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Association of Prostate Cancer and Manganese Superoxide Dismutase AA Genotype Influenced by Presence of Occult Cancer in Control Group

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Abstract

OBJECTIVES—To investigate whether the inclusion of occult cancer in the control group can influence the association of prostate cancer and the polymorphism of manganese superoxide dismutase (MnSOD).

METHODS—Prostate specimens and sera were obtained from 194 deceased men who did not have a history of prostate cancer. Eighteen-core biopsy specimens and whole-mount sections were evaluated histologically. The MnSOD genotype of the specimens was determined by polymerase chain reaction restriction fragment length polymorphism analysis.

RESULTS—Tumors were present in 57 of the prostates, and biopsy detected 33 (including 1 false-positive finding). It detected 17 (1 false-positive finding) and missed 14 tumors in the subgroup of 135 specimens with a prostatic-specific antigen <4 ng/mL. The MnSOD AA genotype was associated with prostate cancer found in the step-sectioned specimens vs the control group in whom the absence of occult prostate cancer had been verified. However, no association was found if the control group consisted of subjects with negative biopsy results from the overall group or the subgroup with a prostatic-specific antigen level of <4 ng/mL.

CONCLUSIONS—The MnSOD AA genotype was associated with prostate cancer in our study; however, contamination of occult prostate cancer in the control group reduced the power of analysis and might yield seemingly negative results. Epidemiologic studies should strive to include control groups with a verified absence of occult cancer.

Autopsy and clinical data have demonstrated that >30% of men without a history of prostate cancer (PCa) have occult PCa, and the risk increases with age. Data from the Prostate Cancer Prevention Trial demonstrated a cancer incidence of 15.2% in the control group on biopsy.² The inclusion of these cancer cases in the control group of conventional epidemiologic studies could result in false-negative results. Most control subjects are selected on the basis of an absent history of PCa or a low prostate-specific antigen (PSA) level (<4 ng/mL). Biopsies are not usually done to validate the absence of PCa. Clinical data have suggested that nearly one half of localized low-grade PCa cases will progress to clinically significant cancer during the next 20 years.³ Therefore, the inclusion of 15%–30% of occult PCa cases in a control group would reduce the power of analysis. The use of autopsy cases in which the cancer status in the prostate can be ascertained by histologic examination provides a unique opportunity to examine the

effect of the inclusion of occult cancer in the control group for epidemiologic studies on the association of PCa and manganese superoxide dismutase (MnSOD) genotypes.

PCa is the most prevalent nonskin malignancy in the U.S. male, and it is estimated that 1 in 6 men probably will develop invasive PCa in his lifetime.⁴ The etiology of PCa is multifactorial, and it is suspected that environmental factors contribute to most PCa cases.⁵ Epidemiologic studies have correlated PCa with prostatitis and sexually transmitted disease.⁶ Inflammation invariably produces reactive oxygen species, which can damage both mitochondrial and nuclear DNA.⁷ Oxidative damage to nuclear DNA can result in the arrest or induction of transcription, the induction of signal transduction pathways, replication errors, or genomic instability, which could result in carcinogenesis.⁸ MnSOD is present in the mitochondria and plays an important role in the protection from reactive oxygen species-mediated DNA damage by converting superoxide radical to oxygen and hydrogen peroxide.⁹ Human MnSOD gene has a polymorphism at codon 16, which encodes for either alanine (A) or valine (V).¹⁰ Recent studies have suggested that the MnSOD AA genotype is associated with PCa in smokers¹¹ or with a low antioxidant status.^{12,13} However, some studies reported no association.¹⁴ In the present study, we investigated the association of MnSOD genotypes and PCa in prostates obtained from deceased men, for which the cancer status could be verified by complete histopathologic evaluation.

MATERIAL AND METHODS

The present study used 194 consecutive prostate glands from deceased men >45 years of age, collected as a part of a larger study on the prevalence of PCa.¹ The prevalence of PCa in men <45 years old was very low, and specimens from these men were excluded. The prostates removed at autopsy were obtained from the University Hospital (Syracuse, NY), the Onondaga County Medical Examiner (Syracuse, NY), and the National Disease Research Interchange (Philadelphia, PA). Only age, race, and cause of death were recorded. This study used samples from the decedents and received exemption from institutional review. The decedents had no known history of PCa. The total PSA levels were measured in the sera collected within 24 hours of death.¹⁵

All 194 prostate glands underwent 18-core biopsy regimens performed as previously described.¹ In brief, biopsies were taken ex vivo by an experienced urologist (G.P.H.) in a manner similar to the method used for clinical transrectal ultrasound-guided biopsies. The biopsies were taken from both sides of the glands in a symmetric fashion from the mid-peripheral, lateral-peripheral, and central zones at the level of the apex, mid-gland, and base. The entire prostate was fixed, step sectioned at 5-mm intervals, embedded in paraffin, cut into 5- μ m sections, and stained with hematoxylineosin. Histologic evaluation was performed, and the presence or absence of PCa on the biopsies and final pathologic examination was compared.

DNA was extracted from the autopsied prostatic tissues using QIAamp DNA Blood Mini Kits (Qiagen, Valencia, CA) according to the manufacturer's instructions. The MnSOD gene was polymerase chain reaction-amplified with the following primer pairs: 5'-GTGCTTTCTCGTCTTCAGCA-3' and 5'-AACGCCTCCTGGTACTTCTCC-3'. The MnSOD genotypes were determined by polymerase chain reaction restriction fragment length polymorphism analysis (BsaWI). To assess genotyping reproducibility, 25% of the samples were randomly selected for repeated determination, and all genotypes were as initially determined. The laboratory personnel were unaware of the pathologic status of the specimens during genotyping.

A test of 2 independent proportions was performed using Pearson's χ^2 test, likelihood ratio, or Fisher's exact method, using the software package JMP, version 3.2.1 (SAS Institute, Cary,

NC). The 95% confidence interval was determined at the level of $\alpha = 0.05$ for a type I error. The Cochran-Armitage test of trend was used to determine whether a relationship existed between the MnSOD genotypes and PCa. Pearson's χ^2 test was applied to the Hardy-Weinberg equilibrium.

RESULTS

This study included 194 prostates from 178 white, 7 African American, 1 Hispanic, and 8 unknown-origin men. Their median age was 66 years (interquartile range 56–74). The pathologic evaluation identified PCa in 57 specimens (29.4%). The median age was 72 years (interquartile range 61–79) and 64 years (interquartile range 55–72) in the PCa and PCa-free group, respectively. Table 1 lists the number of PCa cases detected or missed by biopsy in the overall specimens or specimens with a PSA level of ≥ 4 or < 4 ng/mL. Biopsy detected 17 tumor cases in 135 specimens (12.5%) from those with a PSA level < 4 ng/mL. Table 2 lists the prevalence of occult cancer in various subgroups selected according to PSA status and/or biopsy results. Despite a PSA level of < 4 ng/mL and negative biopsy results, 11.9% of the prostates had undetected PCa. The biopsy regimens significantly reduced the proportion of undetected PCa cases in the overall group and the PSA < 4 ng/mL subgroup (Table 2).

When we compared the MnSOD AA genotype prevalence between the histologically verified PCa cases ($n = 57$) vs those glands with no PCa ($n = 137$), the results demonstrated an association between MnSOD AA genotype and PCa ($P = 0.049$), and the A allele might be a risk factor ($P_{trend} = 0.048$) (Table 3). However, if the control group was selected according to the biopsy-negative findings (occult cancer rate 15.5%), no association was found ($P = 0.115$), and the trend was not significant ($P_{trend} = 0.120$). When the control group was selected according to a PSA level < 4 ng/mL and negative biopsy finding (occult cancer rate 11.9%), the association ($P = 0.131$) and trend ($P_{trend} = 0.153$) were not significant (Table 3).

COMMENT

The results of the present study have shown that an association exists between PCa and the polymorphism of MnSOD in the autopsy cases in which the control group had been verified to be free of occult PCa. However, this association might be masked in the studies in which the “cancer-free” control group contains cases of occult PCa. Therefore, the effect of occult PCa might be overlooked in some epidemiologic studies. Our results support the hypothesis that the MnSOD AA genotype and PCa are associated and that reactive oxygen species are involved in the carcinogenesis of the prostate.⁸

The use of a PSA level of < 4 ng/mL as a criterion will reduce the inclusion of occult PCa, and this can be reduced further when cancer cases are excluded by biopsy. Biopsy regimens detect large tumors and reduce the proportion of occult cancer in the control group and, consequently, reduce the effect of occult cancer in case-control studies. Therefore, it is recommended that, at the least, only those whose PSA level is < 4 ng/mL and have negative biopsy results are selected as control subjects for epidemiologic studies of PCa. In most case-control studies for PCa, the control subjects have consisted of men who did not have a history of PCa.^{11,12,14} Only one recent (2007) study used controls who had had at least one PCa screening with PSA determination.¹³ To our knowledge, none of the published case-control studies for MnSOD polymorphism required negative biopsy findings for inclusion in the control group. This principle can be applied to other case-control studies of PCa and genetic polymorphism.

Our study had several limitations. Autopsy studies have reported that the incidence of occult PCa increases with increasing age. It is expected that 30%–50% of men > 60 years old have occult PCa.¹ Some of the young men in the cancer-free group in our study could have developed

PCa had they lived to an older age. Although the inclusion of these young men in our study might have caused an overrepresentation of the MnSOD AA genotype in the control group, our results still showed an association. In addition, the sample size of this exploratory study was relatively small, which might have limited the power of the analysis. Additional studies with larger sample sizes and investigating other risk factors associated with PCa will clarify some of these issues.

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Table 1
Ability of biopsy regimens to detect tumors

Selection Criteria	PSA <4 ng/mL (n = 135)		PSA ≥4 ng/mL n = 59)	
	PCa (+)	PCa (-)	PCa (+)	PCa (-)
Biopsy findings				
Positive (n = 33)	16	1	16	0
Negative (n = 161)	14	104	11	32
Total	30	105	27	32

PSA = prostate-specific antigen; PCa = prostate cancer.

Table 2

Presence of undetected tumors using varying selection criteria

Selection Criteria	Total	Occult PCa	<i>P</i> Value	<i>P</i> Value
None	194	57 (29.4)	Referent	
PSA <4 ng/mL	135	30 (22.2)	0.148	Referent
Negative biopsy	161	25 (15.5)	0.002	0.140
PSA <4 ng/mL and negative biopsy	118	14 (11.9)	<0.001	0.030
Whole mount section	137	0 (0.0)	<0.001	<0.001

Abbreviations as in Table 1.

Data in parentheses are percentages.

Null hypothesis tested using Pearson's χ^2 test; likelihood-ratio test yielded similar *P* values.

Table 3
Proportion of MnSOD genotypes in PCa and control groups by various methods of diagnosis

Selection Criteria (% Undetected PCa)	MnSOD	Control* (No. Undetected PCa)	Detected PCa	OR (95% CI)	P Value
Whole mount section (0.0%)	VV	37 (0)	9	1.00 (ref.)	
	VA	59 (0)	24	1.67 (0.71–3.93)	0.244 [†]
	AA	41 (0)	24	2.41 (1.00–5.75)	0.049 [†]
<i>P_{trend}</i> Biopsy (15.5%)	VV	42 (5)	4	1.00 (ref.)	0.048 [†]
	VA	68 (9)	15	2.32 (0.75–7.08)	0.198 [‡]
	AA	51 (11)	13	2.68 (0.84–8.37)	0.115 [‡]
<i>P_{trend}</i> PSA <4.0 ng/mL and biopsy (11.9%)	VV	33 (2)	7	1.00 (ref.)	0.120 [†]
	VA	45 (6)	18	1.89 (0.72–4.92)	0.201 [†]
	AA	40 (6)	18	2.12 (0.80–5.56)	0.131 [†]
<i>P_{trend}</i>					0.153 [†]

MnSOD = manganese superoxide dismutase; OR = odds ratio; CI = confidence interval; ref. = referent.

* Control group contained undetected PCa by methods of diagnosis.

[†] Null hypothesis tested by Pearson's χ^2 test; Likelihood-ratio test yielded similar *P* values; *P_{trend}* calculated using Cochran Armitage test.

[‡] Null hypothesis tested by 2-tailed Fisher's exact test.