancer susceptibility genes, including *ELAC2/HPC2*,[7] 2′-5′-oligoadenylate-dependent RNase L (*RNASEL/HPC1*),[8] and macrophage scavenger receptor 1 (*MSR1*).[9] Two of these genes, *RNASEL* and *MSR1*, have been shown to have a major role in inflammation and innate immunity. *RNASEL* is a constitutively expressed latent endonuclease involved in the antiviral and pro-apoptotic activities of the interferon-inducible 2-5A system[10,11] linked to the first reported hereditary prostate cancer (*HPC1*) locus at 1q24-25 in 2002.[8] Several gene variations (G265X, 471_474delAAAG, and R462Q) were reported to be associated with the disease,[12–14] whereas functional studies demonstrated loss of the normal allele in prostate tumors from patients carrying germ line mutations,[8,13] and reduced RNase L activity.[5] *MSR1* (8p22) encodes a homotrimeric class A macrophage scavenger receptor that is able to bind chemically modified molecules ranging from bacteria to modified lipoproteins,[15] and several common and rare missense variants in this gene were observed to be associated with the disease.[2,9]

However, confirmation of these results has been difficult with only weak consensus among studies. Our group evaluated the role of putative susceptibility alleles within these genes in prostate cancer, and although we found no significant association with prostate cancer risk overall, we observed certain effects for *MSR1* IVS7delinsTTA and *RNASEL* R462Q when stratified by race, family history, or disease severity.[16] These conflicting associations may be explained by genetic heterogeneity of prostate cancer, incomplete penetrance, or environmental factors. An alternate reason may be inclusion of subjects with clinically insignificant tumors, diagnosed based on prostate-specific antigen (PSA) screening, decreasing the likelihood of identifying meaningful genetic associations.

The incidence of prostate cancer in India is very low.[17] However, in the absence of screening practices for prostate cancer, most patients are diagnosed at an advanced stage. Therefore, an attempt was made to study the role of *RNASEL* and *MSR1* in the low-risk Indian-Asian population. We genotyped DNA samples from 113 sporadic cases with advanced prostate cancer and 245 age-matched controls from North India.

Material and Methods

Study Population and Data Collection

We recruited study participants through the Sanjay Gandhi Post Graduate Institute of Medical Sciences (SGPGI) Hospital, Lucknow, India, between 2003 and 2005 as part of an ongoing prostate cancer case-control study. We obtained case status and clinical information by reviewing medical records. Prostate cancer was diagnosed primarily based on lower urinary tract symptoms, clinical impression of the prostate upon digital rectal examination, and high PSA level. We excluded patients who had a prior diagnosis of cancer at any other site. We obtained confirmation of diagnosis either from prostate biopsy or pathologic examination of a prostatectomy specimen. The median age at diagnosis for the patients was 64.8 years (range, 46 to 86 years). Median Gleason score at diagnosis was 8.0. A total of 28% of patients were diagnosed with low-grade (<7) and 72% with high-grade (≥7) disease. Ninety-nine percent of cases had late-stage (T3/T4) disease and 72% had bone metastases at diagnosis. Median PSA level was 26.6 ng/mL. Controls (N = 245), seen for routine checkups and other acute illnesses, were ascertained concurrently with the cases and were matched for age. Controls were specifically screened for prostate cancer by PSA tests, and were excluded from the study if they had an elevated PSA (≥4 ng/mL), abnormal digital rectal examination, or previous cancer diagnosis. The median age for the controls at the time of ascertainment was 63.2 years (range, 44 to 85 years). This study has Institutional Review Board approval at SGPGI and Cornell.

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PCR Analysis and Mutation Screening

We extracted DNA from blood samples using standard methods. All coding sequences, exon-intron boundaries, and 5′- and 3′-untranslated regions of *RNASEL* and *MSR1* were amplified using gene-specific primers. PCR was performed in a final volume of 25 μL containing, 20 ng genomic DNA, 200 μM of each dNTP, and 0.2 μM of each primer (Sigma-Genosys, St. Louis, MO) and 1 U TaqGold (ABI, Foster City, Calif) using an ABI 9700 thermacycler (primers sequence and PCR conditions are available upon request). We analyzed DNA alterations using DHPLC on a WAVE Fragment Analysis System (Transgenomic, Cambridge, Conn) as previously described.[13] PCR products demonstrating aberrant melting profile were sequenced using the Big Dye Terminator Chemistry (ABI) and an ABI 3100 Genetic Analyzer. *RNASEL* and *MSR1* common SNPs were further genotyped using Assay-by-Design Taqman primers/probes assays (ABI) and the ABI PRISM 7900 HT Sequence Detection System as previously described.[16] We computed allele frequencies, Hardy Weinberg Equilibrium (HWE), and haplotype estimates in STATA v. 8.01 using standard approaches (STATA, College Station, TX).

Results

Allelic Distribution

Tables 1 and 2 summarize genetic analyses results on *RNASEL* and *MSR1* in Asian-Indian men. For *RNASEL*, we identified a total of 7 novel sequence variants (3 exonic and 4 intronic) and 1 previously reported common exonic variant. One of these variants is a novel, 4-base pair insertion/duplication mutation, 147_150dupCAAT, detected in two control individuals. The mutation at codon 51 in exon 2 results in premature truncation in codon 53 (Val51GlnfsX2). This mutation was not present in prostate cancer patients, and individuals homozygous for the mutation were not identified. For *RNASEL*, the most common alteration was D541E, which was found in approximately 70% of the cases and controls. The previously reported common variant R462Q was not observed in this sample of Asian-Indian men.

For *MSR1*, we identified a total of 4 novel variants (2 intronic and 2 exonic) and 2 previously reported variants (−14,637 A>G at position −33 of the 5′-uncoding region, and the common P275A exonic change). In addition, we examined three previously reported common non-coding sequence variants, promoter −14,742 A>G, IVS5-59 C>A, and a 3-bp deletion/insertion of TTA in intron 7 (delinsTTA). Tables 1 and 2 list the location and frequency of each of the sequence variants. For the common variants, allele frequencies ranged from 3% to 18%. The remainder previously unreported variants in *RNASEL* and *MSR1* were relatively rare, with allele frequencies of 1% or less. Overall there were no significant differences in allele frequencies between cases and controls. Allele frequencies for all sequence variants were in Hardy-Weinberg proportions except for I181K, which demonstrated a nonsignificant trend towards higher 181K homozygosity in cases compared with controls.

Haplotype Analysis

Haplotype analysis of the three most common sequence variants in *MSR1* using the EM algorithm estimated that three major haplotypes account for approximately 90% of all haplotypes in Asian Indians (Table 3). The haplotypes frequencies in controls were not significantly different from the haplotype frequencies in cases.

Discussion

We comprehensively analyzed the distribution of mutations and sequence variants of *RNASEL* and *MSR1* and evaluated the relationship of the most common gene variations with prostate cancer risk in a sample of Asian-Indian study subjects from North India, with advanced

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disease. Our findings demonstrate that there are significant differences in the spectrum of mutations and sequence variants in *MSR1* and *RNASEL* in this group compared with previous reports of the frequency of these alleles in non-Asian populations.[18,19] Our results also show that common alleles are more likely to be present in all three groups of Asian Indians, European Americans, and blacks, whereas rare alleles are more commonly detected in a single racial group. Before this report, *RNASEL* and *MSR1* genes were not substantially studied outside Western populations. Thus, to our knowledge, our research is the first to investigate the role of these genes in prostate cancer risk, grouping the lowest risk racial group with unambiguously significant disease for prostate cancer.

For *RNASEL*, we identified a total of 8 variants, 7 of which have not been previously reported. One of these variants was a novel 4-base pair truncating mutation, 147_150dupCAAT, detected solely in two controls. Their ages at the time of study ascertainment were 68 and 72 years, respectively. In *RNASEL*, the only previously published sequence variant found was D541E, which was present in approximately 75% of the cases and controls. The allele frequency we observed in our Asian-Indian control population for the *RNASEL* D541E common polymorphisms (48%) was similar to the previously reported frequency for European Americans (44%) in the United States (Table 1).[19,20] Interestingly, the *RNASEL* R462Q was not observed at all among Asian-Indian men, and therefore is unlikely to explain prostate cancer in this group, unless a significant inverse association with disease was conferred by the Arg462 allele.

For *MSR1*, we identified a total of 6 common variants, 4 of which were previously reported by Xu *et al.*,[21] and 3 rare sequence variants. The −14,637 A>G (5′UTR −33 A>G) was previously observed in a single Swedish family with hereditary prostate cancer.[23] This variation was present in approximately 3% of our study population and was not associated with prostate cancer risk. Of the 4 unpublished variations, 2 were in intronic changes and 2 were in exons. Of this, I181K missense change was relatively common in our sample population with an allele frequency of 3% and 4% among cases and controls, respectively. Allele frequencies for common variants in *MSR1* (−14,742 G>A, IVS5-59 C>A, and IVS7 delinsTTA) varied slightly from the frequencies reported for European-American and black men in other studies in the United States,[22,24] whereas P275A allele frequency in Asian Indians was significantly higher (18%) compared with the frequency observed in blacks (7%)[24] and European Americans (6%).[21,22] This discrepancy in allele frequencies may be due to heterogeneity between the populations studied, or small sample sizes used to estimate these frequencies in some studies. In the present study, allele frequencies for additional 10 very rare alleles in, *RNASEL* and *MSR1* ranged from 0% to 1%.

In the present study we examined 358 prostate cancer cases and controls. Screening for prostate cancer (e.g., PSA determination or digital rectal examination) is extremely rare in India. Therefore, the Indian series represent an unselected, unscreened series of men who were diagnosed with prostate cancer. The present study did not identify an association between the common *RNASEL* D541E variant or *MSR1* common sequence variants and prostate cancer risk. These results are in agreement with our previous data and most previous reports[16,18,20,21] that observed no significant association between patients and controls for *MSR1* or *RNASEL* common variants. However, one comprehensive meta-analysis of *RNASEL* common polymorphisms found a small effect (less than 1.5-fold) of D541E variant on prostate cancer risk in whites only.[20] Meta analysis for *MSR1* common variants, however, using data from all eight published studies revealed no evidence for an association of *MSR1* common variants with prostate cancer risk in either whites or blacks.[18] Although no significant association has been found with prostate cancer risk overall in European or African Americans, certain effects for *MSR1* IVS7 delinsTTA and *RNASEL* R462Q were observed when stratified by race, family history, or disease severity.[16] Association studies stratified by disease clinico-pathologic

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characteristics failed to detect any effect of the common SNPs examined in this study (data not shown); however this can also be due to the small sample size and lack of power to detect significant effects.

Haplotype analysis of the five common SNPs in *MSR1* in Asian Indians provides no support for *MSR1* as a risk factor for prostate cancer. We could not test for an association between disease genotype and disease stage because all our cases except for one had advanced-stage T3/T4 disease. Statistical tests for an association between haplotypes and prostate cancer risk by disease grade were all insignificant, although prostate cancer individuals harboring the P275A and IVS7 delinsTTA haplotypes tended to have less aggressive disease and no metastasis (Table 3).

Several strengths and limitations should be considered when interpreting the results presented here. We recruited both prostate cancer cases and control study subjects according to standard selection criteria in a single health system that draws from a wide geographic area in North India. Consistent medical records obtained from this health system also allowed consistent diagnostic data from cases, and systematic evaluation of prostate cancer absence in controls. Moreover, the availability of study subjects with clinically significant tumors allowed evaluation of these genes in a relatively homogeneous disease subset, enhancing the probability of identifying significant genetic associations. Another strength of our approach was the use of gene scanning as well as automated genotyping techniques for uncovering all existing variations in *RNASEL* and *MSR1* in this population, ensuring a high call rate and accuracy. As noted above, a primary limitation of the present study was the low statistical power to detect effects of small magnitude.

In summary, we have characterized the spectrum of sequence variations in *RNASEL* and *MSR1* in Asian Indians, and report novel variants including a deleterious truncating mutation, 147_150dupCAAT, in *RNASEL*. Most of these mutations are rare and we were not able to provide evidence that they have a role in prostate cancer in Asian Indians. Analysis results for the common SNPs, both individually and through haplotypes, provided no evidence for the association of *MSR1* or *RNASEL* with prostate cancer in Asian Indians. However, the present results provide information about the spectrum of *MSR1* and *RNASEL* variants in a previously unstudied, low-risk population.

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Table 1

Allele frequencies in candidate prostate cancer genes

Gene	Nucleotide Sequence Variant	Amino Acid Change	Allele Frequency (Total No. of Individuals)					
			Asian-Indian		Black[§]		European-American[§]	
			Controls	Cases	Controls	Cases	Controls	Cases
MSR1	−14,742 A>G	None	0.120 (230)	0.107 (112)	0.269 (130)[‡]	0.253 (93)	0.105 (343)	0.105 (530)
	−14,637 A>G	None	0.026 (227)	0.036 (112)	—	—	—	—
	IVS5 −59C>A	None	0.086 (234)	0.054 (111)	0.012 (123)[‡]	0.006 (85)	0.059 (314)	0.050 (484)
	IVS7 delinsTTA	None	0.174 (233)	0.183 (103)	0.264 (110)[‡]	0.218 (78)	0.057 (281)[‡]	0.045 (447)
	823 C>G	P275A	0.175 (240)	0.180 (111)	0.07 (333)[/]	0.05 (131)	0.06 (488)[¶]	0.06 (496)[¶]
	542 T>A[†]	I181K	0.039 (244)	0.040[*] (112)	—	—	—	—
RNASEL	1385G>A	R462Q	—	—	0.119 (126)	0.159 (88)	0.361 (321)	0.370 (505)
	1623 T>G	D541E	0.477 (242)	0.535 (113)	NT	NT	0.44 (508)[**]	0.45 (929)[**]

* Deviations from Hardy-Weinberg proportions based on χ^2.

† Previously unpublished missense variant.

‡ Significant difference in frequency compared with Asian-Indian controls.

§ Allele frequencies are based on Rennert *et al.*[15] unless indicated otherwise:

/ Miller *et al.*,[24]

¶ Wang *et al.*,[22]

** Wang *et al*,[19] Li and Ti[20].

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Table 2

Prevalence of rare *RNASEL* and *MSR1* sequence variants in prostate cancer patients and controls

Gene	Sequence Variant (Base Pair)	Amino Acid Change	Controls (N = 245) [No. (%)]	Cases (N = 113) [No. (%)]
MSR1	171 C>T	Y57Y	—	1
	IVS2-10 A>G	None	5 (2%)	3 (3%)
	IVS7+35 G>A	None	—	2 (2%)
RNASEL	147_159dupCAAT	V51QfsX2	2 (1%)	—
	1078 A>G	K360E	1 (1%)	—
	1260 C>T	F420F	1 (1%)	—
	IVS1+59 A>G		4 (2%)	3 (3%)
	IVS4+77 T>C		3 (1%)	1 (1%)
	IVS5-27 T>C		2 (1%)	—
	3′UTR2272 G>A		1 (1%)	1

* Deviates significantly from HWE (P <0.05) None deviate from HWE.

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Table 3

MSR1 haplotype frequencies by case status, tumor grade, and metastasis

Haplotype	Controls (N = 245)	Cases (N = 113)	Grade <7 (N = 32)	Grade ≥7 (N = 81)	No Mets (N = 32)	Mets (N = 81)
1.1.1.1.1	0.649	0.657	0.580	0.684	0.626	0.675
1.1.1.1.2	0.020	0.023	0.032	0.020	0.049	0.013
1.1.1.2.1	0.037	0.040	0.031	0.044	0.031	0.044
1.1.2.1.1	0.019	0.028	0.016	0.032	0.033	0.025
1.1.2.1.2	0.156	0.145	0.185	0.133	0.182	0.124
2.1.1.1.1	0.034	0.046	0.109	0.023	0.044	0.046
2.1.1.1.2	—	—	—	—	—	—
2.2.1.1.1	0.084	0.051	0.045	0.054	0.031	0.053

Haplotype key: allele 1: promoter −14,742 A>G (haplotype codes: 1 = A, 2 = G); allele 2: intron 5 C>A (haplotype codes: 1 = C, 2 = A); allele 3: intron7 delinsTTA (haplotype codes: 1 = del, 2 = TTA ins); allele 4: MSR1 542 T>A (haplotype codes: 1 = T, 2 = A); allele 5: MSR1 823 C>G (haplotype codes: 1 = C, 2 = G).