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Report

Common Sequence Variants of the Macrophage Scavenger Receptor 1 Gene Are Associated with Prostate Cancer Risk

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Rare germline mutations of macrophage scavenger receptor 1 (*MSR1*) gene were reported to be associated with prostate cancer risk in families with hereditary prostate cancer (HPC) and in patients with non-HPC (Xu et al. 2002). To further evaluate the role of *MSR1* in prostate cancer susceptibility, at Johns Hopkins Hospital, we studied five common variants of *MSR1* in 301 patients with non-HPC who underwent prostate cancer treatment and in 250 control subjects who participated in prostate cancer–screening programs and had normal digital rectal examination and PSA levels (<4 ng/ml). Significantly different allele frequencies between case subjects and control subjects were observed for each of the five variants (*P* value range .01–.04). Haplotype analyses provided consistent findings, with a significant difference in the haplotype frequencies from a global score test ($P = .01$). Because the haplotype that is associated with the increased risk for prostate cancer did not harbor any of the known rare mutations, it appears that the observed association of common variants and prostate cancer risk are independent of the effect of the known rare mutations. These results consistently suggest that *MSR1* may play an important role in prostate carcinogenesis.

The macrophage scavenger receptor 1 (*MSR1* [MIM 153622]) gene was recently identified as a candidate gene for prostate cancer susceptibility, from a systematic search for prostate cancer (MIM 176807) genes at 8p, using multiple approaches such as linkage, direct sequencing, and association studies (Xu et al. 2002). Analysis of the *MSR1* gene sequence in members of families with hereditary prostate cancer (HPC) identified six rare missense mutations (Pro36Ala, Ser41Tyr, Val113Ala, Asp174Tyr, Gly369Ser, and His441Arg) and one nonsense mutation (Arg293X). A family-based linkage and association test provided statistical evidence that these mutations cosegregate with prostate cancer ($P = .0007$). Further examination of these mutations in a collection of patients with non-HPC and unaffected men revealed that they were either not observed or were observed less frequently in men without prostate cancer. For white men, the rare *MSR1* mutations were detected in 4.4% of cases, compared to 0.8% in unaffected men ($P = .009$); for African American men, these values were 12.50% and 1.82%, respectively ($P = .01$). These results provide genetic evidence that *MSR1* may play an important role in prostate cancer susceptibility in both African American men and men of European descent.

The MSR1 protein, a Class A scavenger receptor, is a multidomain trimeric molecule composed of identical protein chains. It has two functional isoforms (Type I and Type II) and one nonfunctional isoform (Type III), generated by alternative splicing of a single 11-exon mRNA (Kodama et al. 1990; Emi et al. 1993). This macrophage-specific receptor is capable of binding a highly diverse array of polyanionic ligands, ranging from gram negative and positive bacteria and oxidized LDL

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Table 1

Pairwise Linkage Disequilibrium (Correlation Coefficient) in Patients With Prostate Cancer and in Unaffected Control Subjects

	PRO3	INDEL1	IVS5-59	P275A	INDEL7
PRO3		.99[a]	.59[a]	.03	.04
INDEL1	.98[a]		.58[a]	.03	.04
IVS-59	.69[a]	.71[a]		.08	.08
P275A	.06	.05	.06		.86[a]
INDEL7	.03	.02	.07	.73[a]	

NOTE.—Estimates in the upper right are for control subjects and in the lower left are for case subjects.

[a] Indicates $P < .00001$, which was based on the permutation tests of exact test statistics using GDA.

to silica, and, correspondingly, has been linked to a wide variety of normal and pathological processes, including inflammation, innate and adaptive immunity, oxidative stress, and apoptosis (Platt and Gordon 2001). Although the exact role of *MSR1* in prostate carcinogenesis is unknown, some or all of these processes have been implicated in the development of prostate cancer (De Marzo et al. 1999; Nelson et al. 2001). Recent findings showing that the degree of macrophage infiltration is associated with prostate cancer prognosis strengthen the link between *MSR1* and prostate cancer (Lissbrant et al. 2000; Shimura et al. 2000).

Besides the seven rare *MSR1* mutations we reported elsewhere (Xu et al. 2002), we identified four additional common sequence variants (>10%) after sequencing the PCR products of all 11 exons, exon-intron junctions, promoter region, and 5′ and 3′ UTRs of *MSR1* in the germline DNA of probands from each of the 159 families with HPC (i.e., at least three first-degree relatives affected with prostate cancer). Together with the common missense change we identified elsewhere (Xu et al. 2002), there are five common sequence variants in our sequenced regions.

To evaluate the role of *MSR1* sequence variants in prostate cancer susceptibility as it relates to the general population, we focused this study on the common sequence variants and patients with prostate cancer who do not meet criteria for HPC. Because of the limited number of African Americans in our study, all the following analyses were restricted to men of European descent. Five common sequence variants were genotyped in 301 patients with prostate cancer and in 250 unaffected control subjects. The case subjects were recruited from the patients who underwent prostate cancer treatment at Johns Hopkins Hospital and did not meet the criteria for HPC ($N = 317$). The diagnosis of prostate cancer was confirmed by pathology reports, and the mean age at diagnosis was 59.3 years. Sixteen patients were excluded from the current study because of insufficient DNA. The control subjects were recruited from volunteers participating in prostate cancer–screening programs at Johns Hopkins Hospital who had a normal digital rectal examination and PSA (<4 ng/ml) ($N = 256$). The mean age at examination was 58.5 years. Six control subjects were excluded from the current study because of insufficient DNA. All subjects in this study gave full informed consent.

The five sequence variants genotyped in this study include an SNP in the promoter region (PRO3), a 15-bp insertion/deletion of "GAATGCTTTATTGTA" in intron 1 (INDEL1), an SNP in intron 5 (IVS5-59), a missense change in exon 6 (P275A), and a 3-bp insertion/deletion of "TTA" in intron 7 (INDEL7). The positions of these sequence variants are listed in table 2. Genotyping of the three SNPs (PRO3, IVS-59, and P275A) was performed using the MassARRAY system (SEQUENOM). Genotyping of the two insertion/deletions (INDEL1 and INDEL7) was performed using the 3700 DNA Analyzer (Applied Biosystems). The primer information and detailed PCR conditions for these sequence variants are available on the authors' Web site.

All five sequence variants were in Hardy-Weinberg equilibrium in control subjects, on the basis of 10,000 permutations of the Fisher probability test statistic (Weir 1996), as implemented in the software Genetic Data Anal-

Table 2

Frequencies of MSR1 Sequence Variants in Patients with Prostate Cancer and Unaffected Control Subjects

SNP, (POSITION),[a] AND GENOTYPE	NO. (%) OF SUBJECTS WITH GENOTYPE	
	Control Subjects	Case Subjects
PRO3 (-14,742):		
AA	214 (85.6)	235 (78.1)
AG	34 (13.6)	58 (19.3)
GG	2 (.8)	8 (2.6)
INDEL1[b] (-14,458):		
−/−	211 (85.4	233 (78.4)
-/+	33 (13.4)	58 (19.5)
+/+	3 (1.2)	6 (2.0)
IVS5-59 (22,788):		
CC	232 (92.8)	262 (87.3)
CA	18 (7.2)	34 (11.3)
AA	0 (.0)	4 (1.3)
P275A (22,850):		
CC	209 (83.6)	271 (90.3)
CG	38 (15.2)	28 (9.3)
GG	3 (1.2)	1 (.3)
INDEL7 (34,504):[c]		
−/−	204 (82.9)	264 (88.9)
−/+	41 (16.7)	33 (11.1)
+/+	1 (.4)	0 (.0)

[a] Positions (bp) are based on the initiation codon (ATG) from MSR1 genomic DNA (NT_015280).

[b] "+" and "−" denote with and without the 15-bp sequence "GAATGCTTTATTGTA," respectively.

[c] "+" and "−" denote with and without the 3-bp sequence "TTA," respectively.

Table 3

Allele Frequencies in Patients with Prostate Cancer and Unaffected Control Subjects

Allele	Allele Frequencies (%)		χ^2 Test for Allele (*P* Values)
	Control Subjects	Case Subjects	
PRO3 "G"	7.6	12.3	.01
INDEL1 "+"[a]	7.9	11.8	.04
IVS5-59 "A"	3.6	7.0	.02
P275A "C"	91.2	95.0	.01
INDEL7 "−"[b]	91.3	94.4	.04

[a] "+" denotes the presence of the 15-bp sequence "GAATGCTTTATTGTA."

[b] "−" denotes the absence of the 3-bp sequence "TTA."

ysis (GDA). The first three sequence variants (PRO3, INDEL1, and IVS5-59) were in strong linkage disequilibrium (LD), because the tests for pairwise LD among them were all highly significant (all $P < .00001$), again on the basis of 10,000 permutations of the exact test statistic (Weir 1996), as implemented in GDA. The last two variants also had strong LD between them ($P < .00001$). There was no LD between the blocks of the first three variants and the last two variants (all $P > .2$). The estimates of pairwise LD between all these sequence variants in case subjects and control subjects, as measured by correlation coefficients and implemented in SAS/Genetics, are presented in table 1.

The allele frequencies of the five sequence variants were all significantly different between case subjects and control subjects, on the basis of a χ^2 test for allele frequencies, with 1 df (table 2; table 3). Specifically, the frequency of allele "G" of PRO3 ($P = .01$), 15-bp insertion (+) of INDEL1 ($P = .04$), allele "A" of IVS5-59 ($P = .02$), allele "C" of P275A ($P = .01$), and deletion (−) of INDEL7 ($P = .04$), were higher in case subjects than in control subjects, respectively. To estimate the prostate cancer risk of these variants, we performed the tests by grouping three genotypes into two genotypes as shown in table 4. Except for the INDEL7, there were significant elevated risks for prostate cancer among the sequence variants, even when adjusted for age.

The haplotype analysis of these five sequence variants using the EM algorithm (Excoffier and Slatkin 1995) estimated that four major haplotypes account for >96% of all haplotypes (table 5). The haplotype frequencies were significantly different between case subjects and control subjects, with a *P* value of .011 from 10,000 simulations of global score tests, as implemented in haplo.score (Schaid et al. 2002). When specific haplotypes were examined, the haplotype "G (+) A C (−)" of these five variants (in the order of PRO3, INDEL1, IVS5-59, P275A, and INDEL7) had a significantly higher frequency in prostate cancer patients (6.6%) than in control subjects (2.6%), with a *P* value of .004 (on the basis of 10,000 simulations). It is worth noting that this haplotype did not harbor any of the rare mutations (Arg293X, His441Arg, Val113Ala, and Ile54Val) (Xu et al. 2002). For example, all eight occurrences of Arg293X and three occurrences of His441Arg resided on the haplotype "A (−) C C (−)." Therefore, it appears that the significant association between the common *MSR1* sequence variants and prostate cancer risk is independent of the impact of the known rare *MSR1* mutations.

Caution should be taken when interpreting these findings. Although the significant differences in allele and haplotype frequencies between patients with prostate cancer and unaffected control subjects could be due to the prostate cancer risk associated with these polymorphisms, it could also be due to other reasons, such as a type I error or population stratification. Regarding type I error, all the reported significance levels were nominal *P* values and were not adjusted for multiple comparisons. If we considered that at least 15 tests were performed in this report, and that the commonly suggested Bonferoni correction was used, none of the tests was significant at $P = .05$. However, the Bonferoni correction is not optimal in this case; not all of these tests were independent because of the LD between these polymorphisms and the dependence between allele and haplotype. Regarding population stratification, the results, as a case-control study, are always subject to this potential confounder: that is, the different genotype frequencies observed may partially reflect different genetic backgrounds in case subjects and control subjects. Although great attention was paid in the study design and analysis,

Table 4

Odds Ratio (OR) Estimates for Prostate Cancer, Adjusting for Age

Variant: Genotypes	OR (95% CI)
PRO3:	
AA	1.00
AG/GG	1.81 (1.15–2.85)
INDEL1:	
−/−	1.00
+/− or +/+	1.73 (1.10–2.72)
IVS-59:	
CC	1.00
CA/AA	1.93 (1.07–3.50)
P275A:	
CG/GG	1.00
CC	1.75 (1.05–2.94)
INDEL7:	
−/−	1.00
+/− or +/+	1.54 (.94–2.52)

Note.—OR estimates are adjusted for age.

Table 5

Haplotype Frequencies of MSR1 Sequence Variants in Patients with Prostate Cancer and Unaffected Control Subjects

	Frequency (95% CI)		*P* values (empirical)
Haplotype[a]	Control Subjects	Case Subjects	Haplotype Specific
A (−) C C (−)	83.6% (80.3–86.9)	81.8% (78.7–84.9)	.44
G (+) A C (−)	2.6% (1.2–4.0)	6.6% (4.6–8.6)	.004
A (−) C G (+)	6.5% (4.3–8.6)	4.0% (2.4–5.5)	.06
G (+) C C (−)	3.7% (2.1–5.4)	4.1% (2.5–5.7)	.77
Global			.011

[a] Haplotype of five SNPs (in the order of PRO3, INDEL1, IVS-59, P275A, and INDEL7)

including the restriction to subjects of European descent only, we cannot rule out the possibility of population stratification.

Each of these five sequence variants could have an important impact on MSR1 function. For example, the SNP in the promoter region and the 15-bp ins/del polymorphism could affect transcription of the *MSR1* gene. The missense change of Pro275Ala could affect the function of the MSR1 protein, because it changes a conserved residue in the first Gly-X-Y repeat of the collagenous domain of the protein. However, because all five sequence variants were associated with prostate cancer risk, and the risk haplotype "G (+) A C (−)" included all the risk alleles for each variant, it is difficult to dissect genetically which variant(s) are the most important changes. It is possible that one or more of these variants are associated with prostate cancer risk. Future functional analyses using various combinations of these variants may help to confirm these findings and provide insight into the function of each variant.

The results from this study and the study of *MSR1* rare mutations (Xu et al. 2002) suggest that rare mutations and common sequence variants of *MSR1* confer differential risks of prostate cancer. Although the rare *MSR1* mutations tend to impose relatively high risk of prostate cancer, common *MSR1* sequence variants within the same major genes tend to have a relatively low risk of prostate cancer. Similar observations were observed in two other major prostate cancer susceptibility genes, *HPC2/ELAC2* (MIM 605367) and *RNASEL* (MIM 180435) (Tavtigian et al. 2001; Carpten et al. 2002). For example, two rare mutations (E265X and M1I) of the ribonuclease L gene (RNASEL) were rare and observed mainly in families with HPC; another common sequence variant (Arg462Gln) was frequent in population and imposed intermediate risk to prostate cancer (Wang et al. 2002).

In summary, the significant differences in the allele and haplotype frequencies between patients with non-HPC and unaffected control subjects observed in this study suggest that common *MSR1* sequence variants are associated with prostate cancer risk in the general population. Together with evidence that the rare *MSR1* mutations are associated with increased prostate cancer susceptibility in patients with HPC and with non-HPC (Xu et al. 2002), the role of MSR1 and macrophages in prostate carcinogenesis is implicated. However, independent studies are extremely important to support these findings, given the complexity of prostate cancer.

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Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

Center for Human Genomics, http://www.wfubmc.edu/genomics/ (for authors' Web site)
GDA: Software for the Analysis of Discrete Genetic data, http://lewis.eeb.uconn.edu/lewishome/software.html
haplo.score, http://www.mayo.edu/statgen/software/
Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for prostate cancer [MIM 176807], *HPC2/ELAC2* [MIM 605367], *RNASEL* [MIM 180435], and *MSR1* [MIM 153622])

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