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Research Article

Use of the Cytokinesis-Blocked Micronucleus Assay to Detect Gender Differences and Genetic Instability in a Lung Cancer Case–Control Study

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Abstract

Background: Although tobacco exposure is the predominant risk factor for lung cancer, other environmental agents are established lung carcinogens. Measuring the genotoxic effect of environmental exposures remains equivocal, as increases in morbidity and mortality may be attributed to coexposures such as smoking.

Methods: We evaluated genetic instability and risk of lung cancer associated with exposure to environmental agents (e.g., exhaust) and smoking among 500 lung cancer cases and 500 controls using the cytokinesis-blocked micronucleus (CBMN) assay. Linear regression was applied to estimate the adjusted means of the CBMN endpoints (micronuclei and nucleoplasmic bridges). Logistic regression analyses were used to estimate lung cancer risk and to control for potential confounding by age, gender, and smoking.

Results: Cases showed significantly higher levels of micronuclei and nucleoplasmic bridges as compared with controls (mean ± SEM = 3.54 ± 0.04 vs. 1.81 ± 0.04 and mean ± SEM = 4.26 ± 0.03 vs. 0.99 ± 0.03, respectively; $P < 0.001$) with no differences among participants with or without reported environmental exposure. No differences were observed when stratified by smoking or environmental exposure among cases or controls. A difference in lung cancer risk was observed between nonexposed male and female heavy smokers, although it was not statistically significant ($I^2 = 64.9\%$; P value for Q statistic = 0.09).

Conclusions: Our study confirms that the CBMN assay is an accurate predictor of lung cancer and supports the premise that heavy smoking may have an effect on DNA repair capacity and in turn modulate the risk of lung cancer.

Impact: Identifying factors that increase lung cancer risk may lead to more effective prevention measures.
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Introduction

Many occupational and environmental exposures, such as asbestos, arsenic, chromium, radon, and polycyclic aromatic compounds (1) are established lung carcinogens. Assessing genetic instability as a marker of exposure is well documented for these compounds as well as a large number of agents, such as pesticides (2–4), heavy metals (5–8), styrene (9–12), ionizing radiation (13–17), common beauty salon products (18–20), and antineoplastic drugs (21–23). Measuring the genotoxic effect of environmental exposures in humans remains equivocal as increases in morbidity and mortality may also be attributed to coexposures or lifestyle factors, such as smoking and alcohol consumption (24–28).

It is well known that cancer results from the accumulated genetic events that have the potential to be detected cytogenetically (29, 30). Chromosome aberrations are a result of errors in DNA repair in response to exposure to the clastogenic agent (31). The cytokinesis-blocked micronucleus (CBMN) assay in human lymphocytes is one of the most frequently used methods for measuring DNA damage (i.e., chromosome aberrations; ref. 32). The CBMN assay is a multi-endpoint assay that measures not only chromosome damage [i.e., micronuclei reflecting chromosome breaks; nucleoplasmic bridges (NPB) reflecting chromosome rearrangements; and nuclear buds (NBUD) reflecting gene amplification] but also other cellular events (such as apoptosis and necrosis). Compared with other cytogenetic assays, quantification of micronuclei confers several advantages, including speed and ease of analysis, no requirement for metaphase cells and reliable identification of cells that have completed only 1 nuclear division. This prevents confounding effects caused by differences in cell division kinetics because

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expression of the micronuclei is dependent on completion of nuclear division (33).

We have previously reported that the CBMN assay is a sensitive and specific predictor of lung cancer risk (34, 35), and we further found an increase in lung cancer risk for participants that self-reported exposure to asbestos, fiberglass, wood dust, solvents, bleach, and pesticides (36–38). On the basis of these findings, we conducted the current study to evaluate the extent of genetic instability (using the CBMN assay) in lung cancer cases and controls exposed to environmental agents (namely asbestos, fiberglass, wood dust, solvents, paints, exhaust, and bleach) and the effect of smoking on modulating such genetic instability.

Materials and Methods

Study population

Lung cancer cases and matched controls in this study were from an ongoing molecular epidemiologic lung cancer risk assessment study at the University of Texas MD Anderson Cancer Center (Houston, TX). Cases ($n = 500$) were consecutive patients with newly diagnosed, previously untreated, histologically confirmed lung cancer with no age, gender, ethnicity, tumor histology, or disease stage restrictions. Healthy controls ($n = 500$) were recruited from Kelsey-Seybold Clinics (Houston, TX). Controls were matched to the cases on age (±5 years) and gender. Medical history, family history of cancer, smoking habits, and occupational history were obtained through an interviewer-administered risk-factor questionnaire and by review of an institutional electronic patient history database. Institutional Review Board's approval at both MD Anderson Cancer Center and Kelsey-Seybold was obtained for this study. Upon receiving informed consent, a 10-mL blood sample was drawn into heparinized tubes from all study participants.

Lymphocyte cultures for the CBMN assay

The CBMN assay was conducted using the cytochalasin B technique described by Fenech and Morley (39). Duplicate lymphocyte cultures were prepared for each study subject. Each culture contained 2.0×10^6 cells in 5 mL RPMI-1640 medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, 10% FBS, and 2 mmol/L L-glutamine (Life Technologies/Invitrogen) and 1% phytohemagglutinin (Remel). At 44 hours after initiation, the cells were blocked in cytokinesis by adding cytochalasin B (Sigma; final concentration 4 μg/mL). The total incubation time for all cultures was 72 hours. After incubation, the cells were fixed in 3:1 methanol: glacial acetic acid, dropped onto clean microscopic slides, air-dried, and stained with Giemsa stain. For each sample, 1,000 binucleated cells were scored blindly using a Nikon E-400 light optical microscope following the scoring criteria and recommendations from The International Collaborative Project on Micronucleus Frequency in Human Populations (HUMN Project) to measure binucleated cells with micronuclei (BN-MN) and binucleated cells with nucleoplasmic bridges (BN-NPB; refs. 40–42).

Exposure assessment

Trained interviewers administered a risk-factor questionnaire through a structured personal interview, and participants were classified as never smokers (smoked fewer than 100 cigarettes in their lifetimes), former smokers (quit more than 1 year before lung cancer diagnosis or interview date), and current smokers. Smoking groups were further categorized into light (pack-years $\leq$ 20), moderate (20 < pack-years $\leq$ 40), and heavy (>40 pack-years). Participants were asked to select from a list of environmental exposures (e.g., asbestos, wood dust, pesticides, fiberglass, toluene, fertilizers, diesel fumes, paints, solvents, and exhaust) they may have handled, used, or been in contact with for at least 8 hours a week for a year or more on the job or while working on a hobby. Exposures to asbestos, wood dust, and pesticides were classified by self-report as well as from occupations and industries categorized as having a risk of exposure to these agents. Other exposures such as fibers, benzene, toluene/xylene, dry cleaning fluids, vehicle exhaust, glues, bleach, and plastics were based on the self-reported responses only. In our current study, we focused on the 7 major environmental agents reported by the participants namely: asbestos, fibers, solvents, paints, wood dust, exhaust, and bleach.

Statistical analysis

Pearson χ^2 test was used to test for differences between cases and controls for categorical variables: gender, smoking history, environmental exposures (i.e., asbestos, fiber, bleach, wood dust, solvents, paints, and exhaust). Student 2-sample t test was used to test for differences between cases and controls for continuous variables: age and CBMN outcome variables (i.e., BN-MN and BN-NPB). The correlation between the CBMN outcomes was evaluated with Pearson correlation coefficient within cases and controls separately. Principal components analysis (PCA), with the varimax orthogonal method, was used to reduce the number of exposure variables to develop a CBMN outcome exposure score to investigate interaction or effect modification between number of chromosome aberrations and environmental exposures (43). BN-MN and BN-NPB were examined separately for all 7 environmental agents; the 4 modified exposure factors from the PCA (i.e., chemicals, fibers, particulates, and bleach); those with at least 1 of 7 environmental agents; and those without any of the 7 environmental agents. Univariate OR and 95% confidence intervals (CI) were used to estimate the risk of lung cancer. Unconditional multivariable logistic regression analysis was used to control for potential confounding by age, gender, and smoking when appropriate. Linear regression was applied to estimate the adjusted means of the CBMN assay outcomes. Tests for heterogeneity were conducted to evaluate differences in

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the odds of lung cancer between categories. All statistical tests were 2-sided and P values of 0.05 or less were considered statistically significant. All analyses were conducted using Statistical Analysis System (SAS) software (version 9.2; SAS Institute Inc.).

Results

The study population

Table 1 displays the demographic characteristics of the cases and controls. No statistically significant differences were observed between the cases and controls in terms of age, gender, and environmental exposure. The mean age of cases and controls were 63 (SD ±1.12) years and 62 years (SD ±1.12), respectively ($P = 0.31$). Fifty-one percent of cases and 46% of controls self-reported at least 1 environmental exposure ($P = 0.11$). Approximately 84% of the cases were ever smokers as compared with 66% for the controls ($P < 0.001$). Cases reported significantly heavier smoking histories than controls. When stratified by smoking intensity (i.e., light, moderate, or heavy), approximately 16% of the cases were light smokers, 23% were moderate smokers, and 43% were heavy smokers. For controls, approximately 22% were light smokers, 17% were moderate smokers, and 27% were heavy smokers ($P < 0.001$). Pack-years of smoking were significantly higher in ever smoker cases as compared with ever smoker controls with 45.5 pack-years compared with 37.5 pack-years, respectively ($P < 0.001$).

For exposure to the environmental agents (i.e., asbestos, fiber, bleach, sawdust, solvents, paints, and exhaust), 53% of participants self-reported no environmental exposure, 23% reported 1 of 7 environmental exposures, 10.5% reported 2 of 7 environmental exposures, and 13.5% reported 3 or more of the environmental exposures. Asbestos (19%) and solvents (18%) were the 2 most self-reported environmental exposures followed by exhaust (16%), paints (13.5%), bleach (13%), wood dust (12%), and fiberglass (8.5%).

Genetic instability by smoking and environmental exposure

We analyzed exposure separately for (i) all 7 environmental agents; (ii) as modified exposure categories based on factors from the PCA (i.e., chemicals, fibers, particulates, and bleach); and (iii) as those with at least 1 of 7 environmental agents. We did not find statistically significant differences in chromosome damage for those without environmental exposure or with exposure to any of the 3 exposure categories. Because of sample size, we used those with at least 1 of 7 environmental exposures as the final environmental exposure category in our analyses.

Table 2 presents the distribution of the CBMN outcomes for all cases and controls stratified by smoking and self-reported environmental exposure to at least 1 of 7 environmental agents. Overall cases showed

Table 1. Demographic characteristics by case–control status

	Case ($N = 500$)	Control ($N = 500$)	P value*
Age (mean ± SD)	62.50 ± 1.12	61.80 ± 1.12	0.310
Age group (%)			
Age ≤ 50 y	80 (16.00)	65 (13.00)	0.209
Age > 50 y	420 (84.00)	435 (87.00)	
Gender (%)			
Male	245 (49.00)	246 (49.20)	0.949
Female	255 (51.00)	254 (50.80)	
Smoking (%)			
Never smokers	91 (18.20)	171 (34.20)	<0.001
Ever smokers	409 (84.00)	329 (65.80)	
Smoking intensity[a] (%)			
Light (0 < pack-years ≤ 20)	79 (15.80)	109 (21.80)	<0.001
Moderate (20 < pack-years ≤ 40)	117 (23.40)	86 (17.20)	
Heavy (40 < pack-years)	213 (42.60)	134 (26.80)	
Smoking pack-years[b] (mean ± SE)	45.56 ± 1.43	37.53 ± 1.58	<0.001
Environmental exposure[c] (%)			
Without environmental exposure	243 (48.60)	269 (53.80)	0.114
With at least 1 environmental exposure	257 (51.40)	231 (46.20)	

*P values were obtained from Pearson χ^2 test (categorical variables) and the Student 2-sample t test (continuous variables).
[a]Smoking intensity, light smokers (0 < pack-years ≤ 20), moderate smokers (20 < pack-years ≤ 40), heavy smokers (40 < pack-years).
[b]Smoking pack-years, years smoked times the average number of cigarettes per day divided by 20.
[c]Self-reported exposure to at least 1 of 7 environmental agents (i.e., asbestos, fiberglass, wood dust, solvents, paints, exhaust, and bleach).

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Table 2. CBMN outcomes for all cases and controls by smoking[a] and environmental exposure[b]

ALL	Case ($N = 500$)	*P* value	Control ($N = 500$)	*P* value
BN-MN				
Overall	3.54 ± 0.04		1.81 ± 0.04	<0.001
Smoking[a]				
Never smoker	3.56 ± 0.10		1.83 ± 0.07	
Light smokers	3.42 ± 0.11		1.76 ± 0.08	
Moderate smokers	3.60 ± 0.09		1.77 ± 0.09	
Heavy smokers	3.53 ± 0.07		1.84 ± 0.08	
		$P_{trend} = 0.69$		$P_{trend} = 0.95$
Environmental exposure[b]				
Without exposure	3.58 ± 0.07	0.24	1.79 ± 0.06	0.85
With at least 1 exposure	3.48 ± 0.11		1.81 ± 0.06	
BN-NPB				
Overall	4.26 ± 0.03		0.99 ± 0.03	<0.001
Smoking[a]				
Never smoker	4.20 ± 0.08		0.99 ± 0.05	
Light smokers	4.37 ± 0.09		0.97 ± 0.06	
Moderate smokers	4.20 ± 0.07		1.04 ± 0.07	
Heavy smokers	4.27 ± 0.05		0.98 ± 0.06	
		$P_{trend} = 0.89$		$P_{trend} = 0.75$
Environmental exposure[b]				
Without exposure	4.31 ± 0.05	0.17	1.05 ± 0.04	0.06
With at least 1 exposure	4.21 ± 0.05		0.94 ± 0.04	

[a]Smoking intensity, light smokers (0 < pack-years ≤ 20), moderate smokers (20 < pack-years ≤ 40), heavy smokers (40 < pack-years); means adjusted by age, gender, and environmental exposures.

[b]Self-reported exposure to at least 1 of 7 environmental exposures (i.e., asbestos, fiberglass, wood dust, solvents, paints, exhaust, and bleach; means adjusted by age, gender, and smoking.

significantly higher levels of genetic instability (BN-MN mean ± SEM = 3.54 ± 0.04; BN-NPB mean ± SEM = 4.26 ± 0.03) as compared with controls (BN-MN mean ± SEM = 1.81 ± 0.04; BN-NPB mean ± SEM = 0.99 ± 0.03; $P < 0.001$). Among the cases, no statistically significant differences were observed when data were stratified by smoking (BN-MN: $P_{trend} = 0.69$; BN-NPB: $P_{trend} = 0.89$) or environmental exposure (BN-MN: $P = 0.24$; BN-NPB: $P = 0.89$); nor for controls by smoking (BN-MN: $P_{trend} = 0.95$; BN-NPB: $P_{trend} = 0.75$). Similarly, among the controls, no significant differences were observed when stratified by environmental exposure BN-MN ($P = 0.85$), however, a borderline significant difference was observed for those without exposure (BN-NPB mean ± SEM = 1.05 ± 0.04) compared with those with at least 1 environmental exposure (BN-NPB mean ± SEM = 0.94 ± 0.04; $P = 0.06$). In addition, among the cases, we did not observe significant differences in the micronuclei and NPBs distribution when stratified by tumor histology, or disease stage, thus reducing the probability of this being a disease marker (data not shown).

To ensure quality control, 20% of the slides were randomly selected for blind rescoring and the intraclass correlation (ICC) was ICC = 0.96 and ICC = 0.97 for the micronuclei and the NPBs, respectively, thus indicating that the agreement between the original and the rescored data were not attributed to random chance.

Genetic instability by gender

Table 3 displays the distribution of CBMN outcomes among males stratified by smoking and self-reported environmental exposure. Male cases showed significantly higher levels of genetic instability (BN-MN mean ± SEM = 3.52 ± 0.06; BN-NPB mean ± SEM = 4.29 ± 0.05) as compared with controls (BN-MN mean ± SEM = 1.83 ± 0.05; BN-NPB mean ± SEM = 0.98 ± 0.04). There was a marginal statistically significant difference in the BN-MN among male cases stratified by smoking ($P_{trend} = 0.07$). No statistically significant differences in BN-MN or BN-NPB were observed among males when stratified by environmental exposure. Similarly, no significant findings were observed among male controls when stratified by smoking or environmental exposure.

Table 4 presents the distribution of CBMN outcomes for female cases and controls stratified by smoking and self-reported environmental exposure. Overall female cases displayed significantly higher levels of genetic instability (BN-MN mean ± SEM = 3.55 ± 0.06; BN-NPB mean ±

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Table 3. CBMN outcomes for male cases and controls by smoking[a] and environmental exposure[b]

Males	Case ($N = 500$)	*P* value	Control ($N = 500$)	*P* value
BN-MN				
Overall	3.52 ± 0.06		1.83 ± 0.05	<0.001
Smoking[a]				
Never smoker	3.35 ± 0.17		1.85 ± 0.11	
Light smokers	3.31 ± 0.17		1.92 ± 0.12	
Moderate smokers	3.82 ± 0.13		1.65 ± 0.13	
Heavy smokers	3.50 ± 0.09		1.85 ± 0.09	
		$P_{trend} = 0.07$		$P_{trend} = 0.38$
Environmental exposure[b]				
Without exposure	3.49 ± 0.11	0.93	1.87 ± 0.09	0.45
With exposure	3.50 ± 0.09		1.78 ± 0.07	
BN-NPB				
Overall	4.29 ± 0.05	0.98 ± 0.04		<0.001
Smoking[a]				
Never smoker	4.12 ± 0.13		0.90 ± 0.08	
Light smokers	4.48 ± 0.13		0.89 ± 0.08	
Moderate smokers	4.24 ± 0.10		1.04 ± 0.09	
Heavy smokers	4.30 ± 0.07		1.05 ± 0.07	
		$P_{trend} = 0.71$		$P_{trend} = 0.07$
Environmental exposure[b]				
Without exposure	4.30 ± 0.08	0.82	1.03 ± 0.09	0.22
With at least 1 exposure	4.27 ± 0.07		0.93 ± 0.05	

[a]Smoking intensity, light smokers (0 < pack-years ≤ 20), moderate smokers (20 < pack-years ≤ 40), heavy smokers (40 < pack-years); means adjusted by age and environmental exposures.

[b]Self-reported exposure to at least 1 of 7 environmental exposures (i.e., asbestos, fiberglass, wood dust, solvents, paints, exhaust, and bleach; means adjusted by age and smoking intensity.

SEM = 4.23 ± 0.05) as compared with controls (BN-MN mean ± SEM = 1.79 ± 0.05; BN-NPB mean ± SEM = 1.01 ± 0.04). No differences were observed among female cases stratified by smoking (BN-MN: $P_{trend} = 0.42$; BN-NPB: $P_{trend} = 0.73$) and marginal statistical significance when stratified by environmental exposure (BN-MN: $P = 0.08$; BN-NPB: $P = 0.08$). As with male controls, there were no statistically significant observations among the female controls when stratified by smoking or environmental exposure.

Risk of lung cancer associated with smoking and environmental exposure

Table 5 presents the OR and 95% CI associated with smoking and self-reporting environmental exposure. Both moderate and heavy smoking was associated with an increased risk for lung cancer regardless of exposure status: risk for lung cancer in absence of an environmental exposure (light smokers: OR = 1.07, 95% CI, 0.64–1.78; moderate smokers: OR = 2.60, 95% CI, 1.55–4.35; and heavy smokers: OR = 3.05, 95% CI, 1.82–4.85; $P_{trend} < 0.001$) and those with environmental exposure (light smokers: OR = 0.93, 95% CI, 0.54–1.60; moderate smokers: OR = 2.56, 95% CI, 1.55–4.21; and heavy smokers: OR = 3.15, 95% CI, 2.03–4.89; $P_{trend} < 0.001$).

Gender differences

When stratified by gender, male heavy smokers with environmental exposure had a nearly 3-fold increased risk of lung cancer (OR = 2.68; 95% CI, 1.41–5.11) as compared with male light (OR = 1.20; 95% CI, 0.56–2.58) and male moderate smokers (OR = 1.67; 95% CI, 0.80–3.46; $P_{trend} < 0.001$). Interestingly, males without a history of environmental exposure showed a borderline risk for lung cancer when stratified by smoking; however, these findings were not statistically significant (Table 5).

Among females without an environmental exposure, we observed an increased risk of lung cancer in association with smoking (light smokers: OR = 1.26, 95% CI, 0.68–2.35; moderate smokers: OR = 2.68, 95% CI, 1.41–5.09; and heavy smokers: OR = 4.25, 95% CI, 2.30–7.88; $P_{trend} < 0.001$). Similar observations were noted among female light and moderate smokers with environmental exposure. In contrast, we found an inverse relationship with female heavy smokers with environmental exposure. Although female heavy smokers in this group had an increased risk of lung cancer (OR = 2.67; 95% CI, 1.39–5.11), the risk was lower than exposed female moderate smokers (OR = 3.71; 95% CI, 1.78–7.70; $P_{trend} = 0.27$; Table 4). We evaluated possible heterogeneity between these observed risks using the Q statistic and

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Table 4. CBMN outcomes for female cases and controls by smoking[a] and environmental exposure[b]

Females	Case ($N = 500$)	*P* value	Control ($N = 500$)	*P* value
MN-BN				
Overall	3.55 ± 0.06		1.79 ± 0.05	<0.001
Smoking[a]				
Never smoker	3.69 ± 0.13		1.81 ± 0.08	
Light smokers	3.51 ± 0.14		1.61 ± 0.11	
Moderate smokers	3.41 ± 0.12		1.89 ± 0.13	
Heavy smokers	3.58 ± 0.11		1.85 ± 0.12	
		$P_{trend} = 0.42$		$P_{trend} = 0.27$
Environmental exposure[b]				
Without exposure	3.63 ± 0.08	0.08	1.75 ± 0.07	0.26
With at least 1 exposure	3.41 ± 0.10		1.88 ± 0.09	
NPB-BN				
Overall	4.23 ± 0.05		1.01 ± 0.04	<0.001
Smoking[a]				
Never smoker	4.24 ± 0.08		1.04 ± 0.06	
Light smokers	4.28 ± 0.09		1.05 ± 0.09	
Moderate smokers	4.16 ± 0.07		1.03 ± 0.01	
Heavy smokers	4.25 ± 0.05		0.86 ± 0.09	
		$P_{trend} = 0.73$		$P_{trend} = 0.13$
Environmental exposure[b]				
Without exposure	4.30 ± 0.06	0.08	1.04 ± 0.05	0.18
With at least 1 exposure	4.12 ± 0.08		0.92 ± 0.07	

[a]Smoking intensity, light smokers (0 < pack-years ≤ 20), moderate smokers (20 < pack-years ≤ 40), heavy smokers (40 < pack-years); means adjusted by age and environmental exposures.

[b]Self-reported exposure to at least 1 of 7 environmental exposures (i.e., asbestos, fiberglass, wood dust, solvents, paints, exhaust, and bleach; means adjusted by age and smoking intensity.

associated I^2 value (44) and observed no significant heterogeneity between observed risks of lung cancer of female heavy smokers with and without exposure ($I^2 = 4.4\%$; P value for Q statistic = 0.31).

When comparing both genders, we observed no difference between nonexposed male and female moderate smokers (OR = 2.31; 95% CI, 0.96–5.53; OR = 2.68; 95% CI, 1.41–5.11, respectively); yet there was a difference between nonexposed male and female heavy smokers (OR = 1.86; 95% CI, 0.90–3.88; OR = 4.25; 95% CI, 2.30–5.11, respectively). Upon evaluation of heterogeneity, we found a borderline significant ($I^2 = 64.9\%$; P value for Q statistic = 0.09) level of heterogeneity between these 2 subgroups.

Discussion

In this study, we evaluated the extent of genetic instability associated with exposure to environmental agents and smoking among lung cancer cases and controls using the CBMN assay. We previously reported that the CBMN assay is a sensitive predictor of lung cancer risk (34, 35). Our current study confirms our previous findings in a larger well-designed case–control study that included never smokers and further stratified by smoking intensity (i.e., light, moderate, and heavy).

Although we did not find significant differences in chromosomal damage in cases or controls with or without environmental exposure, the mean levels of chromosomal damage were higher in cases, even after adjusting for age, gender, and smoking. The observed genetic damage was persistently higher in cases after further stratification by gender and adjustment for age, smoking, and environmental exposure; thus, providing further support to the use of the CBMN assay as a highly predictive biomarker for lung cancer. To address the odds of lung cancer risk, we stratified by gender and smoking and found no differences in nonexposed male and female moderate smokers. For estimated risk of lung cancer, we found a difference between nonexposed male heavy smokers and nonexposed female heavy smokers, although it was not statistically significant ($I^2 = 64.9\%$; P value for Q statistic = 0.09). Because of the subgroup analyses and issues with multiple comparisons, chance variability may account for such findings. However, other factors such as interindividual differences in the genetic susceptibility and other coexposures may also play a role.

According to the 2012 American Cancer Society estimates, cancer of the lung and bronchus in both males and females each represent 14% of the new cancer diagnoses in the United States (116,470 and 109,690,

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Table 5. ORs and 95% CIs for smoking[a] and environmental exposures[b] and lung cancer risk

All[c]	Case ($N = 500$)	Control ($N = 500$)	OR (95% CI)
Without exposure			
Never smoker	61 (12.20)	111 (22.20)	1.00 (Referent)
Light smokers	37 (7.40)	63 (12.60)	1.07 (0.64–1.78)
Moderate smokers	55 (11.00)	39 (7.80)	2.60 (1.55–4.35)
Heavy smokers	90 (18.00)	56 (11.20)	3.05 (1.82–4.85)
			$P_{trend} < 0.001$
With exposure			
Never smoker	30 (6.00)	60 (12.00)	0.93 (0.54–1.60)
Light smokers	42 (8.40)	46 (9.20)	1.78 (1.05–3.02)
Moderate smokers	62 (12.40)	47 (9.40)	2.56 (1.55–4.21)
Heavy smokers	123 (24.60)	78 (15.60)	3.15 (2.03–4.89)
			$P_{trend} < 0.001$
Males[d]	**Case ($N = 245$)**	**Control ($N = 246$)**	**OR (95%CI)**
Without exposure			
Never smoker	21 (8.57)	34 (13.82)	1.00 (Referent)
Light smokers	10 (4.08)	22 (8.94)	0.74 (0.29–1.86)
Moderate smokers	20 (8.16)	14 (5.69)	2.31 (0.96–5.53)
Heavy smokers	36 (14.69)	31 (12.60)	1.86 (0.90–3.88)
			$P_{trend} = 0.081$
With exposure			
Never smoker	12 (4.90)	28 (11.38)	0.70 (0.29–1.66)
Light smokers	23 (9.39)	31 (12.60)	1.20 (0.56–2.58)
Moderate smokers	33 (13.47)	32 (13.01)	1.67 (0.80–3.46)
Heavy smokers	90 (36.73)	54 (21.95)	2.68 (1.41–5.11)
			$P_{trend} < 0.001$
Females[d]	**Case ($N = 255$)**	**Control ($N = 254$)**	**OR (95% CI)**
Without exposure			
Never smoker	40 (15.69)	77 (30.31)	1.00 (Referent)
Light smokers	27 (10.59)	41 (16.14)	1.26 (0.68–2.35)
Moderate smokers	35 (13.73)	25 (9.84)	2.68 (1.41–5.09)
Heavy smokers	54 (21.18)	25 (9.84)	4.25 (2.30–7.88)
			$P_{trend} < 0.001$
With exposure			
Never smoker	18 (7.06)	32 (12.60)	1.07 (0.53–2.14)
Light smokers	19 (7.45)	15 (5.91)	2.44 (0.12–5.30)
Moderate smokers	29 (11.37)	15 (5.91)	3.71 (1.78–7.70)
Heavy smokers	33 (12.94)	24 (9.45)	2.67 (1.39–5.11)
			$P_{trend} = 0.027$

[a]Smoking intensity, light smokers ($0 <$ pack-years ≤ 20), moderate smokers ($20 <$ pack-years ≤ 40), heavy smokers ($40 <$ pack-years).
[b]Self-reported exposure to at least 1 of 7 environmental exposures (i.e., asbestos, fiberglass, wood dust, solvents, paints, exhaust, and bleach.
[c]Model adjusted by age and gender.
[d]Model adjusted by age.

respectively). Although the incidence rate in males has been decreasing since the mid-1980s, the incidence rate in females has just begun to decrease. Between 2004 and 2008 the incidence rate in males decreased by 1.9% per year, whereas the rate in females has only decreased by 0.3% per year (45).

In terms of cancer risk, the concept that females have an increased risk of lung cancer compared with males remains debatable. Several studies have reported that females exhibit an excess risk of lung cancer (46–48); however, other studies refute these findings (49–55). In a recent study in never smokers, Lo and colleagues (56)

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found males with a family history of lung cancer in first degree relatives had a slightly higher increased risk as compared with females with a family history of lung cancer in first-degree relatives. However, in the same study, females with a history of hormone replacement therapy (HRT) displayed a protective effect, thus suggesting hormonal differences in the development of lung cancer.

The obvious biologic differences between males and females are hormonal, yet the role of HRT and risk of lung cancer remains controversial (57). Studies have shown a decreased risk (58–63), whereas others have shown an increased risk of lung cancer (64–68). However, evidence for the role of estrogen receptors (ER) in human lung studies is rapidly accumulating and providing evidence on the role of ERs (ER-α and ER-β) in the pathogenesis of lung cancer (68). Both ER-α and ER-β have been shown in lung cancer tissue and cell lines and could hypothetically contribute to lung carcinogenesis (69–79). Although biologic differences between the sexes remain unequivocal, lung cancer development may also be attributed to areas such as growth factor receptors mutagen sensitivity, and differences in DNA repair capacity (80, 81).

Because only 10% to 15% of long-term smokers develop lung cancer in their lifetime, it is believed that genetic variation in sensitivity to carcinogen induced damage, as well as variation in DNA damage and repair capacity, may play a critical role in the development of lung cancer (82–84). DNA repair is a critical biologic defense mechanism that maintains the strength of the human genome by repairing damage from endogenous and exogenous sources (82). Wei and colleagues (85) were among the first to show an association between suboptimal DNA repair and an increased risk in lung cancer. Their findings were later confirmed in other studies (82, 86), which additionally found that pack-years smoked was also an independent predictor of lung cancer risk and further noted higher DNA repair in heavier smoking cases and lower repair in women compared with men (86).

Hill and colleagues (87) investigated gender differences in genetic damage induced by tobacco-specific nitrosamine (NNK), using the mutagen-sensitivity assay, which reflects an individual's sensitivity to mutagens as well as measures the DNA repair capacity in the individual (88). Although we did not find a difference in the number of chromosome aberrations between males and females in our study, their findings that chromosome aberrations were higher in females than in males ($P = 0.02$) support the idea that such biologic differences may contribute to the increase in lung cancer among females. Further their data suggest that a female's reduced capacity for repair of NNK-induced genetic damage may make them more sensitive to the mutagen effects (87). Considering the biologic differences between the sexes helps substantiate our finding that female heavy smokers exhibited an increased odds of lung cancer when compared with their male heavy smoker counterparts and could be due, in part, to suboptimal DNA repair. However, it does not explain why we found nonexposed female heavy smokers had an over a 4-fold increased risk of lung cancer compared with all other categories of female participants, including exposed females.

In our test for heterogeneity, we did not find a statistically significant difference between female heavy smokers (OR = 2.67; 95% CI, 1.39–5.11) and female moderate smokers (OR = 3.71; 95% CI, 1.78–7.70; $I^2 = 4.4\%$; P value for Q statistic = 0.31) with exposure, however, our statistically significant test for trend in this category ($P_{trend} = 0.027$) gives us pause to reflect on why the trend reverses between the moderate and heavy smokers. The concept that a low level of an exposure is deleterious at higher levels and confers a protective effect, also known as the Hormesis Hypothesis, introduces a novel concept that may explain our intriguing findings. Hormetic responses occur at the cellular, organ, individual, and population levels and reflect a general response to environmental induced stress/damage in which low/modest stress induces a prosurvival response (88). Given our results, we speculate that long-term [chronic] exposure to tobacco smoke and environmental agents may act hormetically to up regulate on the DNA repair mechanisms, and thus decrease the risk of lung cancer.

Hormesis is a conceptual model used to characterize a dose–response relationship between a chemical dose and a biologic response. Hormesis uses a U-shaped dose–response relationship to represent an initial protective effect at low exposure concentrations due to overcompensation to a disruption in homeostasis (89). Wang and colleagues (90) found that at low concentrations BDE-47 promoted cell proliferation with no obvious DNA damage or cell apoptosis, whereas at high concentrations BDE-47 inhibited cell proliferation and induced reactive oxygen species (ROS) production, which can lead to apoptosis.

In our study, we cannot rule out the suggestion that long-term, low levels of an environmental exposure could act hormetically with heavy smoking to decrease the risk of lung cancer in females and might explain the differences we found between exposed females who are moderate and heavy smokers. Several studies reported that heavy smokers have a more robust repair capacity (85, 91). In a recent study using data from the diesel exhaust in miners study (DEMS), Silverman and colleagues (92) evaluated exposure to diesel exhaust and lung cancer mortality in a nested case–control setting and found a similar association between smoking intensity and attenuated risk of lung cancer. Among the heaviest smoking participants, lung cancer risk decreased with increased levels of diesel exposure and the risk associated with smoking intensity was modified by diesel exposure. As with our study, the authors speculate that these findings may be due to biologic responses such as more efficient DNA repair, enhanced detoxification, or saturation of metabolic activation (92).

Limitations in our study necessitate consideration. First, we used a hospital-based case recruitment system, therefore, our study is not population-based and selection

bias may occur. Second, we were not able to measure environmental exposure quantitatively. Environmental exposure was based on a self-reported ever or never response, and therefore recall and reporting bias is a possibility. That said, several studies have validated the use of self-report as a viable means for assessing chemical-related exposures (93–95). Thus, if misclassification of exposure occurred it would be nondifferential and bias the risk of lung cancer toward the null (96). Arguably the strength of this study is that it confirms, in a larger sample size with measured smoking intensity, the CBMN assay is a rapid and accurate predictor of lung cancer. Moreover, our study further supports the premise that heavy smoking may have an effect on DNA repair capacity and in turn modulate the risk of lung cancer. The recent findings from the first study with quantitative data on historical diesel exposure and an adequate sample size to evaluate an exposure–response relationship between diesel exhaust, smoking intensity, and risk of lung cancer support our findings. Future studies are needed to confirm and determine the underlying mechanisms involved in the observed gender differences and risk of lung cancer.

Disclosure of Potential Conflicts of Interest

C.J. Etzel has Ownership Interest (including patents) in Patent on CBMN assay. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

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Development of methodology: M.K. McHugh, M.S. Lopez, M.R. Spitz, C. J. Etzel, R.A. El-Zein
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): M.S. Lopez, M.R. Spitz, R.A. El-Zein
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.K. McHugh, C.-H. Ho, M.R. Spitz, R.A. El-Zein
Writing, review, and/or revision of the manuscript: M.K. McHugh, M.R. Spitz, C.J. Etzel, R.A. El-Zein
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M.S. Lopez, M.R. Spitz, R.A. El-Zein
Study supervision: M.R. Spitz, C.J. Etzel, R.A. El-Zein

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Use of the Cytokinesis-Blocked Micronucleus Assay to Detect Gender Differences and Genetic Instability in a Lung Cancer Case–Control Study

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