Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2013 January ; 22(1): 135–145. doi:
10.1158/1055-9965.EPI-12-0435.

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

Michelle K. McHugh¹, Mirtha S. Lopez¹, Chung-Han Ho¹, Margaret R. Spitz², Carol J. Etzel², and Randa A. El-Zein¹

¹Department of Epidemiology, The University of Texas MD Anderson Cancer Center, Houston, TX 77030

²Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX 77030

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 co-exposures 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 to controls (mean $\pm$ SEM=3.54 $\pm$ 0.04 vs. 1.81 $\pm$ 0.04 and mean $\pm$ SEM=4.26 $\pm$ 0.03 vs. 0.99 $\pm$ 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 non-exposed 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.

Keywords

Lung cancer; CBMN assay; DNA damage; gender differences

Correspondence to: Randa El-Zein, MD, PhD, Department of Epidemiology, The University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030 (relzein@mdanderson.org). Phone: 713-745-2539. Fax: 713-792-8261.

Conflict of Interest: All authors report no conflict of interest with regard to the manuscript submitted.

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 co-exposures 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-block micronucleus (CBMN) assay in human lymphocytes is one of the most frequently used methods for measuring DNA damage (i.e., chromosome aberrations) (32). The CBMN assay is a multi-endpoint assay that measures not only chromosome damage (i.e., micronuclei (MN) 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 to 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 one nuclear division. This prevents confounding effects caused by differences in cell division kinetics because 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) (37, 38). Based on 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. 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 in Houston, Texas. 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 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 performed 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% fetal bovine serum, and 2 mmol/L L-glutamine (Life Technologies/Invitrogen, Carlsbad, CA) and 1% phytohemagglutinin (Remel, Lenexa, KS). At 44 hours after initiation, the cells were blocked in cytokinesis by adding cytochalasin B (Sigma, St. Louis, MO; final concentration 4µg/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, 1000 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) (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 one year before lung cancer diagnosis or interview date) and current smokers. Smoking groups were further categorized into light (pack years ≤ 20), moderate ($20 < \text{pack years} \leq 40$), 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 seven major environmental agents reported by the participants namely: asbestos, fibers, solvents, paints, wood dust, exhaust, and bleach.

Statistical Analysis

Pearson's chi square 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's two 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's 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 seven environmental agents; the four modified exposure factors from the PCA (i.e., chemicals, fibers, particulates and bleach); those with at least one of the seven environmental agents; and those without any of the seven environmental agents. Univariate Odds Ratios (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 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 performed using Statistical Analysis System (SAS) software (version 9.2; SAS Institute Inc., Cary, NC).

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 one environmental exposure ($P=0.11$). Approximately 84% of the cases were ever smokers as compared to 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 to 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 one of the seven environmental exposures, 10.5% reported two of the seven environmental exposures, and 13.5% reported 3 or more of the environmental exposures. Asbestos (19%) and solvents (18%) were the two 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: 1) all seven environmental agents; 2) as modified exposure categories based on factors from the PCA (i.e., chemicals, fibers, particulates and bleach); and 3) as those with at least one of the seven environmental agents. We did not find statistically significant differences in chromosome damage for those without environmental exposure or with exposure to any of the three exposure categories. Due to sample size we used those with at least one of the seven 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 one of the seven environmental agents. Overall cases showed significantly higher levels of genetic instability (BN-MN-mean $\pm$ SEM=3.54 $\pm$ 0.04; BN-NPB mean $\pm$ SEM=4.26 $\pm$ 0.03) as compared to controls (BN-MN mean $\pm$ SEM=1.81 $\pm$ 0.04; BN-NPB mean $\pm$ SEM=0.99 $\pm$ 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 $\pm$ SEM=1.05 $\pm$ 0.04) compared to those with at least one environmental exposure (BN-NPB mean $\pm$ SEM=0.94 $\pm$ 0.04) (P =0.06). In addition, among the cases, we did not observe significant differences in the MN 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 MN and the NPBs

respectively, thus indicating that the agreement between the original and the rescored data was 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 $\pm$ SEM=3.52 $\pm$ 0.06; BN-NPB-mean $\pm$ SEM=4.29 $\pm$ 0.05) as compared to controls (BN-MN mean $\pm$ SEM=1.83 $\pm$ 0.05; BN-NPB mean $\pm$ SEM=0.98 $\pm$ 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 $\pm$ SEM=3.55 $\pm$ 0.06; BN-NPB mean $\pm$ SEM=4.23 $\pm$ 0.05) as compared to controls (BN-MN mean $\pm$ SEM=1.79 $\pm$ 0.05; BN-NPB mean $\pm$ SEM=1.01 $\pm$ 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 three-fold increased risk of lung cancer (OR = 2.68, 95% CI 1.41–5.11) as compared to 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 border line 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 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 non-exposed 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 non-exposed 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 two 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 utility 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 non-exposed male and female moderate smokers. For estimated risk of lung cancer, we found a difference between non-exposed male heavy smokers and non-exposed female heavy smokers, although it was not statistically significant ($I^2=64.9\%$; p-value for Q statistic=0.09). Due to the subgroup analyses and issues with multiple comparisons, chance variability may account for such findings. However, other factors such as inter-individual differences in the genetic susceptibility and other co-exposures 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, 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 while 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 to 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 et al. (56) found males with a family history of lung cancer in first degree relatives had a slightly higher increased risk compared to 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 biological 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), while 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 10–15% 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 biological defense mechanism that maintains the strength of the human genome by repairing damage from endogenous and exogenous sources (82). Wei et al. (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 to men (86).

Hill et al. (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 biological differences may contribute to the increase in lung cancer amongst 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 biological differences between the sexes helps substantiate our finding that female heavy smokers exhibited an increased odds of lung cancer when compared to their male heavy smoker counterparts and could be due, in part, to suboptimal DNA repair. However, it does not explain why we found non-exposed female heavy smokers had an over a four-fold increased risk of lung cancer compared to 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 where low/modest stress induces a pro-survival 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 biological response. Hormesis utilizes 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 et al., (90) found that at low concentrations BDE-47 promoted cell proliferation with no obvious DNA damage or cell

apoptosis while 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 et al. (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 biological 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 non-differential and bias the risk of lung cancer towards 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.

Acknowledgments

We would like to acknowledge Dr. Terrance J. Kavanagh at the University of Washington, Department of Environmental and Occupational Health Sciences for his insightful comments.

GRANT SUPPORT: This work was supported by the National Cancer Institute at the National Institutes of Health (K07CA093592 to C.J.E., CA55769 and CA127219 to M.R.S., and CA123208 and CA131327 to C.J.E.), and by a National Cancer Institute at the National Institutes of Health cancer prevention fellowship (R25T CA57730 to M.K.M.), Shine Chang, Ph.D., Principal Investigator.

REFERENCES

1. Checkoway, H.; Pearce, N.; Kriebel, D. Research methods in occupational epidemiology. 2nd ed.. Oxford: Oxford UP; 2004.
2. Sailaja N, Chandrasekhar M, Rekhadevi PV, Mahboob M, Rahman MF, Vuyyuri SB, et al. Genotoxic evaluation of workers employed in pesticide production. *Mutat Res.* 2006; 609(1):74–80. [PubMed: 16887377]
3. Atherton KM, Williams FM, Egea Gonzalez FJ, Glass R, Rushton S, Blain PG, et al. DNA damage in horticultural farmers: a pilot study showing an association with organophosphate pesticide exposure. *Biomarkers.* 2009; 14(7):443–451. [PubMed: 19863182]

4. Zeljezic D, Vrdoljak AL, Lucas JN, Lasan R, Fucic A, Kopjar N, et al. Effect of occupational exposure to multiple pesticides on translocation yield and chromosomal aberrations in lymphocytes of plant workers. *Environ Sci Technol*. 2009; 43(16):6370–6377. [PubMed: 19746739]
5. Hengstler JG, Bolm-Audorff U, Faldum A, Janssen K, Reifenrath M, Gotte W, et al. Occupational exposure to heavy metals: DNA damage induction and DNA repair inhibition prove co-exposures to cadmium, cobalt and lead as more dangerous than hitherto expected. *Carcinogenesis*. 2003; 24(1): 63–73. [PubMed: 12538350]
6. Palus J, Rydzynski K, Dziubaltowska E, Wyszynska K, Natarajan AT, Nilsson R. Genotoxic effects of occupational exposure to lead and cadmium. *Mutat Res*. 2003; 540(1):19–28. [PubMed: 12972055]
7. Danadevi K, Rozati R, Saleha Banu B, Hanumanth Rao P, Grover P. DNA damage in workers exposed to lead using comet assay. *Toxicology*. 2003; 187(2–3):183–193. [PubMed: 12699907]
8. Cebulska-Wasilewska A, Panek A, Zabinski Z, Moszczynski P, Au WW. Occupational exposure to mercury vapour on genotoxicity and DNA repair. *Mutat Res*. 2005; 586(2):102–114. [PubMed: 16125447]
9. Vodicka P, Koskinen M, Stetina R, Soucek P, Vodickova L, Matousu Z, et al. The role of various biomarkers in the evaluation of styrene genotoxicity. *Cancer Detect Prev*. 2003; 27(4):275–284. [PubMed: 12893075]
10. Slysokova J, Dusinska M, Kuricova M, Soucek P, Vodickova L, Susova S, et al. Relationship between the capacity to repair 8-oxoguanine, biomarkers of genotoxicity and individual susceptibility in styrene-exposed workers. *Mutat Res*. 2007; 634(1–2):101–111. [PubMed: 17855160]
11. Vodicka P, Tvrdik T, Osterman-Golkar S, Vodickova L, Peterkova K, Soucek P, et al. An evaluation of styrene genotoxicity using several biomarkers in a 3-year follow-up study of hand-lamination workers. *Mutat Res*. 1999; 445(2):205–224. [PubMed: 10575431]
12. Manini P, De Palma G, Andreoli R, Marczyński B, Hanova M, Mozzoni P, et al. Biomarkers of nucleic acid oxidation, polymorphism in, expression of, hOGG1 gene in styrene-exposed workers. *Toxicol Lett*. 2009; 190(1):41–47. [PubMed: 19563869]
13. Maffei F, Angelini S, Forti GC, Violante FS, Lodi V, Mattioli S, et al. Spectrum of chromosomal aberrations in peripheral lymphocytes of hospital workers occupationally exposed to low doses of ionizing radiation. *Mutat Res*. 2004; 547(1–2):91–99. [PubMed: 15013703]
14. Kopjar N, Garaj-Vrhovac V. Assessment of DNA damage in nuclear medicine personnel—comparative study with the alkaline comet assay and the chromosome aberration test. *Int J Hyg Environ Health*. 2005; 208(3):179–191. [PubMed: 15971857]
15. Jovicic D, Milacic S, Milic N, Bukvic N, Vukov TD. Chromosomal aberrations in subjects exposed to ionizing radiation. *J Environ Pathol Toxicol Oncol*. 2009; 28(1):75–82. [PubMed: 19392657]
16. Lalic H. Cytogenetic monitoring of medical staff professionally exposed to Gamma and X radiation. *Neoplasma*. 2005; 52(4):307–313. [PubMed: 16059647]
17. Engin AB, Ergun MA, Yurtcu E, Kan D, Sahin G. Effect of ionizing radiation on the pteridine metabolic pathway and evaluation of its cytotoxicity in exposed hospital staff. *Mutat Res*. 2005; 585(1–2):184–192. [PubMed: 15998597]
18. Galiotte MP, Kohler P, Mussi G, Gattas GJ. Assessment of occupational genotoxic risk among Brazilian hairdressers. *Ann Occup Hyg*. 2008; 52(7):645–651. [PubMed: 18596021]
19. Costa S, Coelho P, Costa C, Silva S, Mayan O, Santos LS, et al. Genotoxic damage in pathology anatomy laboratory workers exposed to formaldehyde. *Toxicology*. 2008; 252(1–3):40–48. [PubMed: 18721846]
20. Cavallo D, Ursini CL, Setini A, Chianese C, Cristaudo A, Iavicoli S. DNA damage and TNFalpha cytokine production in hairdressers with contact dermatitis. *Contact Dermatitis*. 2005; 53(3):125–129. [PubMed: 16128749]
21. Ursini CL, Cavallo D, Colombi A, Giglio M, Marinaccio A, Iavicoli S. Evaluation of early DNA damage in healthcare workers handling antineoplastic drugs. *Int Arch Occup Environ Health*. 2006; 80(2):134–140. [PubMed: 16761155]

22. Sasaki M, Dakeishi M, Hoshi S, Ishii N, Murata K. Assessment of DNA damage in Japanese nurses handling antineoplastic drugs by the comet assay. *J Occup Health*. 2008; 50(1):7–12. [PubMed: 18285639]
23. Rekha Devi PV, Sailaja N, Chandrasekhar M, Mahboob M, Rahman MF, Grover P. Genotoxicity assessment in oncology nurses handling anti-neoplastic drugs. *Mutagenesis*. 2007; 22(6):395–401. [PubMed: 17855733]
24. Consonni D, Boffetta P, Andersen A, Chang-Claude J, Cherrie JW, Ferro G, et al. Lung cancer mortality among European rock/slag wool workers: exposure-response analysis. *Cancer Causes Control*. 1998; 9(4):411–416. [PubMed: 9794173]
25. Chiaze L Jr, Watkins DK. Epidemiology studies of synthetic vitreous fibers: methods used and current studies. *Regul Toxicol Pharmacol*. 1994; 20(3 Pt 2):S58–S67. [PubMed: 7724856]
26. Chiaze L Jr, Watkins DK, Fryar C. Adjustment for the confounding effect of cigarette smoking in an historical cohort mortality study of workers in a fiberglass manufacturing facility. *J Occup Environ Med*. 1995; 37(6):744–748. [PubMed: 7670922]
27. Wong O, Musselman RP. An epidemiological and toxicological evaluation of the carcinogenicity of man-made vitreous fiber, with a consideration of coexposures. *J Environ Pathol Toxicol Oncol*. 1994; 13(3):169–180. [PubMed: 7722884]
28. Berrigan D. Respiratory cancer and exposure to man-made vitreous fibers: a systematic review. *Am J Ind Med*. 2002; 42(4):354–362. [PubMed: 12271483]
29. Ross NA, Wolfson MC, Dunn JR, Berthelot JM, Kaplan GA, Lynch JW. Relation between income inequality and mortality in Canada and in the United States: cross sectional assessment using census data and vital statistics. *BMJ*. 2000; 320(7239):898–902. [PubMed: 10741994]
30. El-Zein R, Vral A, Etzel CJ. Cytokinesis-blocked micronucleus assay and cancer risk assessment. *Mutagenesis*. 2011; 26(1):101–106. [PubMed: 21164189]
31. Christmann M, Tomicic MT, Roos WP, Kaina B. Mechanisms of human DNA repair: an update. *Toxicology*. 2003; 193(1–2):3–34. [PubMed: 14599765]
32. Fenech M. Biomarkers of genetic damage for cancer epidemiology. *Toxicology*. 2002; 181–182:411–416.
33. Fenech M. The in vitro micronucleus technique. *Mutat Res*. 2000; 455(1–2):81–95. [PubMed: 11113469]
34. El-Zein RA, Schabath MB, Etzel CJ, Lopez MS, Franklin JD, Spitz MR. Cytokinesis-blocked micronucleus assay as a novel biomarker for lung cancer risk. *Cancer Res*. 2006; 66(12):6449–6456. [PubMed: 16778224]
35. El-Zein RA, Fenech M, Lopez MS, Spitz MR, Etzel CJ. Cytokinesis-blocked micronucleus cytome assay biomarkers identify lung cancer cases amongst smokers. *Cancer Epidemiol Biomarkers Prev*. 2008; 17(5):1111–1119. [PubMed: 18483333]
36. McHugh MK, Kachroo S, Liu M, D'Amelio AM Jr, Dong Q, Hong WK, et al. Assessing environmental and occupational risk factors for lung cancer in Mexican-Americans. *Cancer Causes Control*. 2010; 21(12):2157–2164. [PubMed: 20809339]
37. Spitz MR, Hong WK, Amos CI, Wu X, Schabath MB, Dong Q, et al. A risk model for prediction of lung cancer. *J Natl Cancer Inst*. 2007; 99(9):715–726. [PubMed: 17470739]
38. Etzel CJ, Kachroo S, Liu M, D'Amelio A, Dong Q, Cote ML, et al. Development and validation of a lung cancer risk prediction model for African-Americans. *Cancer Prev Res (Phila Pa)*. 2008; 1(4):255–265.
39. Fenech M, Morley AA. Measurement of micronuclei in lymphocytes. *Mutat Res*. 1985; 147(1–2):29–36. [PubMed: 3974610]
40. Fenech M. Cytokinesis-block micronucleus cytome assay. *Nat Protoc*. 2007; 2(5):1084–1104. [PubMed: 17546000]
41. Fenech M. Micronucleus frequency in human lymphocytes is related to plasma vitamin B12 and homocysteine. *Mutat Res*. 1999; 428(1–2):299–304. [PubMed: 10518002]
42. Fenech M, Chang WP, Kirsch-Volders M, Holland N, Bonassi S, Zeiger E. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. *Mutat Res*. 2003; 534(1–2):65–75. [PubMed: 12504755]
43. Jolliffe, IT. Principle component analysis. New York: Springer; 2002.

44. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. 2003; 327(7414):557–560. [PubMed: 12958120]
45. [cited 2012 March 2]] American Cancer Society: Cancer Facts and Figures 2012. 2012. Available from: <http://www.cancer.org/Research/CancerFactsFigures/CancerFactsFigures/cancer-factsfigures-2012>
46. Brownson RC, Chang JC, Davis JR. Gender and histologic type variations in smoking-related risk of lung cancer. *Epidemiology*. 1992; 3(1):61–64. [PubMed: 1313311]
47. Risch HA, Howe GR, Jain M, Burch JD, Holowaty EJ, Miller AB. Are female smokers at higher risk for lung cancer than male smokers? A case-control analysis by histologic type. *Am J Epidemiol*. 1993; 138(5):281–293. [PubMed: 8395141]
48. Zang EA, Wynder EL. Differences in lung cancer risk between men and women: examination of the evidence. *J Natl Cancer Inst*. 1996; 88(3–4):183–192. [PubMed: 8632492]
49. Henschke CI, Yip R, Miettinen OS. Women's susceptibility to tobacco carcinogens and survival after diagnosis of lung cancer. *JAMA*. 2006; 296(2):180–184. [PubMed: 16835423]
50. Prescott E, Osler M, Hein HO, Borch-Johnsen K, Lange P, Schnohr P, et al. Gender and smoking-related risk of lung cancer. The Copenhagen Center for Prospective Population Studies. *Epidemiology*. 1998; 9(1):79–83. [PubMed: 9430273]
51. Halpern MT, Gillespie BW, Warner KE. Patterns of absolute risk of lung cancer mortality in former smokers. *J Natl Cancer Inst*. 1993; 85(6):457–464. [PubMed: 8445673]
52. Bain C, Feskanich D, Speizer FE, Thun M, Hertzmark E, Rosner BA, et al. Lung cancer rates in men and women with comparable histories of smoking. *J Natl Cancer Inst*. 2004; 96(11):826–834. [PubMed: 15173266]
53. Bach PB, Kattan MW, Thornquist MD, Kris MG, Tate RC, Barnett MJ, et al. Variations in lung cancer risk among smokers. *J Natl Cancer Inst*. 2003; 95(6):470–478. [PubMed: 12644540]
54. Agudo A, Ahrens W, Benhamou E, Benhamou S, Boffetta P, Darby SC, et al. Lung cancer and cigarette smoking in women: A multicenter case-control study in Europe. *International Journal of Cancer*. 2000; 88(5):820–827.
55. Kreuzer M, Boffetta P, Whitley E, Ahrens W, Gaborieau V, Heinrich J, et al. Gender differences in lung cancer risk by smoking: a multicentre case-control study in Germany and Italy. *British Journal of Cancer*. 2000; 82(1):227–233. [PubMed: 10638994]
56. Lo YL, Hsiao CF, Chang GC, Tsai YH, Huang MS, Su WC, et al. Risk factors for primary lung cancer among never smokers by gender in a matched case-control study. *Cancer Causes Control*. 2012
57. Chakraborty S, Ganti AK, Marr A, Batra SK. Lung cancer in women: role of estrogens. *Expert Rev Respir Med*. 2010; 4(4):509–518. [PubMed: 20658912]
58. Schabath MB, Wu X, Vassilopoulou-Sellin R, Vaporciyan AA, Spitz MR. Hormone replacement therapy and lung cancer risk: a case-control analysis. *Clin Cancer Res*. 2004; 10(1 Pt 1):113–123. [PubMed: 14734459]
59. Ramnath N, Menezes RJ, Loewen G, Dua P, Eid F, Alkhaddo J, et al. Hormone replacement therapy as a risk factor for non-small cell lung cancer: results of a case-control study. *Oncology*. 2007; 73(5–6):305–310. [PubMed: 18493157]
60. Schwartz AG, Wenzlaff AS, Prysak GM, Murphy V, Cote ML, Brooks SC, et al. Reproductive factors, hormone use, estrogen receptor expression and risk of non small-cell lung cancer in women. *J Clin Oncol*. 2007; 25(36):5785–5792. [PubMed: 18089876]
61. Chen KY, Hsiao CF, Chang GC, Tsai YH, Su WC, Perng RP, et al. Hormone replacement therapy and lung cancer risk in Chinese. *Cancer*. 2007; 110(8):1768–1775. [PubMed: 17879370]
62. Blackman JA, Coogan PF, Rosenberg L, Strom BL, Zauberman AG, Palmer JR, et al. Estrogen replacement therapy and risk of lung cancer. *Pharmacoepidemiol Drug Saf*. 2002; 11(7):561–567. [PubMed: 12462132]
63. Kreuzer M, Gerken M, Heinrich J, Kreienbrock L, Wichmann HE. Hormonal factors and risk of lung cancer among women? *Int J Epidemiol*. 2003; 32(2):263–271. [PubMed: 12714547]
64. Taioli E, Wynder EL. Re: Endocrine factors and adenocarcinoma of the lung in women. *J Natl Cancer Inst*. 1994; 86(11):869–870. [PubMed: 8182770]

65. Adami HO, Persson I, Hoover R, Schairer C, Bergkvist L. Risk of cancer in women receiving hormone replacement therapy. *Int J Cancer*. 1989; 44(5):833–839. [PubMed: 2583865]
66. Smith JR, Barrett-Connor E, Krititz-Silverstein D, Wingard DL, Al-Delaimy WK. Hormone use and lung cancer incidence: the Rancho Bernardo cohort study. *Menopause*. 2009; 16(5):1044–1048. [PubMed: 19387414]
67. Slatore CG, Chien JW, Au DH, Satia JA, White E. Lung cancer and hormone replacement therapy: association in the vitamins and lifestyle study. *J Clin Oncol*. 2010; 28(9):1540–1546. [PubMed: 20159813]
68. Chlebowski RT, Schwartz AG, Wakelee H, Anderson GL, Stefanick ML, Manson JE, et al. Oestrogen plus progestin and lung cancer in postmenopausal women (Women's Health Initiative trial): a post-hoc analysis of a randomised controlled trial. *Lancet*. 2009; 374(9697):1243–1251. [PubMed: 19767090]
69. Fasco MJ, Hurteau GJ, Spivack SD. Gender-dependent expression of alpha and beta estrogen receptors in human nontumor and tumor lung tissue. *Mol Cell Endocrinol*. 2002; 188(1–2):125–140. [PubMed: 11911952]
70. Omoto Y, Kobayashi Y, Nishida K, Tsuchiya E, Eguchi H, Nakagawa K, et al. Expression, function, and clinical implications of the estrogen receptor beta in human lung cancers. *Biochem Biophys Res Commun*. 2001; 285(2):340–347. [PubMed: 11444848]
71. Kawai H, Ishii A, Washiya K, Konno T, Kon H, Yamaya C, et al. Estrogen receptor alpha and beta are prognostic factors in non-small cell lung cancer. *Clin Cancer Res*. 2005; 11(14):5084–5089. [PubMed: 16033821]
72. Ishibashi H, Suzuki T, Suzuki S, Niikawa H, Lu L, Miki Y, et al. Progesterone receptor in non-small cell lung cancer—a potent prognostic factor and possible target for endocrine therapy. *Cancer Res*. 2005; 65(14):6450–6458. [PubMed: 16024650]
73. Marquez-Garban DC, Chen HW, Fishbein MC, Goodglick L, Pietras RJ. Estrogen receptor signaling pathways in human non-small cell lung cancer. *Steroids*. 2007; 72(2):135–143. [PubMed: 17276470]
74. Schwartz AG, Prysak GM, Murphy V, Lonardo F, Pass H, Schwartz J, et al. Nuclear estrogen receptor beta in lung cancer: expression and survival differences by sex. *Clin Cancer Res*. 2005; 11(20):7280–7287. [PubMed: 16243798]
75. Kaiser U, Hofmann J, Schilli M, Wegmann B, Klotz U, Wedel S, et al. Steroid-hormone receptors in cell lines and tumor biopsies of human lung cancer. *Int J Cancer*. 1996; 67(3):357–364. [PubMed: 8707409]
76. Weinberg OK, Marquez-Garban DC, Fishbein MC, Goodglick L, Garban HJ, Dubinett SM, et al. Aromatase inhibitors in human lung cancer therapy. *Cancer Res*. 2005; 65(24):11287–11291. [PubMed: 16357134]
77. Stabile LP, Davis AL, Gubish CT, Hopkins TM, Luketich JD, Christie N, et al. Human non-small cell lung tumors and cells derived from normal lung express both estrogen receptor alpha and beta and show biological responses to estrogen. *Cancer Res*. 2002; 62(7):2141–2150. [PubMed: 11929836]
78. Hersherberger PA, Vasquez AC, Kanterewicz B, Land S, Siegfried JM, Nichols M. Regulation of endogenous gene expression in human non-small cell lung cancer cells by estrogen receptor ligands. *Cancer Res*. 2005; 65(4):1598–15605. [PubMed: 15735050]
79. Radzikowska E, Langfort R, Giedronowicz D. Estrogen and progesterone receptors in non small cell lung cancer patients. *Ann Thorac Cardiovasc Surg*. 2002; 8(2):69–73. [PubMed: 12027790]
80. Rivera MP. Lung cancer in women: the differences in epidemiology, biology and treatment outcomes. *Expert Rev Respir Med*. 2009; 3(6):627–634. [PubMed: 20477352]
81. Kiyohara C, Ohno Y. Sex differences in lung cancer susceptibility: a review. *Gend Med*. 2010; 7(5):381–401. [PubMed: 21056866]
82. Spitz MR, Wei Q, Dong Q, Amos CI, Wu X. Genetic susceptibility to lung cancer: the role of DNA damage and repair. *Cancer Epidemiol Biomarkers Prev*. 2003; 12(8):689–698. [PubMed: 12917198]
83. Christiani DC. Smoking and the molecular epidemiology of lung cancer. *Clin Chest Med*. 2000; 21(1):87–93. viii. [PubMed: 10763091]

84. Li D, Firozi PF, Wang LE, Bosken CH, Spitz MR, Hong WK, et al. Sensitivity to DNA damage induced by benzo(a)pyrene diol epoxide and risk of lung cancer: a case-control analysis. *Cancer Res.* 2001; 61(4):1445–1450. [PubMed: 11245449]
85. Wei Q, Cheng L, Amos CI, Wang LE, Guo Z, Hong WK, et al. Repair of tobacco carcinogen-induced DNA adducts and lung cancer risk: a molecular epidemiologic study. *J Natl Cancer Inst.* 2000; 92(21):1764–1772. [PubMed: 11058619]
86. Shen H, Spitz MR, Qiao Y, Guo Z, Wang LE, Bosken CH, et al. Smoking, DNA repair capacity and risk of nonsmall cell lung cancer. *Int J Cancer.* 2003; 107(1):84–88. [PubMed: 12925960]
87. Hill CE, Affatato AA, Wolfe KJ, Lopez MS, Hallberg CK, Canistro D, et al. Gender differences in genetic damage induced by the tobacco-specific nitrosamine NNK and the influence of the Thr241Met polymorphism in the XRCC3 gene. *Environ Mol Mutagen.* 2005; 46(1):22–29. [PubMed: 15887211]
88. Calabrese EJ, Iavicoli I, Calabrese V. Hormesis: why it is important to biogerontologists. *Biogerontology.* 2012
89. Calabrese EJ, Baldwin LA. The frequency of U-shaped dose responses in the toxicological literature. *Toxicol Sci.* 2001; 62(2):330–338. [PubMed: 11452146]
90. Wang L, Zou W, Zhong Y, An J, Zhang X, Wu M, et al. The hormesis effect of BDE-47 in HepG(2) cells and the potential molecular mechanism. *Toxicol Lett.* 2012; 209(2):193–201. [PubMed: 22233939]
91. Deng L, Kimmel M, Foy M, Spitz M, Wei Q, Gorlova O. Estimation of the effects of smoking and DNA repair capacity on coefficients of a carcinogenesis model for lung cancer. *Int J Cancer.* 2009; 124(9):2152–2158. [PubMed: 19123470]
92. Silverman DT, Samanic CM, Lubin JH, Blair AE, Stewart PA, Vermeulen R, et al. The Diesel Exhaust in Miners Study: A Nested Case-Control Study of Lung Cancer and Diesel Exhaust. *J Natl Cancer Inst.* 2012
93. Koutros S, Lynch CF, Ma X, Lee WJ, Hoppin JA, Christensen CH, et al. Heterocyclic aromatic amine pesticide use and human cancer risk: results from the U.S. Agricultural Health Study. *Int J Cancer.* 2009; 124(5):1206–1212. [PubMed: 19058219]
94. Blair A, Tarone R, Sandler D, Lynch CF, Rowland A, Wintersteen W, et al. Reliability of reporting on life-style and agricultural factors by a sample of participants in the Agricultural Health Study from Iowa. *Epidemiology.* 2002; 13(1):94–99. [PubMed: 11805592]
95. Hoppin JA, Yucel F, Dosemeci M, Sandler DP. Accuracy of self-reported pesticide use duration information from licensed pesticide applicators in the Agricultural Health Study. *J Expo Anal Environ Epidemiol.* 2002; 12(5):313–318. [PubMed: 12198579]
96. Rothman, KJ.; Greenland, S. *Modern Epidemiology.* 2nd Edition. Philadelphia: Lippencott-Raven; 1998.

Table 1

Demographic characteristics by case-control status

	Case (N=500)	Control (N=500)	* <i>P</i> -value
Age (Mean±SD ^a)	62.50 ±1.12	61.80 ±1.12	0.310
Age Group (%)			
Age 50 years of age	80 (16.00)	65 (13.00)	0.209
Age>50 years of age	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 ^β (%)			
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 [£] (Mean±SE)	45.56 ±1.43	37.53 ±1.58	<0.001
Environmental Exposure [¥] (%)			
Without environmental exposure	243 (48.60)	269 (53.80)	0.114
With at least one environmental exposure	257 (51.40)	231 (46.20)	

* *P*-values were obtained from Pearson's chi square test (categorical variables) and the Student's two sample t-test (continuous variables)

^a SD, Standard Deviation

^β Smoking intensity, light smokers (0<pack-years 20), moderate smokers (20<pack-years 40), heavy smokers (40<pack-years)

[£] Smoking pack-years, years smoked times the average number of cigarettes per day divided by 20

[¥] Self-reported exposure to at least one of seven environmental agents (i.e., asbestos, fiberglass, wood dust, solvents, paints, exhaust, and bleach)

Table 2CBMN outcomes for all cases and controls by smoking^a and environmental exposure^β

ALL	Case (N=500)	P-value	Control (N=500)	P-value
BN-MN^μ				
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	
	<i>P_{trend}</i> = 0.69		<i>P_{trend}</i> = 0.95	
Environmental exposure ^β				
Without exposure	3.58 ±0.070	0.24	1.79 ±0.06	0.85
With at least one 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	
	<i>P_{trend}</i> = 0.89		<i>P_{trend}</i> = 0.75	
Environmental exposure ^β				
Without exposure	4.31 ±0.05	0.17	1.05 ±0.04	0.06
With at least one exposure	4.21 ±0.05		0.94 ±0.04	

^aSmoking 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

^βSelf-reported exposure to at least one of seven environmental exposures (i.e., asbestos, fiberglass, wood dust, solvents, paints, exhaust, and bleach; means adjusted by age, gender and smoking

^μBN-MN, binucleated cells with micronuclei

[Ⓜ]NPB binucleated cells with nucleoplasmic bridges

Table 3CBMN outcomes for male cases and controls by smoking^a and environmental exposure^β

MALES	Case (N=500)	P-value	Control (N=500)	P-value
BN-MN^μ				
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	
	<i>P_{trend}</i> = 0.07		<i>P_{trend}</i> = 0.38	
Environmental exposure ^β				
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	
	<i>P_{trend}</i> = 0.71		<i>P_{trend}</i> = 0.07	
Environmental exposure ^β				
Without exposure	4.30 ±0.08	0.82	1.03 ±0.09	0.22
With at least one exposure	4.27 ±0.07		0.93 ±0.05	

^aSmoking 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

^βSelf-reported exposure to at least one of seven environmental exposures (i.e., asbestos, fiberglass, wood dust, solvents, paints, exhaust, and bleach; means adjusted by age and smoking intensity)

^μBN-MN, binucleated cells with micronuclei

[¥]BN-NPB, binucleated cells with nucleoplasmic bridges

Table 4CBMN outcomes for female cases and controls by smoking^a and environmental exposure^β

FEMALES	Case (N=500)	P-value	Control (N=500)	P-value
MN-BN^μ				
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	
	<i>P_{trend}</i> = 0.42		<i>P_{trend}</i> = 0.27	
Environmental exposure ^β				
Without exposure	3.63 ±0.08	0.08	1.75 ±0.07	0.26
With at least one 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 ±0.010	
Heavy Smokers	4.25 ±0.05		0.86 ±0.09	
	<i>P_{trend}</i> = 0.73		<i>P_{trend}</i> = 0.13	
Environmental exposure ^β				
Without exposure	4.30 ±0.06	0.08	1.04 ±0.05	0.18
With at least one exposure	4.12 ±0.08		0.92 ±0.07	

^aSmoking 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

^βSelf-reported exposure to at least one of seven environmental exposures (i.e., asbestos, fiberglass, wood dust, solvents, paints, exhaust, and bleach; means adjusted by age and smoking intensity)

^μBN-MN, binucleated cells with micronuclei

[¥]BN-NPB, binucleated cells with nucleoplasmic bridges

Table 5Odds Ratios and 95% Confidence Intervals for smoking^a and environmental exposures^b and lung cancer risk

ALL ^μ	Case (N=500)	Control (N=500)	Odds Ratio (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 [€]	Case (N=245)	Control (N=246)	Odds Ratio (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 [€]	Case (N=255)	Control (N=254)	Odds Ratio (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)

ALL ^μ	Case (N=500)	Control (N=500)	Odds Ratio (95%CI [*])
<i>P</i> _{trend} = 0.027			

^α Smoking intensity, light smokers (0<pack-years 20), moderate smokers (20<pack-years 40), heavy smokers (40<pack-years),

^β Self-reported exposure to at least one of seven environmental exposures (i.e., asbestos, fiberglass, wood dust, solvents, paints, exhaust, and bleach,

^μ model adjusted by age and gender,

[€] model adjusted by age,

^{*} CI, confidence interval