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Although prostate cancer is the most common cancer in western countries, risk factors for this disease have not been well characterized. Furthermore, research on genetic susceptibility to prostate cancer is in its infancy. This study builds upon an ongoing project by adding genetic susceptibility markers. Additionally, we are accruing 100 new patients with metastatic disease. Constitutional markers are being evaluated as predictors of prostate cancer risk including: a) polymorphisms within the androgen receptor and 5-α-reductase genes, b) relative expression levels of several mismatch repair genes (*hMSH2* and *hMLH1*) and radiosensitivity related genes (*ATM, GADD45, XRCC1*), and c) frequency of replication errors in tumor and normal DNA. These data will be integrated with epidemiologic and clinical information. Results from this research may identify markers of progression, which could help in the diagnosis and treatment of prostate cancer. Our preliminary findings indicate that decreased mismatch repair gene expression may be associated with increased risk of prostate cancer. These results suggest that DNA damage-repair pathways may be involved in prostate carcinogenesis. Incorporation of more data from this study, including the metastatic patients, will allow us to confirm this finding and further explore the molecular basis of the underlying mechanisms of prostate cancer etiology.

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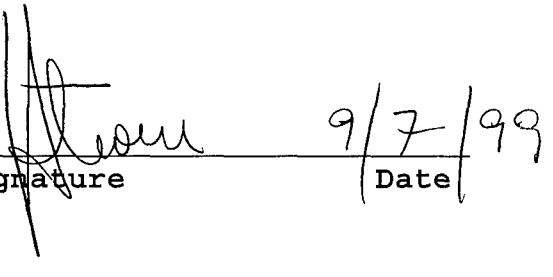

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A Molecular Epidemiologic Case-Case Study of Prostate Cancer Susceptibility

INTRODUCTION

Although prostate cancer is the most common cancer in the western countries, risk factors contributing to the development and progression of this disease have not been well characterized. Furthermore, research on genetic susceptibility to prostate cancer is in its infancy. This study builds upon an ongoing NCI-funded project by adding a panel of genetic susceptibility markers. Additionally, we are accruing 100 new patients with metastatic disease. We are evaluating constitutional markers of genetic susceptibility as predictors of prostate cancer risk including: a) polymorphisms within the androgen receptor and 5-α-reductase genes, b) relative expression levels of several mismatch repair genes (*hMSH2* and *hMLH1*) and radiosensitivity related genes (*ATM, GADD45, XRCC1*), and c) frequency of replication errors in tumor and normal DNA. These genetic susceptibility marker data will be integrated into epidemiologic and clinical information. Results from this research may identify markers of progression, both epidemiologic and molecular, which could help in the diagnosis and treatment of prostate cancer. ***By adding patients with metastatic disease, a new set of markers, and by using an ongoing study protocol, data collection instruments, and data management tools currently in operation, we are incorporating patients with the entire spectrum of disease in a time- and cost-effective manner.***

STATEMENT OF WORK

Task 1 **Subject Recruitment.** (Months 1-24)

A. Identify and enroll 100 prostate cancer patients with metastatic disease from UTMDACC.

Our recruitment protocol from the ongoing NCI-funded study has been modified for recruiting metastatic patients. To date 47 men with metastatic prostate cancer (38 Caucasian, 5 African-Americans and 4 Hispanics) have been enrolled in this study.

B. Complete interviews, anthropometric measurements, and collect blood samples and tissue from study participants.

Data collection instruments have been modified and tested for incorporating data specific to metastatic prostate cancer. Interviews have been completed and blood samples have been collected for all 47 participants.

C. Abstract medical records.

Medical records for all 47 participants have been abstracted.

D. Complete data-entry using protocols developed for the on-going study.

Data entry protocols for the ongoing NCI-funded study have been modified and utilized for this current study. Data for these 47 participants has been data entered according to our protocol.

E. Perform quality control checks using protocols developed for the ongoing study.

Data quality control checks updated. These checks are conducted in accordance with the existing protocols.

We will continue to enroll cases prospectively and to follow-up with all participants to ensure the completion of study components.

Task 2 **Evaluate Constitutional Markers of Genetic Susceptibility.** (Months 1-24)

A. DNA will be extracted from peripheral blood samples (200 from ongoing study and 100 newly-enrolled metastatic cases) by the UTMDACC Institutional Molecular Core.

DNA has been extracted for 120 participants from the ongoing NCI-funded study and for 47 metastatic participants.

B. Complete analysis of polymorphisms related to androgen metabolism (S. Hursting)

Dr. S. Hursting has left our institution, this work is now being done by Dr. P. Thompson (see Appendix 1). Dr. Thompson will become a Co-PI in this grant, replacing Dr. Hursting. Due to this unanticipated change in key personnel, there has been some delay in the analyses of these polymorphisms. These assays have been standardized in Dr. Thompson's laboratory and samples are now in process.

C. Determine expression levels of DNA mismatch repair and radiosensitivity related genes (Q. Wei)

Using a novel multiplex RT-PCR assay, we determined mismatch repair gene expression levels among 70 prostate cancer cases and 97 healthy controls. These results have been summarized in a manuscript prepared for submission to a peer-reviewed journal (see Appendix 2). Overall, the cases had lower expression levels for these genes than did healthy controls. The data below summarize these findings.

Table 1 Mismatch Repair Gene Expression: case-control

Gene	Expression Level*	Number		OR (95% CI)
		Cases (n =70)	Controls (n = 97)	
hMLH1	HT	17	32	1.00
	MT	15	33	0.86 (0.37-2.00)
	LT	38	32	2.24 (1.05-4.75)
hMSH2	HT	8	32	1.00
	MT	25	33	3.03 (1.19-7.70)
	LT	37	32	4.62 (1.87-11.46)

**HT, highest tertile; MT, middle tertile; LT lowest tertile; based on controls levels*

We also used similar techniques to examine for possible differences in radiosensitivity genes, GADD45, XRCC1, and ATM. This manuscript is in preparation.

Table 2 Radiosensitivity Gene Expression: case-control

Gene	Expression Level*	Number		OR (95% CI)
		Cases (n =70)	Controls (n = 97)	
GADD45	HT	7	32	1.00
	MT	16	33	2.22 (0.81-6.10)
	LT	47	32	6.71 (2.64-17.07)
XRCC1	HT	19	32	1.00
	MT	19	33	0.97 (0.44-2.16)
	LT	32	32	1.68 (0.80-3.57)
ATM	HT	11	32	1.00
	MT	14	33	1.23 (0.49-3.12)
	LT	45	32	4.10 (1.80-9.30)

**HT, highest tertile; MT, middle tertile; LT lowest tertile; based on controls levels*

We will continue to obtain blood samples for newly enrolled participants and DNA will be extracted from these samples to allow us to determine gene expression levels for mismatch repair genes as well as radiosensitivity genes. Androgen receptor polymorphisms will be examined, described and compared among our case population.

Task 3 **Determine Microsatellite Instability in Tissue Samples.** (Months 1-24)

A. DNA will be extracted from tumor and normal tissue in a subset of 120 cases (40 insignificant, 40 significant, and 40 metastatic PC).

   The institutional pathologist has identified tumor and normal tissue blocks for 60 cases. Slides have been prepared for DNA extraction and are being processed.

B. Determine the frequency of replication errors.

   The frequency of replication errors will be determined as soon as the samples have been processed for analysis to commence.

For a subset of 120 cases, microsatellite instability will be examined and compared among case groups to determine if there is an association between MIN and prostate cancer progression.

Task 4 **Final Analysis and Preparation of Reports.** (Months 25-30) – N/A

A. Final quality control checks for epidemiologic, clinical, and laboratory data.

B. Analyze data, integrate epidemiologic and clinical data with biomarker results.

C. Prepare and submit a final report.

D. Prepare and submit manuscripts suitable for publication in peer-reviewed journals.

E. Prepare and submit proposal for competitive renewal of this grant.

KEY RESEARCH ACCOMPLISHMENTS

- 47 patients newly diagnosed with metastatic prostate cancer have been accrued for this study
- Epidemiologic data and biological samples (blood and prostate tissue) have been collected for all study participants
- Gene expression assays have been established and utilized.
- 70 samples have been processed for gene expression assays.
- 60 pathological samples (normal and tumor tissue) have been obtained for MIN determination

REPORTABLE OUTCOME

- 1 manuscript (see Appendix 2) has been submitted.
- 1 manuscript is being prepared for publication
- Presentation was made in November 1998 regarding gene expression and prostate cancer risk by Dr. S. Strom to the University of Texas M. D. Anderson Cancer Center Prostate Research Program Group.

CONCLUSIONS

Our preliminary findings indicate that decreased mismatch repair gene expression may be associated with increased risk of prostate cancer. These results suggest that DNA damage-repair pathways may be involved in prostate carcinogenesis. Incorporation of more data from this study, including the metastatic patients, will allow us to confirm this finding and further explore the molecular basis of the underlying mechanisms of prostate cancer etiology.

Abstracts & Manuscripts published

Strom SS, Yamamura Y, Duphorne CM, Spitz MR, Babaian RJ, Pillow PC, Hurting SD. *Phytoestrogen Intake and Prostate Cancer: A case-control study using a new database.* Nutrition & Cancer. *33(1): 20-25, 1999.*

Strom SS, Guan Y, Yamamura Y, Babaian RJ, Spitz MR, Scardino PT, Wei Q. *Reduced Expression of Mismatch Repair Genes in Prostate Cancer Patients.* (submitted)

Spitz MR, Strom SS, Yamamura Y, Troncoso P, Babaian RJ, Scardino PT, Wheeler T, Amos CI, von Eschenbach A, Kagan J. *Epidemiologic Determinants of Clinically Relevant Prostate Cancer.* (submitted)

APPENDIX 1

P. Thompson Biosketch

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel in the order listed on Form Page 2.
Photocopy this page or follow this format for each person.

NAME	POSITION TITLE
Patricia Ann Thompson	**Assistant Professor**

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(S)	FIELD OF STUDY
The University of Texas Health Science Center At San Antonio, San Antonio, Texas	**Ph.D.**	**1993**	**Microbiology**
Angelo State University, San Angelo, Texas	**B.S.**	**1986**	**Biology**

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list, in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List, in chronological order, the titles, all authors, and complete references to all publications during the past three years and to representative earlier publications pertinent to this application. If the list of publications in the last three years exceeds two pages, select the most pertinent publications. DO NOT EXCEED TWO PAGES.

PROFESSIONAL EXPERIENCE

7/99-Present, Assistant Professor, Division of Cancer Prevention, Department of Epidemiology, University of Texas M.D. Anderson Cancer Center, Houston, Texas

3/98-6/99, Molecular Biologist and Staff Scientist, Division of Molecular Epidemiology, National Center for Toxicological Research, Jefferson, Arkansas

6/98-6/99, Adjunct Professor, Department of Surgical Oncology, University of Arkansas Medical School, Little Rock, Arkansas

1/96-3/98, Post-Doc, National Center for Toxicological Research, Jefferson, AR., Drs. Fred Kadubar and Christine Ambrosone

4/94-7/95, Post-Doc, University of Texas Health Science Center at San Antonio, TX, Dr. Michael Berton

8/93-1/94, Instructor of Cell Biology, Department of Life Sciences, University of Texas at San Antonio, San Antonio, TX.

6/93-3/94, Post-Doc, Department of Virology and Immunology Southwest Foundation for Biomedical Research, San Antonio, TX, Dr. Ronald Kennedy

SELECTED PUBLICATIONS

Thompson, P. A. and Krolick., K. A. Acetylcholine receptor-reactive antibodies in experimental Autoimmune myasthenia gravis differing in disease-causing potential: Subsetting by preparative isolectric focusing. Clin. Immunol. Immunopathol., 62:199, 1992.

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Tang, Y. M., Chen, G., Thompson, P. A., Lin, D., Lang, N. P., and Kadlubar, F. F. (In press) Development of an Anti-peptide Antibody that Binds to the C-terminal Region of Human CYP1B1. Drug Metab Disp., (2): 274-280, 1999.

Thompson, P. A., Seyedi, F., Lang, N. P., MacLeod, S. L., Wogan, G. N., Anderson, K. E., Tang,Y-M., Coles, B., and. Kadlubar, F. F. Comparison of DNA Adduct Levels Associated with Exogenous and Endogenous Exposures in Human Pancreas in Relation to Metabolic Genotype. Mutation Res. 424: 263, 1999.

APPENDIX 2

Strom SS, Guan Y, Yamamura Y, Contois JH, Babaian RJ, Spitz MR, Scardino PT, Wei Q. Reduced Expression of Mismatch Repair Genes in Prostate Cancer Patients.

Reduced Expression of Mismatch Repair Genes in Prostate Cancer Patients

Sara S. Strom[1*], Yongli Guan[1], Yuko Yamamura[1], John H. Contois[1], Richard J. Babaian[2], Margaret R. Spitz[1], Peter T. Scardino[3], and Qingyi Wei[1]

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Abbreviations: RT, reverse transcription; PCR, polymerase chain reaction; OR, odds ratio; CI, confidence interval; MIN, microsatellite instability.

Running head: DNA REPAIR GENES AND PROSTATE CANCER

Key words: biomarker; prostate cancer; gene expression; molecular epidemiology; polymerase chain reaction

Abstract

Although prostate cancer is the most common cancer in men, not much is known about its etiology and genetic determinants. We conducted a case-control study of 70 patients and 97 age- and ethnicity-matched controls to test the hypothesis that endogenous exposure to metabolites of carcinogens may cause oxidative DNA damage and thereby increase risk of prostate cancer in individuals with suboptimal DNA repair. Using multiplex reverse transcription-polymerase chain reactions, we were able to simultaneously evaluate the relative expression of two mismatch repair-related genes (*hMSH2* and *hMLH1*) in the peripheral blood lymphocytes of participants to determine if decreased expression of these genes is associated with increased risk of prostate cancer. Gene expression was not correlated with age, smoking, or alcohol use or serum testosterone levels. Risk of prostate cancer increased significantly with decreasing *hMLH1* expression (odds ratio = 4.31, 95% confidence interval = 1.64-11.30), when the lowest and highest tertiles were compared, after adjustment for age, ethnicity, smoking status, and family history of prostate cancer. These results suggest that reduced expression of mismatch repair genes could be a risk factor for prostate cancer that needs further confirmation.

INTRODUCTION

Despite prostate cancer's high incidence, little is known about its etiology or the factors that influence its progression. Although mutations and polymorphisms in androgen receptor genes suggest that male hormones may play a role in the etiology of prostate cancer [1], the genetic determinants of prostate cancer remain largely unknown. Abnormalities in several genes, including tumor suppressor genes such as *p53* [2], *MXI1*, and *PTEN/MMAC1* [3], and cell-cycle related genes such as *RB*, *WAF1/CIP1/p21*, and *p16* [4], have been identified in prostate tumors. As normal cell-cycle arrest is necessary for DNA repair [5], the frequent somatic mutations and genetic alterations in genes controlling cell-cycle progression and cell growth found in prostate cancer tumors suggest that prostate cancer may involve abnormalities in DNA repair. That endogenous exposure to metabolites of carcinogens may cause oxidative DNA damage and thereby increase risk of prostate cancer in individuals with suboptimal DNA repair is therefore an intriguing hypothesis that has never been tested.

It has been suggested that the first step in the development of cancer involves a mutator phenotype [6]. In prostate cancer, the mutator phenotype is frequent, and leads to genetic instability [7] caused by deficiencies in DNA mismatch repair [8,9]. We hypothesize that downregulation of this DNA damage-repair pathway, as measured by reduction in the expression of the genes involved in this pathway, is associated with increased risk of prostate cancer. To test this hypothesis, we conducted a pilot case-control study to evaluate whether genetically determined expression levels of the mismatch repair genes *hMSH2,* and *hMLH1* are associated with prostate cancer risk. Using a modification of a multiplex reverse transcription RT-polymerase chain reaction (PCR) method [10-13], we simultaneously measured the relative

mRNA expression of *hMSH2* and *hMLH1,* in a single reaction in the peripheral blood lymphocytes of 70 newly diagnosed, previously untreated prostate cancer patients and 97 healthy controls. Our results suggested that downregulation of these DNA damage-repair pathways may be associated with increased risk of prostate cancer.

MATERIALS AND METHODS

Study Population

The cases were patients registered at The University of Texas M. D. Anderson Cancer Center or Baylor College of Medicine with histologically confirmed adenocarcinoma of the prostate. Men with metastatic prostate cancer or a previous history of invasive cancer were excluded from the study. The subjects included in this analysis had not had any treatment for prostate cancer other than prostatectomy. These men were participants in an ongoing molecular epidemiologic case-case study, and they were enrolled between 1997 and 1998. The controls were identified from two sources. The first group of participants (75%) was selected from men attending the M. D. Anderson Cancer Center prostate cancer screening program. Men who had prostate-specific antigen levels ≥ 4, an abnormal rectal digital examination, previous history of cancer, or current infection were excluded. The second group of controls (25%) was selected among male members of a large multi-specialty health maintenance organization. Only patients without a history of cancer or urological conditions were included. The cases and controls were matched on age and ethnicity. After informed consent was obtained, each participant donated 10 mL of blood collected in heparinized tubes and completed either a personal or phone interview that assessed demographic and risk-factor information and family history of prostate cancer.

Multiplex RT-PCR

We have found that it is difficult to extract sufficient RNA from blood samples drawn more than 24 hours before processing. Consequently, total RNA was extracted from all the samples in this study within 6 hours of procurement. On each blood sample, we performed a multiplex RT-PCR assay using the β-actin gene as an internal control [10,11] to evaluate simultaneously the expression of *hMSH2* and *hMLH1*. The inclusion of the internal control β-actin allowed us to evaluate contamination of genomic DNA and to normalize variation in the amount of RNA used for cDNA synthesis as well as the amount of PCR product loaded on gels. To amplify these two selected genes, we used our multiplex RT-PCR protocol [12,13]. The strategy for choosing the primers has been previously described [10,11]. The sequences of the primers were 5'-ACACTGTGCCCATCTACGAGG-3' (sense) and 5'-AGGGGCCGGACTCGTCATACT-3' (antisense) for β-actin (GenBank accession no. M10277; starting positions 2147 and 2954, respectively*);* 5'-GTCGGCTTCGTGCGCTTCTTT-3' (sense) and 5'-TCTCTGGCCATCAACTGCGGA-3' (antisense) for *hMSH2* (U03911; starting positions 52 and 460, respectively*);* and 5'-GTGCTGGCAATCAAGGGACCC-3' (sense) and 5'-CACGGTTGAGGCATTGGGTAG-3' (antisense) for *hMLH1* (U07418; starting positions 466 and 660, respectively*).* Briefly, we isolated total RNA with Tri-Reagent (Molecular Research Center, Inc., Cincinnati, OH). Then, cDNA was synthesized by RT with 0.5 μg of random primers (Promega Biotech, Piscataway, NJ), 200 U of Moloney murine leukemia virus reverse transcriptase (United States Biochemical Co., Cleveland, OH), 1 μg of total cellular RNA, 4 μl of 5x RT buffer (250 mM Tris-HCl, pH 8.3; 375 mM KCl; 50 mM dithiotheitol; and 15 mM $MgCl_2$; Life Technologies, Gaithersburg, MD), 0.25 mM each dNTP, 20 units of RNasin

(Promega Biotech), and 6.5 μL of diethyl pyrocarbonate-treated water. The 20-μL reaction mixtures were incubated at room temperature for 10 min and 42°C for 45 min, heated to 90°C for 10 min, and then quickly chilled on ice.

The PCR primer mixture was optimized by experimenting with different combinations of concentrations of each pair of primers to produce a clearly visible band for each of the genes on an agarose gel. The optimal 50 μL PCR mixture contained 3-5 μL of RT reaction mixture, 1x PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 9.0; 1% Triton X-100; and 2.5 mM $MgCl_2$), 0.04 mM each dNTP, 2 U of *Taq* polymerase (Promega Biotech), 25 pM β-actin primers, 125 pM *hMSH2* primers and 20 pM *hMLH1* primers. The mixtures were amplified in the TwinBlock Ericomp DNA Amplification System (SCI, San Diego, CA) by an initial denaturation step of 95°C for 5 min; 29 cycles of denaturation at 95°C for 30 s, primer annealing at 59°C for 30 s, and extension at 72°C for 45 s; and a final elongation step at 72°C for 10 min. This optimal protocol allows the amplification of all genes simultaneously in 29 cycles and gave very consistent results in repeated assays [10]. The assays were performed in batches of six to eight samples with equal numbers of cases and controls. The RT-PCR products were separated by 1.5% agarose gel electrophoresis, stained with 0.5 μg/mL ethidium bromide, visualized with ultraviolet light, and photographed (Fig. 1). The bands on the photos were scanned as digitized images, and the areas under the peaks were calculated in arbitrary units by densitometric analysis with a computerized Digital Imaging System (Model IS-1000; Alpha Innotech Co., San Leandro, CA). The internal standard (β-actin) in each reaction was used as the baseline gene expression of that sample. The relative expression value for each of the target genes amplified in that reaction was calculated relative to the β-actin value. These values were then compared across the samples tested. Reduced expression was verified by repeating the multiplex RT-PCR assay.

Plasma samples were used to measure testosterone levels by enzyme-linked immunosorbent assays and dihydrotestosterone by radioimmunoassay in a subset of participants. Both hormones

were measured by using commercially available kits from Diagnostics Systems Laboratories Inc. (Webster, TX).

Statistical Analysis

Chi-square tests were used to assess demographic variables. The relative expression of each gene (as a continuous variable) among cases and controls was evaluated by using Student's *t*-test. Pearson correlation coefficients were calculated among demographic factors, smoking levels, alcohol consumption, family history of prostate cancer, testosterone levels, and gene expression values. Crude odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by using unconditional logistic regression for demographic, smoking, alcohol consumption, family history of prostate cancer in a father, brother or son, and gene expression variables. Gene expression values were categorized into three groups by tertile of expression level in the controls. Thus, these ORs can be interpreted as the risks of those in the lowest tertile compared with those in the highest tertile of gene expression. Men who smoked more than 100 cigarettes in their lifetimes were considered "ever smokers," and those who had consumed at least one drink per week during the last year were classified as current alcohol users. The rest of the men were grouped together as "Former or never users" of alcohol. The ORs were adjusted for variables shown to be statistically significant in the univariate analysis.

RESULTS

There were 70 cases (mean age, 64.4±7.5 yr) and 97 controls (mean age, 63.1±7.0 yr) in this study (Table 1). These men were primarily non-Hispanic whites (91%). The frequency matching resulted in no statistically significant differences in the distributions of age and ethnicity between cases and controls ($P > 0.05$). A higher percentage of cases (76%) than controls (57%) reported being ever smokers ($P < 0.05$), whereas the prevalence of current drinking was similar in cases

(57%) and controls (53%). More cases (21%) than controls (6%) reported having a first-degree relative (father, brother, or son) with prostate cancer ($P < 0.001$).

Multiplex PCR of the 70 cases and 97 controls revealed variation in the expression levels in both cases and controls as shown in Figure 2. The mean expression level was in the cases than in the controls (Table 2), indicating that there was downregulation of these genes in the cases relative to the controls. The expression levels of both *hMSH2* and *hMLH1* were statistically significantly lower in the cases than in the controls, with the greatest variation in expression being in *hMSH2*. Correlation analysis indicated that expression levels did not increase with age, smoking, or alcohol consumption in either cases or controls (data not shown). We also explored the correlation between serum testosterone and dihydrotestosterone levels and gene expression among those participants for whom data were available. We found low correlation coefficients between hormone levels and the expression levels of the two genes ($r = -0.26$, $P = 0.06$ for *hMSH2* and testosterone to $r = -0.07$, $P = 0.62$ for *hMLH1* and testosterone; data not shown).

Crude and adjusted ORs are shown in Table 3. The adjusted results did not differ substantially from the crude ORs. Only reduced expression of *hMHl1* remained a statistically significant risk factor for prostate cancer (OR = 4.31, $P = 0.003$) when the lowest and highest tertiles were compared.

DISCUSSION

In this case-control study, we evaluated lymphocyte expression of two mismatch repair genes and prostate cancer risk by using a multiplex RT-PCR assay [11,13]. Comparing 70 cases and 97 controls, we found an association between reduced expression of *hMLH1* and prostate cancer risk that was independent of age, ethnicity, smoking, and family history of prostate cancer. Both genes studied showed reduced expression in cases compared with healthy controls, which supports our hypothesis that DNA damage-repair pathways are downregulated in prostate cancer patients. The reduction in expression of these genes varied from individual to individual. This interindividual variability may be genetically determined or epigenetically influenced by cumulative environmental carcinogen exposure [14].

To the best of our knowledge, mutations in *hMLH1* and *hMSH2* in prostate cancer tumor tissue have not been reported, and the expression of these genes in either prostate cancer tumors or the normal lymphocytes of prostate cancer patients has not been examined. However, there are several lines of evidence suggesting that aberrant expression of these genes is involved in human carcinogenesis.

Defective mismatch repair is believed to be responsible for microsatellite instability (MIN), a common genetic alteration observed in many malignancies. Several studies have analyzed MIN in prostate cancer and have reported frequencies ranging from 10 to 65% [7,15]. A possible explanation for this wide range of MIN frequencies could be the selection of tissues and markers and small number of cases. Results from a Japanese study suggested that the frequency of this phenotype is higher in the United States than Japan, reflecting ethnic differences in the molecular etiology of prostate cancer [15]. The MIN frequency is also lower in latent cases than in clinically relevant cases [15]. MIN has also been reported to be high for specific chromosomes, such as 10 and 8 (65% and 75%, respectively). In summary, these studies suggested

involvement of defective mismatch repair in prostate carcinogenesis. Other studies found that low or undetectable expression of *hMLH1* was frequent in some sporadic cancers with the replication error phenotype [16,17]. Methylation of the *hMLH1* promoter is one possible mechanism for inactivation of this mismatch repair gene without mutation. Low expression of these genes may reflect genetically determined expression levels or an epigenetic effect of endogenous and exogenous exposure to unknown carcinogenic agents.

The use of unstimulated peripheral blood lymphocytes as the surrogate tissue in this study has advantages and disadvantages, particularly in measuring gene expression. Although lymphocytes may have been exposed to the same endogenous and exogenous chemical carcinogens as the target organ (the prostate), as blood serves as the carrier, the extent of exposure to endogenous DNA-damaging agents may be different due to local metabolic activities. However, the genetic and epigenetic characteristics of the target tissue may be altered by organ-specific carcinogens, which would make the target tissue inappropriate for determination of genetic susceptibility, whereas the lymphocytes may be spared such alterations. Therefore, using lymphocytes allows evaluation of the contribution of genetic susceptibility to disease etiology. Because DNA repair plays a central role in removing DNA damage and reducing the frequency of mutation, the genetically determined expression levels of DNA repair genes in lymphocytes should correlate with their DNA repair capacity and therefore with risk of cancer induced by DNA-damaging agents. We reported previously that reduced expression of *hMLH1* and *GTBP/hMSH6* in lymphocytes is associated with risk of developing head and neck cancer [12], and that reduced expression of *hPMS2* is associated with risk of developing colon cancer [18]. However, there is concern that factors such as infection and medications may modulate gene expression in lymphocytes. In the study reported here, only participants without active infections reported at the time of blood draw were enrolled. However, future studies should further assess these concerns. Although unstimulated lymphocytes may provide information about the genetic background of gene expression, it is well known that there is

almost no nucleotide excision repair in unstimulated peripheral blood lymphocytes [19]. We have also noticed that the *p27, RAD51,* and PCNA genes were not detectable in unstimulated lymphocytes under our RT-PCR conditions (unpublished data). Therefore, in future studies, stimulated lymphocytes should be used to measure the expression of nucleotide excision repair genes to provide information about cellular response to DNA damage and the status of repair machinery under stress.

In summary, our findings showed that reduced expression of *hMLH1* in lymphocytes was associated with increased risk of prostate cancer. These results suggest that DNA damage-repair pathways may be involved in prostate carcinogenesis. Studies are needed to confirm this finding and to further explore the molecular basis of the underlying mechanism.

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Figure Legend

Figure 1. Detection of expression of *hMSH2 and hMLH1* in a batch of blood samples. This is a gel photo processed by a computerized imaging system. M W, molecular weight marker (øX174 RF DNA/*Hae*III); lanes 1-3, three cases; lanes 4-6, three controls.

Figure 2. Box-and-whiskers plot of the relative expression of 2 MMR genes in cases and controls. The expression level of β-actin (100%) was used as the internal assay control to calculate the relative expression. The line inside is the median, the upper and lower edges of the boxes are the 75^{th} and 25^{th} percentiles, respectively, and the vertical bars above and below the box are the upper and lower quartiles, respectively. The open dots are outlier values.

Table 1. Distribution of Selected Variables in Prostate Cancer Cases and Controls

Variable	Cases		Controls		*P* value*
	Number	(%)	Number	(%)	
Total	70	(100)	97	(100)	
Age (in yr)					
≤ 65	42	(60)	56	(58)	
> 65	28	(40)	41	(42)	0.769
Ethnicity					
Non–Hispanic white	64	(91)	84	(87)	
Others	6	(9)	13	(13)	0.332
Smoking +					
Ever	53	(76)	48	(57)	
Never	17	(24)	35	(43)	0.017
Alcohol use +					
Current user	40	(57)	46	(53)	
Former or never user	30	(43)	40	(47)	0.648
Family history of prostate cancer					
Yes	15	(21)	6	(6)	
No	55	(79)	91	(94)	0.003

* Chi-square test for distribution.
+ Numbers do not add up to total number of the controls because of missing information.

Table 2. Differences in DNA Repair-Related Gene Expression between Cases and Controls

Gene	Mean (%)±Standard Deviation Cases (n=70)	Controls (n=97)	% Difference*	*P* value+
hMSH2	35.6 ± 22.2	44.0 ± 22.0	-19.1	0.016
hMLH1	41.0 ± 13.8	47.8 ± 14.5	-14.2	0.003

*% Difference = [(Expression$_{case}$ - Expression$_{control}$)/ Expression$_{control}$] x 100%

+Two-sided t-test.

Table 3. Logistic Regression Analysis for DNA-Repair Gene Expression Levels

Gene expression level*	Number		Crude OR (95% CI)	Adjusted OR+ (95% CI)
	Cases	Controls		
hMSH2				
HT	17	32	1.00	1.00
MT	15	33	0.86 (0.37-2.00)	0.65 (0.26-1.62)
LT	38	32	2.24 (1.05-4.75)	1.81 (0.80-4.12)
hMLH1				
HT	8	32	1.00	1.00
MT	25	33	3.03 (1.19-7.70)	2.68 (1.00-7.23)
LT	37	32	4.62 (1.87-11.46)	4.31 (1.64-11.30)

* HT, highest tertile; MT, middle tertile; LT, lowest tertile; based on controls levels.

+ Adjusted for age, smoking, and family history of prostate cancer in a logistic regression model