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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. Futhermore, 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 accrued 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, vitamin D receptor; b) relative expression levels of several mismatched repair genes (*h*MSH2 and *h*MLH1) 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 findings indicate that decreased mismatched 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 data obtained form this research to ongoing studies in our department, 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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Table of Contents

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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 expanded ongoing NCI-funded projects by adding a panel of genetic susceptibility markers and by accruing patients with metastatic disease. We evaluated constitutional markers of genetic susceptibility as predictors of prostate cancer risk including: a) polymorphisms within the vitamin D receptor, 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 are being integrated with 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.

STATEMENT OF WORK

Task 1 Subject Recruitment.

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

To the end of the funding period 125 men with metastatic prostate cancer (80% Caucasian, 12% African-Americans and 8% Hispanics) have been enrolled in this study.

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

Interviews have been completed and blood samples have been collected for all participants.

C. Abstract medical records.

Medical records for all newly accrued patients have been abstracted.

D.& E. Complete data-entry and perform quality control edit checks.

Data have been entered in databases developed for this study and edit checks for quality control have been performed.

Task 2 Evaluate Constitutional Markers of Genetic Susceptibility.

A. DNA was extracted from peripheral blood samples by the UTMDACC Institutional Molecular Core.

DNA has been extracted for 200 participants from the ongoing NCI-funded study and for all metastatic participants.

B. Complete analysis of polymorphisms related to androgen metabolism

Polymorphisms in 5-alpha-reductase gene and Vit D receptor gene have been performed. Androgen receptor polymorphism analysis has started but has not yet been completed.

Vitamin D Receptor Polymorphism (VDR).

We analyzed the VDR TaqI restriction fragment length polymorphism in codon 352 in 236 cases and 328 controls by using a polymerase chain reaction (PCR) based method. These data were presented at the American Association for Cancer Research meeting in 2000. (Appendix B). Depending on the presence or absence of the TaqI restriction site, individuals were classified as TT, Tt or tt. We found that among Caucasians, the frequency of the tt genotype was slightly lower among cases than controls (18% vs. 22%). Men homozygous for the tt genotype appeared to have a 23% reduced risk of developing prostate cancer compared with men who were TT or Tt (OR = 0.8, p = 0.3). Men with metastatic disease had a lower frequency of the tt genotype (15%) than did controls (22%) and men with nonmetastatic disease (19%), although the difference was not statistically significant.

Table 1. Distribution of VDR TaqI Genotypes by Stage among 424 Caucasians

	Number (%)			
	TT	Tt	tt	Total
Controls	74 (34.9%)	91 (42.9%)	47 (22.2%)	212
Cases				
Stage B+C	59 (40.2%)	60 (40.8%)	28 (19.0%)	147
Stage D	27 (41.5%)	28 (43.0%)	10 (15.5%)	65
All	86 (40.6%)	88 (41.5%)	38 (17.9%)	212
Total	160 (37.7%)	179 (42.2%)	85 (20.0%)	424

Having a TT or Tt genotype appeared to be a more significant risk factor for metastatic prostate cancer than for non-metastatic disease (OR = 1.6 vs. 1.2). No associations were found between VDR genotype, Gleason score and age at diagnosis. While the number of minority participants was insufficient for meaningful statistical analysis, the results suggest that the distribution of the VDR genotypes differ by ethnicity. This difference in genotype may be associated with the ethnic differences in prostate cancer risk and virulence of disease and warrants further investigation.

Table 2. Association of Genotype and Disease Stage

		Stage B + C		**Stage D**	
	Controls N=212	Cases N=147	OR (95% CI)	Cases N=65	OR (95% CI)
tt	47	28	1.0	10	1.0
Tt	91	60	1.1 (0.6-2.0)	28	1.4 (0.6-3.5)
TT	74	59	1.3 (0.7-2.5)	27	1.7 (0.7-4.2)
(TT+Tt)	165	119	1.2 (0.7-2.1)	55	1.6 (0.7-3.6)

5-α-reductase Polymorphisms

We analyzed this polymorphism in association with known clinical prognostic indicators among 280 Caucasian men with all stages of PC enrolled in ongoing PC studies conducted at the UTMDACC. DNA was isolated from peripheral lymphocytes and genotyped for the single nucleotide polymorphism in the SRD5A2 gene that results in the substitution of Valine for Leucine at codon 89 (V89L). Overall, 8% of our patients had the Leu/Leu genotype. Men with the Leu/Leu genotype tended to be older than those with Val/Val or Val/Leu genotypes (62.5 vs. 61.7 vs. 61.5 years, respectively) and had lower combined Gleason scores compared to those with Val/Val or Val/Leu (6.8 vs. 7.2 vs. 7.1, respectively). We also found that men with metastatic PC (N = 66) were slightly less likely to have the Leu/Leu genotype relative to men who were diagnosed with non-metastatic (N = 214) disease (6% vs. 8%). Data presented at AACR meeting in 2001 (Appendix B)

Table 3. Patient Characteristics by Disease Stage

Characteristic	**Overall N = 280**	**Non-Metastatic N = 214**	**Metastatic N = 66**	**P-value****
Age @ diagnosis	61.7	62.0	60.7	0.2
Education (years)	15.4	15.4	15.2	0.7
Smoking history				
Current	21 (8%)	13 (6 %)	8 (12%)	
Former	79 (28%)	136 (64%)	44 (67%)	
Never	180 (64%)	65 (30%)	14 (21%)	0.1
Family history of PC*				
Yes	57 (20%)	41 (19%)	16 (24%)	
No	223 (80%)	173 (81%)	50 (76%)	0.4
5-alpha-reductase genotype				
Val/Val	133 (48%)	100 (47%)	33 (50%)	
Val/Leu	126 (45%)	97 (45%)	29 (44%)	
Leu/Leu	21 (7%)	17 (8%)	4 (6%)	0.8
Gleason score (mean)	7.1	6.9	8.0	< 0.001

* Family history of PC among first degree relatives (e.g., father, brothers and sons)
** P-values calculated for Non-Metastatic Cases vs. Metastatic Cases

Table 4. Patient Characteristics by Genotype

Characteristic	Val/Val N = 133	Val/Leu N = 126	Leu/Leu N = 21	P-value
Age @ diagnosis	61.7	61.5	62.5	0.8
Education (years)	15.7	15.2	14.1	0.06
Smoking history				
Current	6 (5%)	11 (9%)	4 (19%)	
Former	87 (65%)	78 (62%)	15 (71%)	
Never	40 (30%)	37 (29%)	2 (10%)	0.07
Family history of PC*				
Yes	25 (19%)	29 (23%)	3 (14%)	
No	108 (81%)	97 (77%)	18 (86%)	0.5
Disease stage				
Non-Metastatic	100 (75%)	97 (77%)	17 (81%)	
Metastatic	33 (25%)	29 (23%)	4 (19%)	0.8
Gleason score (mean)	7.2	7.1	6.8	0.3

* Family history of PC among first degree relatives (e.g., father, brothers and sons)

Table 5. Patient Characteristics by Stage & Genotype

	Val/Val		Val/Leu		Leu/Leu	
	Non-Met N=100	Metastatic N = 33	Non-Met N = 97	Metastatic N = 29	Non-Met N = 17	Metastatic N = 4
Age @ diagnosis	62.2	60.4	61.7	60.8	62.7	62.0
Education (years)	15.7	15.5	15.3	15.1	14.0	14.5
Smoking history						
Current	4 (4%)	2 (6%)	5 (5%)	6 (21%)	4 (24%)	0 (0%)
Former	65 (65%)	22 (67%)	59 (61%)	19 (66%)	12 (71%)	3 (75%)
Never	31 (31%)	9 (27%)	33 (34%)	4 (14%)	1 (6%)	1 (25%)
Family history of PC*						
Yes	19 (19%)	6 (18%)	19 (20%)	10 (35%)	3 (18%)	0 (0%)
No	81 (81%)	27 (82%)	78 (80%)	19 (65%)	14 (82%)	4 (100%)
Gleason score (mean)	6.9	8.2	6.8	7.9	6.7	7.3

*Family history of PC among first degree relatives (father, brothers and sons)

In conclusion, these data suggest a role in PC progression for the SRD5A2 V89L polymorphism. Men with the Leu allele (Val/Leu & Leu/Leu genotypes) were diagnosed with lower grade prostate cancer at a later age and were less likely to be diagnosed with metastatic disease than men with the Val/Val or Val/Leu genotype. Larger studies to confirm these findings are necessary.

C. Determine expression levels of DNA mismatch repair and radiosensitivity related genes.

Using a novel multiplex RT-PCR assay, mismatch repair gene expression levels were determined among 70 prostate cancer cases and 97 healthy controls. A manuscript with these results is in press in *The Prostate* (Appendix A). Overall, the cases had lower expression levels for these genes than did healthy controls. The data below summarize these findings.

Table 6. Mismatch Repair Gene Expression: case-control

		Number		
Gene	Expression Level*	Cases (n =70)	Controls (n = 97)	OR (95% CI)
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 the expression of radiosensitivity genes, GADD45, XRCC1, and ATM. (Appendix B)

Table 7. Gene Expression

		Number		
Gene	Expression Level*	Cases (n =70)	Controls (n = 97)	OR (95% CI)
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*

Task 3 **Determine Microsatellite Instability in Tissue Samples.**

DNA has been extracted from tumor and normal tissue in a subset of cases to determine the frequency of replication errors

For identifying microsatellite instability (MSI) at a given locus, we used the definition of MSI recommended at "The International Workshop on Micrisatellite Instability and RER Phenotypes in Cancer Detection and Familial Predisposition" held in Bethesda MD, December, 8-9, 1997. MSI was defined as any length change, due to either insertion or deletion of repeating units, in a microsatellite within a tumor when compared to normal tissue (Boland et al. in press). It was stressed that MSI, as defined above, does not describe a particular tumor phenotype, but refers only to the observation of instability at a given marker. One of our goals in this proposal was to evaluate these markers in order to determine if they are useful in detecting MSI in prostate cancer.

For colorectal cancer, several studies have shown that a subset of colorectal cancers demonstrates the phenomenon of MSI and that such tumors can be divided into three groups according with the frequency of instability (Thibodeau et al., 1998).

The panel of five markers that were recommended by the workshop were two mononucleotide repeats (*BAT25, BAT26*) and three dinucleotide repeats (*D2S123, D5S346, and D17S250*). A tumor was defined as MSI-H if instability was detected in two or more of these markers. They were MSI-L if only one of the five markers displayed instability, and if none of the markers displayed instability, they were they were classified as MSI-L/MSS. We tested this panel of markers on 25 prostate cancers.

For the collection of tissue for the present study, normal and tumor sample were obtained from different regions of the sections of tissue by means of microdissection. Dr. Patricia Truncoso, a pathologist at our institution. DNA was extracted from formalyn fixed tissue using a Qiagen kit for DNA extraction from formalyn fixed tissue. We tested for MSI using the NCI panel. Following PCR the samples were analyzed on an automated ABI model 373 automated DNA sequencer equipped with gene scan software.

We decided to test 25 prostate cancers to obtain preliminary data. Three tumors displayed MSI at one locus and were therefore classified as MSI-L. For two of these, the locus displaying instability was BAT 25, for the remaining tumor, the instability was observed in D17S250. In one of the patients with a tumor displaying instability at the BAT25, had a second tumor which displayed MSI at two loci (BAT 25 and D5S346) and was classified at MSI-H. For the other patient with MSI at the BAT 25 locus, there was only one tumor to examine. We examined a second tumor from the third patient displaying MSI at D17S250, and did not find MSI any of the loci. This later tumor along with the other the remaining tumors which did not display MSI at any of the five loci were classified as MSI-L/MSS.

The overall conclusion from this study is that prostate cancer does not display a high level of microsatellite instability when the NCI panel and criteria described above are used. We observed MSI in at least one tumor for 3/16 cases (19%), with one of these cases having two tumors with MSI (one being MSI-H and one being MSI-L). However, the number of microsatellite markers displaying instability was low relative to tumors from HNPCC where the

number of positive markers was found, on the average, to be around 80% in our hands. The finding that MSI was seen in two tumors from the same patient suggests the possibility of an underlying genetic defect.

Task 4 **Final Analysis and Preparation of Reports.**

A. Marker data have been analyzed and integrated with clinical and epidemiologic and clinical data.

B. We have submitted one manuscript and four abstracts for publication. (Appendix A & B)

C. Data generated from this proposal were included as preliminary data in a SPORE grant on prostate cancer submitted to NIH in June 2000. This proposal has been reviewed and approved for funding.

KEY RESEARCH ACCOMPLISHMENTS

- 125 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 and polymorphism assays have been established and utilized.
- 300 samples have been processed for molecular analyses.
- MIN analysis has been performed in a small sample of tumor and normal tissue.

REPORTABLE OUTCOME

- One manuscript (Appendix A) is in press in The Prostate.
- Two manuscripts are being prepared for publication
- Four abstracts have been presented at different scientific meetings. (Appendix B).

CONCLUSIONS

Our findings suggest 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. Results from the analysis of the genetic polymorphisms would suggest that some of them may play a role in prostate cancer prognosis. Further and larger studies are needed to confirm these findings and to further explore the molecular basis of the underlying mechanisms of prostate cancer etiology and progression.

REFERENCES

Strom SS, Spitz MR, Yamamura Y, Babaian RJ, Scardino PT, Wei Q. Reduced Expression of hMSH2 and hMLH1 and Risk of Prostate Cancer: A Case-control. The prostate 47:1-7, 2001.

Strom SS, Spitz MR, Guan Y, Yamamura Y, Babaian RJ, Scardino P, Wei Q. Reduced Expression of DNA-repair Related Genes in Prostate Cancer. AACR March 1998.

Strom SS, Duphorne CM, Yamamura Y, Hursting SD, Spitz MR. Sources of Phytoestrogens in the American Diet. ASPO March 2000.

Strom SS, Zhang Q, Spitz MR, Yamamura Y, Babaian RJ, Logothetis CJ, Tu S-M, Wei Q. Vitamin D Receptor Polymorphism and Prostate Cancer Risk. AACR March 2000.

Yamamura Y, Thompson PH, Spitz MR, Babaian RJ, Logothetis CJ, Strom SS. Role of 5-alpha Reductase Polymorphisms in Prostate Cancer Progression. AACR March 2001.

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APPENDIX A

PAPER IN PRESS

Reduced Expression of *hMSH2* and *hMLH1* and Risk of Prostate Cancer: A Case-Control Study

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BACKGROUND. Although prostate cancer is the most common incident cancer in men, not much is known about its etiology. We tested the hypothesis that expression levels of *hMSH2* and *hMLH1* in unaffected (normal) tissue play a role in the etiology of prostate cancer.
METHODS. Total RNA was extracted from peripheral blood lymphocytes of subjects ascertained by a case-control study (70 patients and 97 age- and ethnicity-matched controls). A multiplex reverse transcription-polymerase chain reaction assay was used to simultaneously evaluate the relative expression of *hMSH2* and *hMLH1*, using β-actin as the internal control.
RESULTS. The relative gene expression levels of *hMSH2* and *hMLH1* were significantly lower in cases (37 and 41%, respectively) than in controls (44 and 48%, respectively) ($P < 0.05$ for both genes). When compared with the highest tertile of the controls, low expression levels (the middle and lowest tertiles) of *hMLH1* were associated with significantly increased risk of prostate cancer in a dose–response relationship (ORs = 2.68, and 4.31; 95% confidence interval = 1.00–7.23 and 1.64–11.30, respectively) after adjustment for age, ethnicity, smoking status, and family history of prostate cancer.
CONCLUSIONS. These results suggest that reduced expression of *hMLH1* in peripheral lymphocytes may be a risk factor for prostate cancer. However, it cannot be ruled out that the reduced expression we observed may be caused by the disease status. Our finding and the factors that may affect the expression of *hMLH1* need further confirmation in larger prospective studies. *Prostate 47:1–7, 2001.* © 2001 Wiley-Liss, Inc.

KEY WORDS: **biomarker; gene expression; mismatch repair; molecular epidemiology; polymerase chain reaction**

INTRODUCTION

Prostate cancer is the most common incident cancer in men; yet little is known about its etiology. Although mutations and polymorphisms in androgen receptor genes suggest that male hormones may play a role in the etiology of prostate cancer [1], genetic determinants of prostate cancer remain largely unknown. Abnormalities in several genes, including tumor suppressor genes such as *p53* [2], *MXI1* [3] and *PTEN/MMAC1* [4], and cell-cycle related genes such as *RB* [5] and *p16* [6], have been identified in prostate tumors, but their mutation frequencies are relatively rare. As normal cell-cycle arrest is necessary for DNA repair [7],

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

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somatic mutations and genetic alterations in prostate cancer tumors in genes controlling cell-cycle progression and cell growth suggest that abnormalities in DNA repair may be involved in prostate cancer etiology.

It has been suggested that a mutator phenotype may be involved in the development of cancer [8]. This phenotype is characterized by microsatellite instability (MSI) as a result of deficiencies in DNA mismatch repair [9,10]. In prostate cancer, MSI, known as dinucleotide tandem repeats sequences, has been found in up to 45% of tumors [11–13]. Studies suggest that MSI may be an early event in prostate carcinogenesis, but not a marker for progression or prognosis [14,15]. Although MSI is frequently found in prostate cancer, mutations in two major mismatch repair genes *hMSH2* and *hMLH1* are relatively rare, and studies of these genes have been limited to a few established prostate cell lines [16,17]. It has been recently postulated that the methylation status of *hMLH1*, particularly in its promoter region, regulates gene expression and is linked to MSI [18–21]. This finding suggests that aberrant gene expression may be epigenetic and can be caused by factors other than mutations in the genes.

There are no published data on an association between MSI and methylation status of *hMSH2* and *hMLH1* or expression level of these two genes in prostate cancer. Epidemiologically, expression levels of *hMSH2* and *hMLH1* in unaffected (normal) tissues of prostate cancer patients and their role in the etiology of prostate cancer are of interest. To evaluate the expression levels of *hMSH2* and *hMLH1* simultaneously, we modified our previously published multiplex reverse transcription-polymerase chain reaction assay [22,23] to measure the relative expression of these two genes in peripheral blood lymphocytes as a surrogate tissue. We then conducted a pilot case-control study to test the hypothesis that the low expression levels of *hMSH2* and *hMLH1* are associated with increased risk of prostate cancer. In this report, we describe a significant association between low expression of *hMLH1* and increased risk of prostate cancer in a study of 70 prostate cancer patients treated with radical prostectomy and 97 healthy controls.

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 enrolled between 1997 and 1998 in an ongoing molecular epidemiologic case–case study. 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 $\geq$4 mg/ml, an abnormal rectal digital examination, or previous history of cancer were excluded. The second group of controls (25%) was selected from among male members of a large multi-specialty managed care organization. Only subjects without a history of cancer or urological conditions were included as controls. The cases and controls were matched on age ($\pm$5 years) and ethnicity. After written 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

Because we have found that it is difficult to extract sufficient RNA from blood samples drawn more than 24 hr before processing, we extracted total RNA from all the samples that had been processed with the Tri-Reagent, a RNA/DNA/protein isolation reagent (Molecular Research Center, Cincinnati, OH), within 8 hr of procurement in this study. On each sample, we performed a multiplex RT-PCR assay using the β-actin gene as an internal control [22,23] 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 previously published multiplex RT-PCR protocol, in which the strategy for choosing the primers has been described [22,23]. 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, 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 5 × 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 at 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, 1 × 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 with a Perkin-Elmer GeneAmp PCR System 2400 (Foster City, CA) by an initial denaturation step of 95°C for 5 min; 29 cycles of denaturation at 95°C for 30 sec, primer annealing at 59°C for 30 sec, and extension at 72°C for 45 sec; 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 [21]. The assays were performed in batches of 6–8 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 captured as an electronic photo [Fig. 1]. The bands on the photos were then 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 (100%). These values were then compared across the samples tested. Reduced expression was verified by repeating the multiplex RT-PCR assay.

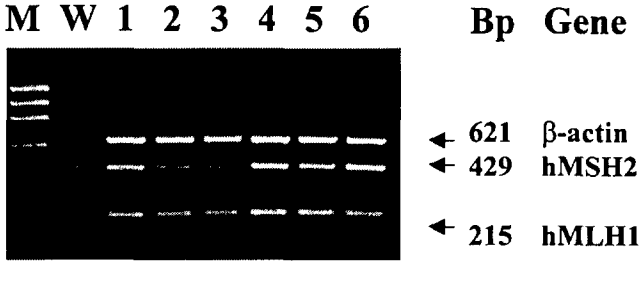

Fig. 1. Detection of expression of *hMSH2* and *hMLH1* in a batch of blood samples. This is a gel photo processed by a computerized imaging system. MW, molecular weight marker (øX174 RF DNA/*Hae*III); lanes 1–3, three cases; lanes 4–6, three controls.

Tumor-Related Measurements

Combined Gleason Score describing the histological differentiation pattern of a tumor was recorded for each patient. Plasma samples were used to measure testosterone levels by enzyme-linked immunosorbent assays and dihydrotestosterone by radioimmunoassay in a subset of case 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 difference in the distribution of demographic variables. The difference in the expression level of each gene (as a continuous variable) between cases and controls was evaluated by using Student's *t*-test. Pearson correlation coefficients were calculated to evaluate correlation among demographic factors, smoking status, alcohol use, family history of prostate cancer, testosterone levels, and gene expression values. Crude odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for demographic, smoking status, family history of prostate cancer in first degree relatives (father, brother or son), and gene expression levels. Men who smoked more than 100 cigarettes in their lifetimes were considered "ever smokers." Gene expression values were then categorized into tertiles based on the expression level of the controls and the ORs were estimated as the risks of those in the lowest tertile compared with those in the highest tertile of gene expression. The ORs were also adjusted for important covariates such as age, smoking status, and family history of prostate cancer in the multivariate logistic regression analysis. To perform the linear trend test, the tertile dummy variables were recoded as one continuous variable (1 to 3 for the lowest to highest tertile) and fitted into a logistic regression model with and without adjustment for the covariates. All statistical tests were two-sided and were performed with Statistical Analysis System software (Version 6; SAS Institute Inc., Cary, NC).

RESULTS

There were 70 cases and 97 controls included in this study (Table I). 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 ($P = 0.769$ and $P = 0.332$, respectively). The mean age of the cases (mean±SD, 64.4±7.5 years) was higher than that of the controls (63.1±7.0 years), but this difference was not statistically significant ($P = 0.252$) (Table II). A higher percentage of cases (76%) than controls (57%) reported being ever smokers ($P = 0.017$), whereas the prevalence of current drinking was similar in cases (57%) and controls (53%) ($P = 0.648$). More cases (21%) than controls (6%) reported having a first-degree relative (father, brother, or son) with prostate cancer ($P < 0.001$) (Table I). Therefore, the important covariates including age, smoking status, and family history of cancer were further adjusted for in multivariate logistic regression analysis.

For both cases and controls, there were considerable variations in the expression levels of *hMSH2* and *hMLH1*, but the mean expression level was statistically significantly lower in the cases than in the controls for both *hMSH2* and *hMLH1* ($P = 0.016$ and $P = 0.003$, respectively) (Table II). The expression levels of these two genes were not significantly correlated with age or smoking status, in either the cases or the controls (data not shown), suggesting that these factors did not have an effect on the expression levels. Furthermore, there

TABLE I. Distribution of Selected Variables in Prostate Cancer Cases and Healthy Controls

	Cases		Controls		
Variable	Number	(%)	Number	(%)	*P* value[a]
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[b]					
Ever	53	(76)	48	(57)	
Never	17	(24)	35	(43)	0.017
Alcohol use[b]					
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

[a]Chi-square test for distribution.
[b]Numbers do not add up to total number of the controls because of missing information.

TABLE II. Differences in Age and Gene Expression of *hMSH2* and *hMLH1* between Prostate Cancer Cases and Healthy Controls

	Mean (%)±SD			
	Cases (n = 70)	Controls (n = 97)	% Difference[a]	*P* value[b]
Age (in yr)	64.4 ± 7.5	63.1 ± 7.0		0.252
Gene				
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

[a]% Difference = $[(\text{Expression}_{case} - \text{Expression}_{control})/\text{Expression}_{control}] \times 100\%$.
[b]Two-sided *t* test.

TABLE III. Logistic Regression Analysis for Gene Expression Levels of *hMSH2* AND *hMLH1* in Prostate Cancer and Healthy Controls

Gene expression level[a]	Number		Crude OR (95% CI)	Adjusted OR[b] (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)
Trend test[b]			$P = 0.023$	$P = 0.101$
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)
Trend test[b]			$P = 0.001$	$P = 0.004$

[a]HT, the highest tertile; MT, the middle tertile; LT, lowest tertile; based on controls levels.
[b]Adjusted for age, smoking status, and family history of prostate cancer in a logistic regression model.

was no correlation between expression levels of these two genes ($r = 0.02$, $P = 0.76$).

We explored the correlation between serum testosterone and dihydrotestosterone levels and gene expression among the cases. We found a borderline correlation between the expression level of *hMSH2* and testosterone ($r = -0.26$, $P = 0.06$) but no correlation between the expression level of *hMLH1* and testosterone ($r = -0.07$, $P = 0.62$). We also explored the relationship between the combined Gleason Score and the expression levels. Cases who had a score 7 and above had similar expression levels of the two genes compared with cases who had a score less than 7 (data not shown). These data indicate that the gene expression was not influenced by hormone level or tumor status.

Crude and adjusted ORs were derived from logistic regression analysis and are shown in Table III. 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 with adjustment for age, smoking status, and family history of prostate cancer.

DISCUSSION

In this case–control study, we evaluated the association between the relative gene expression levels of *hMSH2* and *hMLH1* and prostate cancer risk by using a multiplex RT-PCR assay. We found an association between reduced expression of *hMLH1* and prostate cancer risk that was independent of age, smoking status or family history of prostate cancer. Although the reduction in expression of these genes varied from individual to individual, there was reduced expression of both genes in cases compared with healthy controls. Because the patients were newly diagnosed with prostate cancer and gene expression was measured in peripheral lymphocytes that were unlikely affected by disease status, the findings support our hypothesis that reduced mismatch gene expression may be associated with risk of prostate cancer.

To the best of our knowledge, data on mutations of *hMLH1* and *hMSH2* in prostate cancer tumor tissue have not been previously reported, although some studies of prostate cell lines have exhibited MSI and deficient mismatch repair [16,17]. While there are no data that demonstrate a link between MSI and mutations in *hMLH1* and *hMSH2* or between MSI and the expression levels of these two genes in prostate cancer, there are several lines of evidence that suggest that aberrant expression of these genes may be involved in human carcinogenesis.

It is possible that changes in gene expression can be influenced by both genetic and epigenetic factors and may play a role in carcinogenesis [24], and changes in the expression of *hMLH1* and *hMSH2* may be associated with alterations of mismatch repair function [18,25]. Defective mismatch repair is believed to be responsible for MSI [9,10]. Several studies have analyzed MSI in prostate cancer and have reported frequencies up to 45% [11–13]. Recently, the overall rarity of mutations of *hMLH1* and its relatively frequent aberrant expression seen in tumors are shown to be associated with hypermethylation status

of this gene [18–20]. In contrast, the cancers displaying hMLH1-protein expression were not methylated in the *hMLH1*-promoter region [20]. These data suggest that there is a significant association between the *hMLH1* expression level and genetically or epigenetically controlled level of hypermethylation of its promoter in tumors with MSI. Our data in this report are consistent with previous findings of reduced expression of *hMLH1* in gliomas with MSI [26,27] and in lymphocytes of head and neck cancer [28] and colon cancer patients [29]. Taken together, available data support our finding that low expression of *hMLH1* is a risk factor of cancer and may play a role in the etiology of prostate cancer.

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 processes. 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 activity in unstimulated peripheral blood lymphocytes [30]. We have also noticed that the *p27*, *RAD51*, and *PCNA* genes were not detectable in unstimulated lymphocytes under our RT-PCR conditions (unpublished data). Although we have demonstrated that the expression levels of several nucleotide repair genes in both stimulated and unstimulated lymphocytes were similar [31], the expression levels of *hMLH1* or *hMSH2* in stimulated and unstimulated lymphocytes should be compared in future studies.

In conclusion, our findings show that reduced expression of *hMLH1* in lymphocytes was associated with increased risk of prostate cancer, suggesting that DNA damage-repair pathways may be involved in prostate carcinogenesis. However, it cannot be ruled out that the reduced expression we observed may be caused by the disease status. Our finding and the factors that may affect the expression of *hMLH1* need further confirmation in larger prospective studies with prediagnostic specimen and in prostate tissue. There is also a need to further explore the molecular basis of the underlying mechanism of such low expression. Studies of associations between MSI and expression level or hypermethylation of mismatch repair genes in prostate tumors and between hypermethylation and expression level of mismatch repair genes in normal tissues are critical for illustrating their involvement in the etiology of prostate cancer.

ACKNOWLEDGMENT

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REFERENCES

1. Taplin ME, Bubley GJ, Shuster TD, Frantz ME, Spooner AE, Ogata GK, Keer HN, Balk SP. Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. New Engl J Med 1995;332:1393–1398.
2. Gumerlock PH, Chi SG, Shi XB, Voeller HJ, Jacobson JW, Gelmann EP, deVere, White RW. *p53* Abnormalities in primary prostate cancer: Single-strand conformation polymorphism analysis of complementary DNA in comparison with genomic DNA. The Cooperative Prostate Network. J Natl Cancer Inst 1997;89:66–71.
3. Eagle LR, Yin X, Brothman AR, Williams BJ, Atkin NB, Prochownik EV. Mutation of the *MXI1* gene in prostate cancer. Nat Genet 1995;9:249–255.
4. Whang YE, Wu X, Suzukl H, Reiter RE, Tran C, Vessella RL, Said JW, Isaacs WB, Sawyers CL. Inactivation of the tumor suppressor PTEN/MMAC1 in advanced human prostate cancer through loss of expression. Proc Natl Acad Sci USA 1998;95:5246–5250.
5. Miyoshi Y, Uemura H, Fujinami K, Mikata K, Harada M, Kitamura H, Koizumi Y, Kubota Y. Retinoblastoma gene mutations in primary human prostate cancer. Prostate 1995;27:314–320.
6. Jarrard DF, Bova GS, Ewing CM, Pin SS, Nguyen SH, Baylin SB, Cairns P, Sidransky D, Herman JG, Isaacs WB. Deletional, mutational, and methylation analysis of CDKN2 (p16/MTS1) in primary and metastatic prostate cancer. Genes Chromosomes Cancer 1997;19:90–96.
7. Kastan MB, Zhan Q, El-Deiry WS, Carrier F, Jacks T, Walsh WV, Plunkett BS, Vogelstein B, Fornace AJJr. A mammalian cell cycle checkpoint pathway utilizing *p53* and GADD45 is defective in ataxia-telangiectasia. Cell 1992;71;587–597.
8. Loeb L. Mutator phenotype may be required for mutistage carcinogenesis. Cancer Res 1991;51:3075–3079.
9. Kinzler KW, Vogelstein B. Lessons from hereditary colorectal cancer. Cell 1996;87:159–170.
10. Fishel R, Wilson T. MutS homologs in mammalian cells. Curr Opin Genet Dev 1997;7:105–113.
11. Dahiya R, Lee C, McCarville J, Hu W, Kaur G, Deng G. High frequency of genetic instability of microsatellites in prostatic adenocarcinoma. Int J Cancer 1997;72:762–767.
12. Watanabe M, Shiraishi T, Muneyuki T, Nagai M, Fukutome K, Murata M, Kawamura J, Yatani R. Allelic loss and microsatellite instability in prostate cancers in Japan. Oncology 1998;55:569–574.
13. Perinchery G, Nojima D, Goharderakhshan R, Tanaka Y, Alonzo J, Dahiya R. Microsatellite instability of dinucleotide tandem repeat sequences is higher than trinucleotide, tetranucleotide and pentanucleotide repeat sequences in prostate cancer. Int J Oncol 2000;16:1203–1209.
14. Crundwell MC, Morton DG, Arkell DG, Phillips SM. Genetic instability in incidentally discovered and advanced prostate cancer. Br J Urol Int 1999;123–127.

15. Rohrbach H, Haas CJ, Baretton GB, Hirschmann A, Diebold J, Behrendt RP, Lohrs U. Microsatellite instability and loss of heterozygosity in prostatic carcinomas: Comparison of primary tumors, and of corresponding recurrences after androgen-deprivation therapy and lymph-node metastases. Prostate 1999;40:20–27.
16. Boyer JC, Umar A, Risinger JI, Lipford JR, Kane M, Yin S, Barrett JC, Kolodner RD, Kunkel TA. Microsatellite instability, mismatch repair deficiency, and genetic defects in human cancer cell lines. Cancer Res 1995;55:6063–6070.
17. Rasmussen LJ, Rasmussen M, Lutzen A, Bisgaard HC, Singh KK. The human cyclin B1 protein modulates sensitivity of DNA mismatch repair deficient prostate cancer cell lines to alkylating agents. Exp Cell Res 2000;257:127–134.
18. Kuismanen SA, Holmberg MT, Salovaara R, de la Chapelle A, Peltomaki P. Genetic and epigenetic modification of MLH1 accounts for a major share of microsatellite-unstable colorectal cancers. Am J Pathol 2000;156:1773–1779.
19. Toyota M, Ahuja N, Suzuki H, Itoh F, Ohe-Toyota M, Imai K, Baylin SB, Issa JP. Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. Cancer Res 1999;59:5438–5442.
20. Yanagisawa Y, Akiyama Y, Iida S, Ito E, Nomizu T, Sugihara K, Yuasa Y, Maruyama K. Methylation of the hMLH1 promoter in familial gastric cancer with microsatellite instability. Int J Cancer 2000;85:50–53.
21. Bevilacqua RA, Simpson AJ. Methylation of the *hMLH1* promoter but no *hMLH1* mutations in sporadic gastric carcinomas with high-level microsatellite instability. Int J Cancer 2000;87:200–203.
22. Wei Q, Xu X, Cheng L, Legerski RJ, Ali-Osman F. Simultaneous amplification of four DNA repair genes in lymphocytes by multiplex reverse transcriptase-polymerase chain reaction. Cancer Res 1995;55:5025–5029.
23. Wei Q, Guan Y, Cheng L, Radinsky R, Bar-Eli M, Tsan R, Li L, Legerski RJ. Expression of selected five human mismatch repair genes simutaneously detected in normal and cancer cell lines by a nonradioactive multiplex RT-PCR. Pathobiology 1997;65:293–300.
24. McLeod MC. A possible role in chemical carcinogenesis for epigenetic heritable changes in gene expression. Mol Carcinog 1996;15:241–250.
25. Thibodeau SN, French AJ, Roche PC, Cunningham JM, Tester DJ, Lindor NM, Moslein G, Baker SM, Liskay RM, Burgart LJ, Honchel R, Halling KC. Altered expression of *hMSH2* and *hMLH1* in tumors with microsatellite instability and genetic alterations in mismatch repair genes. Cancer Res 1996;56:4836–4840.
26. Wei Q, Bondy ML, Mao L, Gaun Y, Cheng L, Cunningham J, Fan Y, Bruner JM, Yung WK, Levin VA, Kyritsis AP. Reduced expression of mismatch repair genes measured by multiplex reverse transcription-polymerase chain reaction in human gliomas. Cancer Res 1997;57:1675–1677.
27. Kanamori M, Kon H, Nobukuni T, Nomura S, Sugano K, Mashiyama S, Kumabe T, Yoshimoto T, Meuth M, Sekiya T, Murakami Y. Microsatellite instability and the *PTEN1* gene mutation in a subset of early onset gliomas carrying germline mutation or promoter methylation of the *hMLH1* gene. Oncogene 2000;19:1564–1571.
28. Wei Q, Eicher SA, Guan Y, Cheng L, Xu J, Young LN, Saunders KC, Jiang H, Hong WK, Spitz MR, Strom SS. Reduced expression of *hMLH1* and *hGTBP*: A risk factor for head and neck cancer. Cancer Epidemiol Biomarkers Prev 1998;7:309–314.
29. Soliman AS, Bondy ML, Guan Y, El-Badawi S, Mokhtar N, Bayomi S, Raouf AA, Ismail S, McPherson RS, Abdel-Hakim TF, Beasley RP, Levin B, Wei Q. Reduced expression of mismatch repair genes in colorectal cancer patients in Egypt. Int J Oncol 1998;12:1315–1319.
30. Barret JM, Calsou P, Salles B. Deficient nucleotide excision repair activity in protein extracts from normal human lymphocytes. Carcinogenesis 1995;16:1611–1616.
31. Cheng L, Guan Y, Li L, Legerski JR, Einspahr J, Alberts D, Wei Q. Expression of five nucleotide excision repair genes in human tissues measured simultaneously by multiplex reverse transcrition-polymerase chain reaction. Cancer Epidemiol Biomarkers Prev 1999;8:801–807.

APPENDIX B

ABSTRACTS

mechanisms involved in the regulation of BRCA1 transcription may provide clues regarding the manner in which BRCA1 expression is dysregulated, leading to the development of a neoplastic phenotype.

EPIDEMIOLOGY 2: High and Low Penetrance Genes in Molecular and Genetic Epidemiology

#1234 Reduced expression of DNA-repair related genes in prostate cancer. Strom, SS, Spitz, MR, Guan,Y, Yamamura, Y, Babaian, RJ, Scardino, P, Wei, Q. *University of Texas M.D. Anderson Cancer Center, Houston, TX 77030.*

Although the risk of prostate cancer (PC) associated with exposure to environmental carcinogens remains unclear, it has been hypothesized that endogenous exposure to metabolites may cause oxidative damage to DNA and therefore increase risk of PC. We conducted a case-control study to test the hypothesis that abnormal expressions of five selected genes involved in DNA repair (*GADD45, hMSH2, XRCC1, ATM* and *hMLH1*) are associated with PC risk. In this molecular epidemiologic study, we used a multiplex reverse transcription-polymerase chain reaction assay to simultaneously evaluate the relative expression of these genes in peripheral blood lymphocytes of 90 PC cases and of 97 age-sex matched healthy controls. For each gene the mean expression was significantly lower in cases than in controls ($p<.001$). The risk associated with low expression was statistically significant for *hMLH1* (OR = 4.1; 95% confidence interval (CI) = 2.0–8.3), *ATM* (OR = 2.9;CI = 1.4–5.9), and GADD45 (OR =2.8;CI =1.4–5.6) after adjustment for smoking and family history of PC. These results suggest that low expression of DNA repair related genes may be involved in prostate carcinogenesis. Further studies with a larger number of subjects are warranted to confirm these findings. Supported by grants RO1CA68578 and SPORE CA58204.

#1235 DNA repair gene variants as cancer susceptibility factors. Coleman, M., Shen, M. R., Petersen, G., Jones, I. and Mohrenweiser, H. W. *Lawrence Livermore National Laboratory, Livermore, CA, 94550 USA, Johns Hopkins University, Baltimore MD, 21205*

The repair of DNA damage has a key role in protecting cells from cancer causing agents. Recent reports suggest that individuals with reduced capacity to repair DNA damage have increased susceptibility to breast, lung and skin cancer. We report initial results from a study to estimate the DNA sequence variation in genes encoding proteins of DNA repair as a prelude to molecular epidemiology/ cancer susceptibility studies. 13 different amino acid substitution variants have been identified in resequencing of the exons of 5 nucleotide excision repair (ERCC1, XPA, XPD, XPF, RAD23A), 2 base excision repair (APE, POLB) and 2 double strand break repair/recombination (XRCC3, XRCC1) genes in a group of 12 healthy individuals. Allele frequencies for the different variants range from 0.04 - 0.45; the average variant allele frequency is 0.18. The potential that these variants, and especially the 9 non-conservative amino acid substitutions occuring at amino acid residues that are identical in human and mouse, have reduced repair capacity or fidelity of DNA repair and are associated with increased cancer risk is being addressed by genotyping of lung cancer patients and matched controls with known repair capacity phenotypes. Performed under auspices of the US DOE by LLNL; contract No. W-7405-ENG-48.

#1236 Investigation of BRCA1, ER and CYP17 as candidate genes for low-penetrance breast cancer susceptibility. Dunning, A.M., Healey, C.S., McBride,S., Pharoah, P.D.P., Teare, D., Easton, D.F., & Ponder, B.A.J. *CRC Human Cancer Genetics & Genetic Epidemiology Groups, University of Cambridge, UK.*

We hypothesise that there may be common, low-penetrance alleles which alter breast cancer predisposition in the general population. We are collecting an East Anglian, British population-based, case-control series under 65 years old (Aim: 2000 breast cancer cases and 2000 controls). This is being used in association studies to look for common alleles of candidate genes which might affect risk. To date we have examined several polymorphisms at three loci. We find no genotypes that confer significantly increased risks of breast cancer. The maximum relative risk estimates (95%CI) for each gene [genotype] are: BRCA1[LeuLeu871] 1.23(0.85,1.79), ER [PvuII-/-] 1.33 (0.90,2.01), CYP17 [MspAI-/-] 1.17(0.85,1.62). One rare protective genotype has been observed: BRCA1[ArgArg356] 0.01(0.01,0.56). It is therefore unlikely that any of these genes has a major role in common breast cancer predisposition.

#1237 Different patterns of germline BRCA1 gene mutations detected in the young minority breast cancer patients. Shen, D., Subbarao, M., Chillar, R. and Vadgama, J.V. *Department of Medicine, Charles R. Drew University of Medicine and Science, Los Angeles, CA 90059.*

Minority women, especially African American women in the U.S. have increased rate of incidence and mortality from breast cancer compared with Caucasian women. To uncover the molecular mechanism behind this disproportional distribution, we analyzed the germline mutations of a recently defined breast cancer susceptibility gene BRCA1 in our breast cancer patients. BRCA1 exon 2 and exon 11 mutations were screened by PCR-SSCP and PCR-NIRCA methods, respectively. We screened 10 African American breast cancer patients and our data revealed 2 frameshift mutations (3331 insG and 3654A→TC) and 3 missense sequence variants (3537A→G, 3667A→G and 4009C→T) at exon 11. Each sequence change was confirmed by automatic DNA sequencing. In contrast to the African American patients, from the 15 Hispanic early onset breast cancer patients we screened, no frameshift mutation was observed. However 1 missense sequence variant (3667A→G) was detected. In addition to breast cancer, we also screened 25 other cancer patients. No mutation was detected. However, 3 missense sequence variants were observed. The two frameshift mutations found in African American breast cancer patients have not yet been reported. Our data suggested that young minority breast cancer patients may carry unique BRCA1 gene mutations, which may be distinct from those reported in Caucasian women.

#1238 Germline mutations of BRCA1 and BRCA2 genes in a population-based series of Caucasian and African-American women with breast cancer. Mu, H., Butler, L., Gold, D., King, M-C, and Newman, B. *University of Washington, Seattle, WA 98195, University of North Carolina, Chapel Hill, NC 27599*

Germline mutations in the BRCA1 and BRCA2 genes predispose women to breast cancer and together attribute to over 80% of familial cases. However, their frequency and significance among breast cancer patients in the general population are not well understood. We previously reported preliminary results on BRCA1 mutations of the Carolina Breast Cancer Study, a population-based case-control study of breast cancer among women between 20–74 years who resides in a 24-county area of central and eastern North Carolina. Using a combination of multiplex SSCP analysis, protein truncation test, and direct DNA sequencing, we have completed an extensive screening of the BRCA1 sequence among 126 Caucasian and 88 African-American incidence cases of invasive breast cancer. In addition, the 50 breast cancer cases from this series with the youngest age of diagnosis (≤35 years) or highest-risk families have also been screened for the entire coding region and intron-exon boundaries of the BRCA2 gene. A series of 400 controls, matched for age, race, and residence, were genotyped to distinguish rare disease-associated mutations vs. rare polymorphisms. Multiple distinct mutations in BRCA1 and BRCA2 genes, including novel mutations and founder mutations, have been identified among these patients. A polymorphism in the 3'UTR of the BRCA1 gene is associated with breast cancer in African-American but not in Caucasian women. Overall, our study provides estimates of BRCA1 and BRCA2 mutation frequency and spectrum among Caucasian and African-American women in the general population and indicates considerable variation across populations.

#1239 Breast cancer susceptibility: ATM germline mutations and radiation exposure. Van 't Veer, L.J., Broeks, A., Floore, A.N., Urbanus, J.H.M., Dahler, E., Russell, N.S., Hogervorst, F.B.L., Van Leeuwen, F.E. *Departments of Pathology, Radiotherapy and Epidemiology, The Netherlands Cancer Institute, Amsterdam, The Netherlands.*

Individuals that have inherited one mutated allele of the Ataxia Telangiectasia gene (ATM) are thought to be radiation sensitive and to have a 3.9-fold increased risk for developing breast cancer. In general, radiation exposure is an established risk factor for breast cancer and ATM heterozygotes might have a disproportionally high risk. We are determining the ATM mutation frequency in high-risk breast cancer families, and in case-control studies of women who developed breast cancer subsequent to various levels of ionising radiation (i.e. Hodgkin's disease (n=200), breast cancer (n=250), and mammography (n=300)). Preliminary data reveal that breast cancer patients of 2 out of 22 high-risk families carry ATM germline mutations. Besides, ATM seems to be involved in selected breast cancer patients. The series of analysed patients is currently being extended and an update will be presented.

#1240 Impact of misclassification in studies of gene-environment interaction. Garcia-Closas M, Rothman N, Stewart WF, Lubin JH. *National Cancer Institute, Division of Cancer Epidemiology and Genetics, Rockville, MD 20852 and Department of Epidemiology, Johns Hopkins University, Baltimore, MD 21205.*

There has been increasing interest in studies that evaluate whether a exposure-disease association varies according to a genetic trait, e.g. whether a carcinogen-disease association varies according to polymorphisms in metabolizing enzymes. The authors investigated the impact of exposure misclassification on the sample size required to detect a supra-multiplicative gene-environment interaction, where the genetic and environmental exposures are dichotomomized. The impact of misclassification was evaluated under scenarios with varying exposure and genotype prevalences, magnitude of the interaction and degree of misclassification. Overall, for typical study parameters, sensitivity of exposure assessment had a stronger impact on sample size than specificity. In particular, for common exposures, imperfect sensitivity dramatically increased the required sample size to attain adequate power, calling into question the feasibility of the study. Furthermore, small genotype errors, e.g. 95% sensitivity, substantially increased the required sample size, emphasizing the importance of including quality control samples in order to assess and improve genotype accuracy.

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Sources of phytoestrogens in the American diet: experience from a case-control study.

SS Strom, CM Duphorne, Y Yamamura, SD Hursting, MR Spitz
The Univ. of Texas M.D. Anderson Cancer Center, Houston, TX 77030

Phytoestrogens have been postulated to have anticarcinogenic properties. In a recently published study, we examined prostate cancer (PC) risk and phytoestrogen intake in a group of 190 white American men, and we reported that men with PC consumed smaller amounts of genistein, daidzein, and coumestrol than controls. Here we present the major food sources of these nutrients. Foods were ranked beginning with the main nutrient source. For controls, 75% of the daily average intake of genistein is provided by consuming the equivalent of 1.5 in^3 of tofu, 5 oz of breakfast drinks, 2 teaspoon (tsp) of soybeans, and 1 tsp of imitation bacon bits on a weekly basis. On average, PC cases received half the amount of genistein from tofu and breakfast drinks, none from soybeans, and more than twice the amount from imitation bacon bits. Tofu (1.3 in^3), soybeans (2 tsp), and bacon bits (1 tsp) provided most of the daidzein in the controls as compared to bacon bits (3 tsp), miso soup (1 tsp), and tofu (1 in^3) in cases. Refried/pinto beans provided more than 95% of coumestrol in both groups (equivalent to ½ cup). Main difference in quercetin intake was due to cranberry juice (controls, ½ cup vs. 1 cup in cases), since both groups consumed similar amounts of black tea, onions and apples. Similarly, cases consumed twice the amount of myricetin from cranberry juice (9 oz controls, 19 oz cases) and comparable amounts from tea and red wine. Our data suggest that the food sources of phytoestrogens are similar in cases and controls, but amounts consumed differ. Since many of these foods are already integrated into the American diet, any nutritional interventions developed should capitalize on these phytoestrogen sources.

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null genotype has been reported to be a risk factor for lung cancer. We investigated the association of combined polymorphisms of these genotypes with lung cancer risk in 482 lung cancer patients and 510 controls from Massachusetts General Hospital in Boston. The results showed that for adenocarcinoma (n=243) there was evidence for interaction of these genotypes. The relative risk of the combination of *GSTM1-* and *p53 PP* was calculated using the combined *GSTM1+* and *p53 AA* genotypes as reference. The OR for the combined high risk genotype was 2.10 (95%CI 1.09-3.98). The OR for combined *CYP1A1 V/V+I/I* and *p53 PP* was 2.99 (95%CI 1.17-7.60), using combined *CYP1A1 I/I* and *p53 AA* as reference. There were no significant increases in ORs for the combined null *GSTT1* and *p53 PP* genotype (OR=2.2, 95%CI=0.87, 5.63), nor for the combined *CYP1A1 M2/M2* +M1/M2 and *p53 PP* genotypes (OR=1.84, 95%CI=0.72, 4.72), using the combination of *GSTT1+* with *p53 AA* and the combination of *CYP1A1 M1/M1* with *p53 AA* as respective referents. These results suggest that the *p53 PP* genotype may increase the risk of the *GSTM1-* genotype, and *CYP1A1 V/V* genotype associated with adenocarcinoma of the lung.

#2043 MUTAGEN SENSITIVITY AND DNA REPAIR CAPACITY (DRC) AS RISK FACTORS FOR NON-SMALL CELL LUNG CANCER. Peter Schmezer, N Rajaee-Behbahani, A Risch, S Thiel, W Rittgen, K Kayser, H Dienemann, V Schulz, P Drings, and H Bartsch, *German Cancer Res Ctr, Heidelberg, Germany, and Thoraxklinik Heidelberg-Rohrbach, Heidelberg, Germany*

An alkaline single cell gel electrophoresis assay has been standardised by which mutagen sensitivity and DNA repair capacity (DRC) can be measured in cryopreserved peripheral blood lymphocytes following induction and repair of DNA damage induced by bleomycin. In an ongoing case-control study, we have applied this assay to Caucasian patients with non-small cell lung cancer (n=160) and respective tumor-free hospital controls (n=180). After adjustment for age, gender and smoking status, we found a statistically significant association between increased mutagen sensitivity (OR=4, 95%CI 2.2-7.4), reduced DRC (OR=2.1, 95%CI 1.1-4) and elevated risk for non-small cell lung cancer. Both markers emerged as independent cancer risk factors. We have also tested whether cryopresevation influences mutagen sensitivity and DRC. When fresh lymphocytes were compared with cryopreserved samples, there was no significant influence of cryopreservation time (1 to 371 days) neither on the amount of bleomycin-induced damage nor on the individual DRC as repeatedly measured in four blood donors. We conclude: (i) in comparison to established cytogenetic tests, our new assay is much less time consuming, and mutagen sensitivity and DRC can be assessed as independent endpoints. (ii) because it allows storage of cryopreserved lymphocyte samples, our assay is suitable as a tool in molecular epidemiology. Prospective studies to identify high risk individuals and elucidation of the underlying mechanisms for disease susceptibility are now warranted.

#2044 COMBINED ANALYSIS OF *CYP1A1*, *NAT2*, AND *GSTM1* AS GENETIC RISK FACTORS OF LUNG CANCER. Ingolf Cascorbi, Juergen Brockmoeller, Uta Riedel, Kitty Volkgenannt, Joachim M Mueller, Robert Loddenkemper, and Ivar Roots, *Humboldt Univ Berlin, Berlin, Germany, and Lungenklinik Heckeshorn, Berlin, Germany*

There is strong evidence for association of exposition to precarcinogens such as benzo(a)pyrene or arylamines to the development of lung cancer. Molecular epidemiological studies showed a significant over-representation of activating cytochrome P4501A1 (*CYP1A*) genotypes particularly in Oriental lung cancer patients. GSTM1 deficiency is controversially discussed, however, enhanced DNA-adduct levels are reported for combination of *GSTM1*0* and *CYP1A1*2B*. The role of NAT2 is not fully clear. We aimed to investigate combined effects of these major polymorphic xenobiotic metabolizing enzymes on lung cancer risk in 330 Caucasian cases and 310 controls. Relative risk (RR) for *CYP1A1*2B* (462Ile and 6235C), adjusted for age, gender, and extent of cigarette smoke was 3.75 (95% CL 1.81-7.71; p<0.001). Homozygous rapid *NAT2*4/*4* was also significantly over-represented among cases, RR 2.65, 131-5.35; p<0.01. GSTM1 deficiency, however, did not prove as a host factor. Multiple regression analysis considering combination of activating *CYP1A1*2B* and lack of GSTM1 revealed RR 4.75 (1.31-17.1; p<0.05), combination of *NAT2*4/*4* and *GSTM1*0* disclosed 3.72 (1.32-10.5; p<0.05), suggesting that GSTM1 plays a role only in presence of *CYP1A1* or *NAT2* genotypes coding for high activity. Combined analysis of genotypes of all three polymorphic enzymes revealed an extremely high relative risk of 636, without reaching statistical significance, probably due to the low sample number carrying all at-risk genotypes. However, this molecular epidemiological approach demonstrated clearly that consideration of particular genotype combinations of carcinogen metabolizing enzymes may increase significantly an individual's risk for lung cancer.

#2045 INTERACTION OF TUMOR SIZE AND HER2/NEU EXPRESSION UPON TIME TO RECURRENCE IN INVASIVE DUCTAL CARCINOMA OF THE BREAST. Helen Swede, Kirsten B Moysich, Arthur M Michalek, and Janet S Winston, *Roswell Park Cancer Institute, Buffalo, NY*

This nested case-case study explored the association of HER2, tumor size and recurrence of breast cancer (BC) with the aim of identifying sub-groups concerning the prognostic value of HER2. The study sample included 65 cases with recurrent BC and 91 cases without BC recurrence after a minimum of three years of follow-up. All patients received primary treatment at RPCI between 1982 and 1995. The sample was restricted to cases with primary BC, no prior cancer, invasive ductal carcinoma without distant metastases and who completed a risk factor survey. HER2 expression was detected by immunohistochemistry assay (Mab CB-11) and defined as percentage of cells with positive membrane stains. Compared to non-recurrent cases, recurrent cases had larger tumors (4.0 cm v 2.4 cm, p<.001), greater number of positive axillary lymph nodes (4.4 v 1.6, p<.01), more grade III tumors (90.8% v 68.9%, p<.001) and a higher mean HER2 expression (41.7% v 30.2%, p=.10.) Cox multivariate regression of time to recurrence showed an interaction (p=.07) between tumor size and HER2 expression among node positive cases (n=74) which, by stratification on tumor size, indicated that increased HER2 expression (p<.05) and increased number of positive axillary lymph nodes (p<.05) are associated with shorter time to recurrence in tumors <=2 cm; whereas only increased tumor size (p<.001) is associated with shorter time to recurrence in tumors > 2 cm. Node negative tumors did not show such an interaction. Data were adjusted for age, number of positive axillary lymph nodes and if cytotoxic agents were given. These data suggest that influence of HER2 expression may be exerted relatively more strongly in smaller tumors of node-positive disease.

#2046 ANALYSIS OF A FOUNDER MUTATION OF THE BRCA1 GENE IN WESTERN SWEDEN. Annika Bergman, Margareta Nordling, Zakaria Einbeigi, Jeane Kindblom, Lars-Gunnar Kindblom, Mart Suurkula, Jan Wahlstrom, Arne Wallgren, Per Karlsson, and Tommy Martinsson, *Clin Genetics, Sahlgrenska Univ Hosp, Goteborg, Sweden, Oncology, Sahlgrenska Univ Hosp, Goteborg, Sweden, and Pathology, Sahlgrenska Univ Hosp, Goteborg, Sweden*

Aim: Study geographical distribution of families with BRCA1 mutation nt3166ins5. Describe phenotype and confirm mutational status also in deceased cases. Analyze if and to what extent a specific haplotype is shared among carriers of the mutation. Method: In the cancer counseling clinic of western Sweden (pop. 1.6 mill) app. 204 families have been investigated since May 1995. More than 75% of these families have concern about familial breast and/or ovarian carcinoma. Mutations in the BRCA1/2-genes are assayed with PTT and, and confirmed by DNA-sequencing. In 60 families blood samples have been obtained, and 53 of these were analyzed. We also test for this mutation in archived histological material, so far with 100% agreement with results from blood samples. Haplotypes are constructed from SSTRPs. Results: In 22 families 8 different mutations were found in BRCA1 or BRCA2. 14 families had the same BRCA1 mutation, i,e, insertion of TGAGA at nt3166, yielding a stop codon. The oldest cases originate from the coastal land south and north of Göteborg. This mutation has also been described in other parts of Sweden but the families originates from western Sweden. BRCA1 analysis of deceased individuals and haplotype analyzes of the nt3166ins5 families are ongoing but already we have found one young case of breast cancer without the mutation- a sporadic case. Conclusion: A BRCA1 founder mutation cluster along the Swedish west coast. Since our data is based on referral, the mutation may have a wider geographical distribution. Presently, possible cases are first screened for presence of nt3166ins5. The penetrance for both breast and ovarian cancer seems to be high in referred families. We plan a regional population-based study to further analyse presence of this founder mutation.

#2047 VITAMIN D RECEPTOR POLYMORPHISM AND PROSTATE CANCER RISK. Sara S Strom, Qiang Zhang, Margaret R Spitz, Yuko Yamamura, Richard J Babaian, Christopher J Logothetis, Shi-Ming Tu, and Qingyi Wei, *The Univ of Texas M D Anderson Cancer Ctr, Houston, TX*

Very little is known about the etiology of prostate cancer (PC). Some studies, but not all, have suggested associations between vitamin D receptor (VDR) genotype and the development and progression of PC. To better understand this relationship we analyzed the VDR *TaqI* restriction fragment length polymorphism in codon 352 in 212 Caucasian cases (147 non-metastatic (stage B-C) and 65 metastatic (stage D)) and 212 age-ethnic-matched controls with no clinical evidence of PC. VDR *TaqI* genotype was determined by a PCR-based method. Depending on the absence or presence of *TaqI* restriction site at codon 352 individuals were classified as TT, Tt, or tt. We hypothesized that men homozygous for tt are at decreased risk of PC and that men with this genotype are more likely to be diagnosed at an older age, with non-metastatic disease, and lower Gleason scores. We found the frequency of the tt genotype to be slightly lower among cases than controls (18% vs 22%). Men homozygous for tt genotype appeared to have a 23% reduced risk of developing PC as compared to men who were TT or Tt (Odds Ratio=0.8, p=0.3). Men with metastatic disease had the lowest frequency of tt genotype (15%) as compared to controls (22%) and non-metastatic cases (19%) although not statistically significant. No associations were found between VDR genotype, Gleason score, and age at diagnosis. Our findings do not support an important role for this VDR polymorphism and PC risk. However, more studies to clarify its significance in PC progression are necessary. Supported by grants RO1CA68578 and DAMD 17-98-1-8471.

#2048 THREE COMMON ALLELES OF THE VITAMIN D RECEPTOR (VDR) GENE IN WHITE POPULATIONS. Rowena C Oppenheimer, Diana Garcia, Gerhard A Coetzee, and Sue A Ingles, *Univ of Southern CA, Los Angeles, CA*

Risk of breast and prostate cancer has been associated with polymorphism in the 3' end of the vitamin D receptor (VDR) gene. The functional allelic variant responsible for these associations has not been identified. Rather, association studies have used one of several linked markers in the 3' end of the VDR gene,

(P=0.04). This data supports the idea that longer TA repeats in the 3' UTR of the SRD5A2 gene are associated with more favourable outcome of breast cancer patients.

#2669 The *GSTT1-Null* Genotype Is Associated with a Decreased Susceptibility for Esophageal Carcinoma in a Caucasian Population. Geoffrey Liu, Li-Lian Xu, David P. Miller, Rong Fan, Li Su, John C. Wain, Thomas J. Lynch, and David C. Christiani. *Harvard School of Public Health, Boston, MA, and Massachusetts General Hospital, Boston, MA.*

BACKGROUND: Few studies have evaluated genotypes in Caucasians with esophageal cancers, in comparison to Asian populations of primarily squamous cell histology. *GSTT1-null* and *GSTM1-null* polymorphisms, the *-G463A* polymorphism in the promoter region of the myeloperoxidase (*MPO*) gene, and the *Arg→Pro* (codon 72) *p53* polymorphism were studied in a Caucasian population with esophageal cancer. METHODS: 124 incident cases of esophageal cancer from Massachusetts General Hospital (recruited between 1998 to 2000) and over 748 control subjects consisting of spouses or friends of cancer patients were evaluated for each polymorphism. Results were analyzed using multivariate logistic regression, adjusting for age, gender, smoking status, and pack-years of smoking. RESULTS: The crude and adjusted ORs (95% confidence intervals) for esophageal cancer risk with the *GSTT1-null* genotype were 0.32 (0.16–0.68; p<0.01) and 0.29 (0.13–0.65; p<0.01), respectively. In terms of esophageal cancer risk, heavy smokers and younger individuals appeared to benefit most from carrying the *GSTT1-null* genotype. When compared to each of their respective wildtype alleles, the *GSTM1-null* genotype, the *MPO* variant genotypes (*A/G* and *G/G*), and the *p53* variant genotypes (*Arg/Pro* and *Pro/Pro*) did not directly alter esophageal cancer susceptibility. However, when compared to individuals with both wildtype *GSTT1* and wildtype *p53* genotypes, the OR for the *GSTT1-null, p53* variant individuals was 0.09 (0.01-0.65; p<0.01). The OR for individuals with both *GSTM1-null* and *GSTT1-null* genotypes was 0.22 (0.05-0.97; p=0.05) when compared to individuals with both the wildtype *GSTM1* and wildtype *GSTT1* genotypes. CONCLUSIONS: In this Caucasian population of predominantly esophagheal adenocarcinomas, the mechanisms underlying an unexpected protective effect of the GSTT1-null genotype require further exploration and confirmation.

#2670 Microsomal Epoxide Hydrolase (*mEPHX*) Polymorphisms and Lung Cancer Risk in Caucasians. Hua Zhao, Margaret R. Spitz, Karin M. Gwyn, and Xifeng Wu. *M.D. Anderson Cancer Center, Houston, TX.*

Microsomal epoxide hydrolase (mEPHX) is a critical metabolic enzyme involved in the activation and subsequent detoxification of specific tobacco carcinogens. There are polymorphisms in exon 3 and exon 4 that result in modulated enzymatic activity. The exon 3 polymorphism results in decreased mEPHX metabolic activity, while the exon 4 polymorphism results in increased mEPHX activity. Using a case-control study design, we hypothesized that the *mEPHX* polymorphisms may modulate lung cancer risk. RFLP-PCR assay was used to successfully identify the *mEPHX* polymorphic genotypes in 181 Caucasian lung cancer cases and 163 matched controls (matched on age, gender and smoking history). Our results demonstrated that the variant allele of *mEPHX* exon 4 increased the overall lung cancer risk by 50% (OR = 1.5; 95% CI 0.98 – 2.45). Additionally, the risk estimates were significantly elevated for younger individuals (< 62 years) (OR = 2.2; 95% CI 1.02 - 4.35) and ever smokers (OR = 2.2; 95% CI 1.06 - 4.65). An overall null effect was noted with the variant allele of *mEPHX* exon 3 (OR = 0.9; 95% CI 0.57 - 1.39); however, we found a 50% protective effect (OR = 0.5; 95% CI 0.24 - 1.05) in younger individuals. When we analyzed the exon 3 and exon 4 polymorphisms together, individuals with the high enzymatic activity genotype had an elevated lung cancer risk of 1.75 (OR =1.75; 95% CI 0.97 – 3.17). These findings suggest that the exon 3 and exon 4 polymorphisms of *mEPHX* modulate lung cancer risk. *Supported by NCI Grants CA 74880, CA 55769, and CA 68437.*

#2671 Role of 5-α-Reductase Polymorphisms in Prostate Cancer Prognosis. Yuko Yamamura, Patricia H. Thompson, Margaret R. Spitz, Richard J. Babaian, Christopher Logothetis, and Sara S. Strom. *The University of Texas M.D. Anderson Cancer Center, Houston, TX, and University of Texas M.D. Anderson Cancer Center, Houston, TX.*

Cell growth in the human prostate gland is regulated by androgens. The protein product of the SRD5A2, 5-α-reductase type II gene, is responsible for the conversion of testosterone to the more bioactive form dihydrotestosterone. Several studies have indicated that this gene may play a role in the development of prostate cancer (PC) with cases being less likely to have the Leu/Leu genotype than ethnicity- and age-matched healthy men. We evaluated the association of a SRD5A2 genetic polymorphism with known clinical prognostic indicators among 233 Caucasian men with all stages of PC enrolled in an ongoing PC study conducted at the University of Texas M.D. Anderson Cancer Center. Specifically, DNA from peripheral lymphocytes was isolated and genotyped for the single nucleotide polymorphism in the SRD5A2 gene that results in the substitution of valine for leucine at codon 89 (V89L). Overall, 7.3% of our patients had the Leu/Leu genotype. Although men with the Leu/Leu genotype tended to be younger than those with Val/Val or Val/Leu genotypes (62.4 vs. 61.4 years, respectively), this difference was not statistically significant (p=0.5). There was no association between genotype and combined Gleason score. Similarly, we found no relationship between genotype and having metastatic disease. Our results do not support a role for the V89L mutation in the SRD5A2 gene in PC prognosis. We are in the process of genotyping these same cases for the mis-sense substitution which results in an alanine residue at codon 49 being replaced by threonine (A49T); we believe that this polymorphism may play a more significant role in PC progression. (Supported by NIH grant CA/ES68578 & CA84964, and DOD grant DAMD 17-98-1-84)

#2672 Polymorphism of *GSTT1* and *GSTM1* and Risk of Glioma: A Case-Control Study. Li-E Wang, Melissa L. Bondy, Athanassios P. Kyritsis, Janet L. Bruner, W. K. Alfred Yung, Victor A. Levin, and Qingyi Wei. *The University of Texas M. D. Anderson Cancer Center, Houston, TX, and The University of Texas M.D. Anderson Cancer Center, Houston, TX.*

Brain tumors constitute about 9% of all human cancers, but gliomas account for 90% of these brain tumors. The etiology of brain tumors remains unclear. Certain carcinogenic exposures such as nitrosamines are implicated in the risk of gliomas. The role of genetic polymorphisms in modulating susceptibility to carcinogenic exposures has been well explored for tobacco-related cancers. Glutathione S-transferases (GSTs) are involved in metabolizing a wide range of xenobiotics. We hypothesize that polymorphisms of GSTs modulate risk of gliomas. To test this hypothesis, we conducted a hospital-based case-control study of 304 patients with newly diagnosed gliomas and 352 healthy controls to investigate the roles of *GSTT1* and *GSTM1* polymorphisms in risk of gliomas. The cases and controls were all Caucasians and frequency matched on age and sex. Genomic DNA was extracted from blood samples obtained from each subject. A multiplex polymerase chain reaction assay was used to simultaneously genotype *GSTT1* and *GSTM1* plus the dihydrofolate reductase gene as an internal control. Multivariate logistical regression analysis was performed to estimate adjusted odds ratio (OR) and 95% confidence interval (CI). The results showed that 19.1% of cases were null for *GSTT1* compared with 13.4% of controls, and the difference was statistically significant (*P*=0.046). In contrast, 53.0% of cases were null for *GSTM1* compared with 50.0% of controls and the difference was not statistically significant (*P*=0.449). Although *GSTM1* null genotype was not associated with risk of gliomas, *GSTT1* null genotype was associated with significantly increased risk [OR=1.53 (95% CI=1.00-2.33)] after adjustment for age and sex. These findings suggest that null genotype of *GSTT1* but not *GSTM1* may play a role in the etiology of glioma (Supported by NIH grants CA70334, CA70917 and CA 55261).

#2673 Polymorphisms That Influence Microsomal Epoxide Hydrolase Activity Are Associated with p53 Mutation-positive Gliomas. Eric J. Duell, Pengchin Chen, Karl T. Kelsey, Mei Liu, Rei Miike, John Wiencke, and Margaret Wrensch. *Harvard School of Public Health, Boston, MA, and University of San Francisco, San Francisco, CA.*

Microsomal epoxide hydrolase (mEH) is a major detoxification enzyme that inactivates toxic epoxides by catalyzing their hydration to less toxic dihydrodiols. However, mEH is believed to metabolize some compounds (e.g., polycyclic aromatic hydrocarbons) to more toxic metabolites. Thus, mEH serves as both a detoxifying and toxifying enzyme. Two polymorphisms in mEH are believed to affect enzyme function. Furthermore, mEH is expressed in various regions of the human brain. We investigated the role of mEH polymorphisms in a case-control study of adult glioma in the San Francisco Bay Area (1991-1994). Cases were identified using the Northern California Cancer Center's rapid case ascertainment service, and controls were contacted using random digit dial and frequency matched on age, gender, and ethnicity. mEH genotypes (Tyr113His in exon 3 and His139Arg in exon 4) were determined using PCR-RFLP methods. Based on previous work, genotypes for the two polymorphisms were combined to differentiate individuals with probable low activity from those with probable intermediate or high activities. Gliomas were classified according to p53 mutational status as either mutation-positive (+) or mutation-negative (-). p53 mutational status was determined for exons 5-8 using PCR-SSCP and direct sequencing. For this analysis, there were 10 p53 mutation (+) cases, 49 p53 mutation (-) cases, and 157 controls. Unadjusted and age- and gender-adjusted odds ratios (ORs) and 95 percent confidence intervals (95% CIs) were estimated using logistic regression analysis. Caucasian individuals with probable low mEH activity based on combined genotypes were at a 2-fold increased risk of glioma compared to those with probable high activity (age- and gender-adjusted OR = 1.8, 95% CI = 1.0-3.6). A stronger association between mEH combined genotype (low vs. intermediate or high) and glioma risk was seen comparing p53 mutation (+) cases with controls (adjusted OR = 4.3, 95% CI = 1.0-18.1) than that seen comparing p53 mutation (-) cases with controls (adjusted OR = 1.5, 95% CI = 0.7-3.1). Tobacco smoking did not modify the association of glioma risk and either mEH genotype alone or in combination. Our data suggest that the low activity mEH genotype is more strongly associated with p53 mutation (+) gliomas than with p53 mutation (-) gliomas, and that tobacco smoking does not appear to contribute to this difference.

#2674 Polymorphisms in MPO and NQO1 and Risk of Ovarian Cancer. Sara H. Olson, Melissa D.A. Carlson, Laura Mignone, Susan Harlap, Angie Stone, Maria Winters, Patricia A. Thompson, and Christine B. Ambrosone. *Memorial Sloan-Kettering Cancer Center, New York, NY, National Center for Toxicological Research, Jefferson, AR, and New York University School of Medicine, New York, NY.*

We examined the association of polymorphisms in the genes that code for myeloperoxidase (MPO) and NAD(P)H:quinone oxidoreducatase (NQO1) with