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Advances in Brief

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

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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*[3] 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-Americans 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 freezer 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 Puregene kit (Gentra 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 χ^2 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

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[3] The abbreviations used are: *MSR1*, macrophage scavenger receptor 1; HPC, hereditary prostate cancer; SNP, single nucleotide polymorphism; PSA, prostate-specific antigen; OR, odds ratio; FMHS, Flint Men's Health Study.

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

Mutation	Prostate cancer cases (%) (n = 134)	Unaffected men (%) (n = 340)	Fisher's exact test[a]
Ser41Tyr	1 (0.8)	0 (0)	0.278
Asp174Tyr[b]	9 (6.8)	12 (3.6)	0.143
Gly294Glu	0 (0)	0 (0)	
Pro36Ala	9 (7.0)	27 (8.1)	0.847
Arg293X	1 (0.8)	0 (0)	0.278

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

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, 2 of 9 (22.2%) cases were homozygous for the missense change at this allele. Clinicopathologic 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 3 *Frequencies of common MSR1 sequence variants in African-American men with prostate cancer and unaffected controls*

SNP and genotype	No. (%) of individuals with Genotype: Control subjects (n = 340)	Case subjects (n = 134)	P[a]
PRO3			
AA	125 (37.5)	54 (41.2)	0.244
AG	166 (49.9)	55 (42.0)	
GG	42 (12.6)	22 (16.8)	
INDEL1			
−/−	125 (37.7)	51 (40.2)	0.228
−/+	166 (50.0)	54 (42.5)	
+/+	41 (12.4)	22 (17.3)	
IVS5–59			
CC	329 (99.4)	122 (96.1)	0.020
CA	2 (0.6)	5 (3.9)	
AA	0 (0)	0 (0)	
P275A			
CC	287 (86.2)	118 (90.1)	0.535
CG	43 (12.9)	12 (9.2)	
GG	3 (0.9)	1 (0.8)	
INDEL7			
−/−	168 (50.9)	71 (56.8)	0.478
−/+	134 (40.6)	43 (34.4)	
+/+	28 (8.5)	11 (8.8)	

[a] χ^2 test.

Table 2 *Clinicopathologic features of nine cases with Asp174Tyr missense mutation*

Case	Genotype	Family history of prostate cancer	Age (years)	Serum PSA at diagnosis (ng/ml)	Clinical stage	Gleason Sum[a]	Type of therapy	Pathologic stage[b]	Metastatic disease
1	Homozygous	No	68.3	20.7	T1cNXMO	7	External radiation		No
2[c]	Homozygous	No	71.7	6.2	T2bNXMO	7	External radiation		No
3	Heterozygous	Yes[d]	59.7	5.2	T1cNXMO	7	Radical prostatectomy	T3aNXMX	No
4	Heterozygous	Yes[e]	63.9	157.8	T3cNXM1	9	Hormonal		Yes
5	Heterozygous	No	64.8	1160.0	T2bNXM1	8	Hormonal chemotherapy		Yes
6	Heterozygous	No	68.5	11.0	T1aNXMO	4	External radiation		No
7	Heterozygous	No	61.4	2.0	T2bNXMO	5	Radical prostatectomy	N/A	No
8	Heterozygous	N/A	57.4	0.6	T2aNXMO	6	Radical prostatectomy	T2aNOMX	No
9[f]	Heterozygous	Yes[g]	50.1	N/A	N/A	N/A	N/A	N/A	N/A

[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] Two brothers with prostate cancer.
[f] Serum sample provided but epidemiologic questionnaire not completed.
[g] Father with prostate cancer.

Table 4 *Common sequence variant allele frequencies and age-adjusted OR estimates for prostate cancer among African-American cases and controls*

Allele	Allele frequencies (%)		Fisher's exact test for allele (*P*)	Age-adjusted OR (95% confidence interval)
	Control subjects	Case subjects		
PRO3 "G"	37.5	37.8	0.940	0.84[a] (0.55, 1.29)
INDEL1 "+"[b]	37.4	38.6	0.761	0.88[c] (0.57, 1.36)
IVS5–59 "A"	0.3	2.0	0.020	2.90[d] (0.50, 16.79)
P275A "C"	92.6	94.7	0.312	0.72[e] (0.37, 1.40)
INDEL7 "−"[f]	71.2	74.0	0.457	0.78[g] (0.50, 1.19)

[a] Relative odds for AG or GG genotypes *vs.* AA (referent OR = 1.00).
[b] +, the presence of the 15-bp sequence "GAATGCTTTATTGTA."
[c] Relative odds for +/− or +/+ genotypes *vs.* −/− (referent OR = 1.00).
[d] Relative odds for CA or AA genotypes *vs.* CC (referent OR = 1.00).
[e] Relative odds for CG or GG genotypes *vs.* CC (referent OR = 1.00).
[f] −, the absence of the 3-bp sequence "TTA."
[g] Relative odds for +/− or +/+ genotypes *vs.* −/− (referent OR = 1.00).

associated with each sequence variant, we compared prostate cancer risk for one genotype to the combined risk associated with two other genotypes as described previously (3). In this analysis, although the IVS5-59 variant was associated with an increased risk of prostate cancer (OR = 2.9, 95% confidence interval 0.5–16.79), this finding did not reach statistical significance. Haplotype analyses using the five common polymorphisms for all possible combinations of two, three, four, and five adjacent markers revealed no statistically significant findings (minimum $P = 0.2$ obtained for two marker haplotypes defined by IVS5-59 and P275A). Contrary to the findings of Xu *et al.* (3), evidence for marker–marker linkage disequilibrium was observed for all marker pairings, with values of D′ ranging from 1 for marker pairings PRO3—INDEL1, IVS5–59—P275A, IVS5–59—INDEL7 to 0.42 for the marker pairing INDEL1—INDEL7.

Our data provide some additional evidence for a potential link between prostate cancer and germ-line *MSR1* mutations in African-American men. Xu *et al.* (4) reported the presence of the Asp174Tyr change in 6 of 48 African-American men with non-HPC *versus* only 2 of 110 unaffected African-American men. Indeed, when our data are considered in conjunction with these findings, Asp174Tyr mutations are seen in 15 of 182 (8.2%) cases *versus* only 14 of 450 (3.1%) controls ($P < 0.05$). Furthermore, the presence of homozygosity at Asp174Tyr in 2 cases from our sample, one of whom also carried the Ser41Tyr change, raises interesting questions regarding the impact of multiple germ-line mutations on the biology and function of *MSR1,* as well as the coincident effect on prostate cancer risk.

In general, however, our data provide limited support for an association, in African-American men, between prostate cancer and the five common *MSR1* sequence variants. We evaluated each of the common variants that have been reported previously to confer increased prostate cancer risk among men of European descent (3). Statistically significant differences in allele frequencies, among cases and controls, were observed for only one (IVS5-59) of the five sequence variants (Table 3). However, the overall prevalence of this mutation (IVS5-59) in our sample was sufficiently low (2% of cases *versus* 0.3% of controls) that it may be more appropriately classified as a rare mutation rather than a common sequence variant. This discrepancy notwithstanding, after adjustment for age, none of the sequence variants was associated with a significantly increased risk of prostate cancer (Table 4). The results were similar when the control sample was limited to those men who were >50 years of age with screening PSA value(s) < 4 ng/ml (data not shown). Thus, for the common *MSR1* sequence variants, with the possible exception of IVS5-59, our results in a sample of African-American men are inconsistent with those described previously (3).

Xu *et al.* (3) reported previously that each of the common *MSR1* sequence variants, with the exception of INDEL7, was associated with an elevated risk for prostate cancer. However, a recognized limitation of this study was the potential for population stratification, whereby the observed differences in genotype frequencies may partially reflect differing genetic backgrounds among case and control subjects. In contrast, it is more likely that men in our community-based sample come from similar genetic backgrounds, thereby minimizing population stratification and potentially explaining the lack of an association, in our sample, between common *MSR1* sequence variants and prostate cancer risk. Furthermore, it is important to recognize that the study by Xu *et al.* (3) included only men of European descent, whereas our sample was comprised exclusively of African-American men. As a 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.

There are several limitations to our study. First, we recognize that the relatively small sample size may result in low statistical power for some of our analyses. In addition, selection bias is a potential threat to the validity of all observational studies. Among control subjects in FMHS, <60% of men that completed the initial epidemiological interview participated in the blood draw and clinical examination components of the study. Factors associated with participation in the clinical phases of the project include young age, a family history of prostate cancer, and the presence of urological symptoms (11). Although nonresponse bias is a concern for epidemiological studies of behavioral risk factors, we have no reason to believe that participants and nonparticipants differ systematically with respect to their genetic background.

In conclusion, our analysis of *MSR1* variants in 474 African American men from a community-based study of prostate cancer provides some 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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