Germ-line Mutations of the Macrophage Scavenger Receptor 1 Gene: Association with Prostate Cancer Risk in African-American Men

David C. Miller, S. Lilly Zheng, Rodney L. Dunn, Aruna V. Sarma, James E. Montie, Ethan M. Lange, Deborah A. Meyers, Jianfeng Xu, and Kathleen A. Cooney

Abstract

Both rare germ-line mutations and common sequence variants of the macrophage scavenger receptor 1 (MSR1) gene have recently been implicated as potential prostate cancer susceptibility factors. However, existing studies are limited by the referral-based nature of samples and a paucity of African-American participants. In this context, we evaluated the association of germ-line mutations and common MSR1 sequence variants with prostate cancer risk in a case control study of a community-based sample of 134 African-American men with prostate cancer and 340 unaffected controls. In our sample, the rare Asp174Tyr missense change was identified nearly twice as frequently in men with prostate cancer (6.8%) compared with unaffected controls (3.6%; P = 0.14). Moreover, significantly different allele frequencies between cases and controls were observed for one of the sequence variants, IVS5–59 (P = 0.02). Taken together, our results provide some additional support for the hypothesis that selected, rare MSR1 mutations are associated with increased prostate cancer susceptibility among African-American men.

Introduction

There is a growing body of molecular and genetic epidemiological evidence that implicates the short arm of chromosome 8 (8p22–23) as the location of one or more genes important in the development of adenocarcinoma of the prostate (1, 2). Most recently, the MSR1 gene has been proposed as an etiologic link between germ-line alterations in 8p and prostate carcinogenesis (3, 4). Xu et al. identified several rare germ-line mutations of the MSR1 gene that cosegregated with prostate cancer among families affected with HPC. Moreover, at least one of the germ-line mutations was associated with an increased risk of sporadic prostate cancer among African-American men (4). In a subsequent study of men of European descent, the same authors examined five common sequence variants of MSR1 and reported significantly different allele frequencies for each of the five variants among men diagnosed with prostate cancer compared with unaffected controls. Notably, the association of the common sequence variants with prostate cancer risk was independent of the presence of rare germ-line mutations (3).

The composite results of these studies provide provocative data in support of MSR1 as a prostate cancer susceptibility gene. However, the generalizability of these findings is limited by a lack of African-American participants. Given that African-American men have both a higher incidence and mortality from prostate cancer compared with Caucasian men in the United States, characterization of genetic risk factors in this patient population is an important public health initiative, and further study of a potential role for MSR1 is warranted (5). The aim of this study is to further evaluate the association between genetic variation in the MSR1 gene and prostate cancer susceptibility among African-American men.

Materials and Methods

Subjects. Both cases and controls were recruited as part of the FMHS. Informed consent was obtained from each study participant, and all research protocols were approved by the Institutional Review Board at the University of Michigan Medical School. As described previously, disease-free controls, aged 40–79, were identified from a probability sample of African-American men in the city of Flint, Michigan or in neighboring Beecher Township (Genesee County; Ref. 6). A complete urological history and physical examination, including PSA testing, was performed to exclude the diagnosis of prostate cancer. Participating community urologists used the PSA values in conjunction with other clinical data to determine the need for biopsy; in general, a PSA value of >4 ng/ml indicated the need for biopsy. DNA was available for genetic sequencing for 345 unaffected men; however, the DNA was insufficient for 5 individuals. Thus, our final control sample consists of 340 disease-free African-American males.

Prostate cancer case recruitment from the same community was initiated in 1999 and completed in July 2002. Participation of cases required: (a) an epidemiological interview; (b) a review of the hospital and registry records for information on tumor stage, Gleason Score, prediagnosis PSA, and type of therapy; and (c) provision of a blood sample for DNA and freezeer storage of serum and plasma. After excluding two cases with insufficient DNA, our final case sample included 134 African-American men, aged 40–79, that had been diagnosed with prostate cancer between 1995 and 2002. For both cases and controls, genomic DNA was isolated from whole blood by the use of the Puregen kit (Genta Systems, Inc., Plymouth, MN).

Sequence Analysis. Five common sequence variants and five recently reported rare germ-line mutations were analyzed for 134 cases and 340 unaffected controls. The five rare mutations were identified during screening for sequence variants of MSR1 in germ-line DNA samples from individuals with HPC (4). Four are missense mutations (Ser41Tyr, Asp174Tyr, Gly294Glu, and Pro36Ala), and one is a nonsense change (Arg293X). The five common sequence variants genotyped have been described previously and include an SNP in the promoter sequence (PRO3), a 15-bp insertion/deletion variant in intron 1 (INDEL1), an SNP located in intron 5 (IVS5-59), a missense mutation in exon 6 (P275A), and a 3-bp insertion/deletion in intron 7 (INDEL7; Ref. 3). The method of identification and positions of the five sequence variants have been reported elsewhere (3).

Statistical Analysis. Bivariate comparisons of mutation and allele frequencies among cases and controls were carried with χ² analysis or Fisher’s exact test. Logistic regression models were used to test the association between common variants and disease status. These models were age adjusted to account for the possibility that some of the controls may later become diagnosed as cases. To avoid bias, age was calculated based on the same date for all cases and controls. This date was the most recent follow-up date from the
entire sample, with the exception that age at death was used for the 29 controls that died before this date. This age variable was inserted into the models as an independent covariate. All tests were performed at the 5% significance level and using the SAS System (Cary, NC).

Haplotype-based association studies and calculation of the marker–marker linkage disequilibrium measure D’ (7) were performed using the computer program Dandelion (Green, Langefeld, and Lange, unpublished software) following the methodology described in Mohlke et al. (8). Briefly, a series of likelihood ratio tests were performed comparing the haplotype frequencies between cases and controls, as estimated by the expectation–maximization algorithm, for two, three, four, and five adjacent marker haplotypes (9). Statistical significance was evaluated using a permutation test based on 1000 random permutations of affection status.

Results and Discussion

The mutation frequencies for five nonsynonymous germ-line mutations are summarized in Table 1. In the first report of rare MSR1 mutations and prostate cancer, the Asp174Tyr missense change was reported to occur with increased frequency among African-American men with apparent sporadic prostate cancer (4). In our community-based sample, the Asp174Tyr change was identified roughly twice as frequently in men with prostate cancer (6.8%) than in unaffected controls (3.6%; P = 0.14). In addition, two of 9 (22.2%) cases were homozygous for the missense change at this allele. Clinico-pathologic features for the 9 cases carrying this mutation are summarized in Table 2. Among the 8 patients with available clinical data, 6 (75%) had clinically localized disease at the time of diagnosis. However, 2 cases presented with metastatic disease, with serum PSA levels of 157.8 and 1160 ng/ml, respectively.

Xu et al. also reported that the Asp174Tyr missense change cosegregated with prostate cancer in African-American families affected with HPC. Although formal linkage studies were beyond the scope of this case-control study, it is intriguing that at least three (37.5%) carriers of the Asp174Tyr change reported a history of prostate cancer in a first-degree relative, including one man whose family history fulfills the criteria for HPC (two brothers diagnosed with prostate cancer; Ref. 10).

Given that prostate cancer is, in general, a late-onset disease with a long asymptomatic phase, it is also notable that three of the unaffected men carrying the Asp174Tyr mutation had serum PSA levels in excess of 4 ng/ml, and at least two other unaffected carriers have a history of prostate cancer in a first-degree relative (data not shown). Moreover, the mean age of unaffected men with the Asp174Tyr change was 54.2 years, and 6 (50%) of the individuals are ≥50 years of age. This clinical data raises the possibility that, for a number of men, insufficient time may have elapsed to allow phenotypic expression of the underlying genetic variation. Indeed, misclassification of only a few controls may contribute to the lack of statistical significance for the Asp174Tyr mutation in this study sample.

The relative frequencies of the common MSR1 sequence variants are compared for affected and unaffected men in Table 3. The relative genotype frequencies were similar for cases and controls for each of the common sequence variants with the exception of one nonsynonymous SNP in intron 5 (IVS5-59). For this SNP, heterozygosity (CA versus CC) was significantly more common among affected than unaffected men (P = 0.02). For each of the common sequence variants, the allele frequencies and age-adjusted prostate cancer ORs are summarized in Table 4. To estimate the prostate cancer risk

### Table 1: Rare MSR1 germ-line mutations in African-American men with prostate cancer and unaffected controls

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Prostate cancer cases (% (n = 134))</th>
<th>Unaffected men (% (n = 340))</th>
<th>Fisher’s exact test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ser41Tyr</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
<td>0.278</td>
</tr>
<tr>
<td>Asp174Tyr</td>
<td>9 (6.8)</td>
<td>12 (3.6)</td>
<td>0.143</td>
</tr>
<tr>
<td>Gly294Glu</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Pro366Ala</td>
<td>9 (7.0)</td>
<td>27 (8.1)</td>
<td>0.847</td>
</tr>
<tr>
<td>Arg292X</td>
<td>1 (0.8)</td>
<td>0 (0)</td>
<td>0.278</td>
</tr>
</tbody>
</table>

* a: P’s based on two-sided test
* b: Two unrelated cases were homozygous for the Asp174Tyr mutation.

### Table 2: Clinico-pathologic features of nine cases with Asp174Tyr missense mutation

<table>
<thead>
<tr>
<th>Case</th>
<th>Genotype</th>
<th>Family history of prostate cancer</th>
<th>Age (years)</th>
<th>Serum PSA at diagnosis (ng/ml)</th>
<th>Clinical stage</th>
<th>Gleason Sum</th>
<th>Type of therapy</th>
<th>Pathologic stage</th>
<th>Metastatic disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Homozygous</td>
<td>No</td>
<td>68.3</td>
<td>20.7</td>
<td>T1aNXMO</td>
<td>7</td>
<td>External radiation</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>Homozygous</td>
<td>No</td>
<td>71.7</td>
<td>6.2</td>
<td>T2bNXMO</td>
<td>7</td>
<td>External radiation</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Heterozygous</td>
<td>Yes</td>
<td>59.7</td>
<td>2.5</td>
<td>T1aNXMO</td>
<td>7</td>
<td>Radical prostatectomy</td>
<td>T3aNXMX</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Heterozygous</td>
<td>Yes</td>
<td>63.9</td>
<td>157.8</td>
<td>T2bNXM1</td>
<td>9</td>
<td>Hormonal chemotherapy</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>5</td>
<td>Heterozygous</td>
<td>No</td>
<td>64.8</td>
<td>1160.0</td>
<td>T2bNXM1</td>
<td>8</td>
<td>Hormonal chemotherapy</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>6</td>
<td>Heterozygous</td>
<td>No</td>
<td>68.5</td>
<td>11.0</td>
<td>T1aNXMO</td>
<td>4</td>
<td>External radiation</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>7</td>
<td>Heterozygous</td>
<td>No</td>
<td>61.4</td>
<td>2.0</td>
<td>T2bNXMO</td>
<td>5</td>
<td>Radical prostatectomy</td>
<td>N/A</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>Heterozygous</td>
<td>No</td>
<td>57.4</td>
<td>0.6</td>
<td>T2bNXMO</td>
<td>6</td>
<td>Radical prostatectomy</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>9</td>
<td>Heterozygous</td>
<td>Yes</td>
<td>50.1</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

* a: Pathologic Gleason Sum is reported whenever available; otherwise, biopsy Gleason Sum is reported.
* b: For cases undergoing radical prostatectomy.
* c: Additionally carries Ser41Tyr missense mutation.
* d: Brother with prostate cancer.
* e: Father with prostate cancer.

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Contrary to the findings of Xu et al., the absence of the 3-bp sequence \( \text{GAATGCTTTATTGTA} \) between prostate cancer and germ-line \( MSR1 \) mutations in African-American men was sufficiently low (2% of cases vs. 71.2% of controls) that it may be more appropriately classified as a rare mutation rather than a common sequence variant. This result, it is reasonable that a different conclusion may be reached for African-American men without necessarily compromising the validity and importance of this association in Caucasian men.

In conclusion, our analysis of \( MSR1 \) variants in 474 African-American men from a community-based study of prostate cancer provides additional support for an association between rare germ-line \( MSR1 \) mutations and prostate cancer risk. Specifically, we observed that the Asp174Tyr missense mutation is found nearly twice as frequently among prostate cancer cases compared with controls. Although this difference in mutation frequency did not reach statistical significance in our sample, our findings are nonetheless consistent with the hypothesis that this, and potentially other, rare germ-line mutations may mediate prostate cancer risk among African-American men (4). In addition, the IVS5-59 sequence variant may also modify prostate cancer risk among African-American men, and further investigation into the prevalence and functional significance of this change is warranted. We were unable to demonstrate, in African-American men, an association between four other \( MSR1 \) common sequence variants and prostate cancer risk. This study adds to an expanding body of epidemiological evidence in support of the hypothesis that germ-line \( MSR1 \) mutations are risk factors for prostate cancer. Although the evidence from our study is admittedly modest, the public health burden of prostate cancer in the African-American community warrants further investigation of this potential genetic risk factor.

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**References**

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