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DNA Repair Capacity and Lung Cancer Risk in Never Smokers

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Abstract

Besides secondhand smoke exposure, few other risk factors for lung cancer in lifetime never smokers have been identified. We present the estimates of lung cancer risk associated with suboptimal DNA repair capacity (DRC) measured by the host-cell reactivation assay in lifetime never smokers using data from 219 cases and 309 matched controls enrolled in a case-control study. Suboptimal DRC level (below the control median) conferred a significantly increased lung cancer risk in never smokers [odds ratio, 1.92; 95% confidence interval (95% CI), 1.3–2.9; $P = 0.0024$]. There was a 3.38-fold risk for individuals with DRC below the first quartile (95% CI, 1.8–6.3) compared with individuals with DRC above the third quartile. Secondhand smoke exposure in individuals with DRC below the control median was associated with a 3.81-fold risk of lung cancer (95% CI, 2.3–6.4). A 2.49-fold (95% CI, 1.1–5.6) risk was noted for the joint effects of lung cancer family history in first-degree relatives and suboptimal DRC. Relatives of probands (cases and controls) with lowest DRC (below the first quartile) were significantly more likely to be diagnosed with lung cancer (odds ratio, 2.69; 95% CI, 1.1–6.7) compared with relatives of probands with the most proficient DRC (above the third quartile). Relatives of probands with suboptimal (below the control median) versus proficient DRC also had an earlier age at diagnosis with lung cancer, although the only statistically significant difference was in female relatives (55.4 versus 67.7 years; $P = 0.03$).

Introduction

Risk factors for lung cancer in never smokers are poorly understood. The only established risk factor is second-hand smoke (SHS) exposure (1–5). Although sidestream smoke, to which the involuntary smoker is mostly exposed, is diluted to a variable extent depending on distance from the smoking source and the amount of ventilation, sidestream smoke, compared with mainstream smoke, contains higher concentrations of benzo[*a*]pyrene and other polycyclic aromatic hydrocarbons (~ 10-fold higher; refs. 6, 7) and nitrosamines (6). Benzo[*a*]pyrene is a classic DNA-damaging carcinogen (8, 9), forming bulky DNA adducts

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No potential conflicts of interest were disclosed.

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through covalent binding or oxidation (10) that require repair by the nucleotide excision repair pathway (11). If not repaired, these adducts can block the transcription of an essential gene or cause mutations at hot spots (such as G-to-T transversions in p53; refs. 12, 13). Therefore, inefficient DNA repair capacity (DRC) could play an important role in SHS-induced carcinogenesis.

Wei et al. (14) modified the *in vitro* host-cell reactivation assay to assess DRC for removal of benzo[*a*]pyrene diol epoxide (BPDE) adducts and showed that suboptimal DRC was associated with lung cancer risk, a finding that was confirmed in subsequent studies (15, 16).

Because the majority of lifetime never smokers with lung cancer in our series reported exposure to secondhand tobacco smoke (up to 70%; ref. 17), this analysis focuses on the role of DRC in risk of lung cancer in lifetime never smokers in the context of SHS exposure. We and others have found evidence of familial aggregation of lung cancer among first-degree relatives of never smokers with lung cancer, suggesting a role for genetic susceptibility to lung cancer in lifetime never smokers (18–20). DRC is likely to be constitutive and genetically controlled (21). Therefore, we evaluated the joint effects of cancer family history and DRC on lung cancer risk. We also examined whether the DRC status of study participants was predictive of lung cancer risk among their first-degree relatives.

Materials and Methods

Subject Recruitment.

From September 1995 to December 2003, patients with lung cancer were accrued for an ongoing and previously described molecular epidemiologic study on susceptibility markers for lung cancer from The University of Texas M. D. Anderson Cancer Center. There were no age, gender, ethnic, or stage restrictions. Included in this study were 385 patients (219 with measured DRC) with histologically confirmed, newly diagnosed, and previously untreated lung cancer, who reported themselves to be lifetime never smokers (defined as those who had smoked <100 cigarettes in their lifetime) and who had no history of prior cancer other than nonmelanoma skin cancer. The response rate for case accrual was about 80%. The reasons for refusal to participate included patients too ill, patients referred only for second opinion to The University of Texas M. D. Anderson Cancer Center, or patients unwilling to donate blood for the study and complete the interview. Healthy controls who were also lifetime never smokers ($n = 536$; 309 with measured DRC) without a previous diagnosis of cancer (except for nonmelanoma skin cancer) were recruited from the Kelsey-Seybold Clinics, Houston's largest private multispecialty physician group, which includes a network of 23 clinics and more than 300 physicians in the Houston metropolitan area. Controls were frequency matched to the recruited cases on age (±5 years), gender, and ethnicity. The response rate for controls was about 75% when approached for an interview. The main reasons for declining participation included lack of time or difficulties related to transportation to the clinic. All cases and controls were U.S. residents. This research was approved by The University of Texas M. D. Anderson Cancer Center and Kelsey-Seybold institutional review boards.

Collection of Epidemiologic Data.

After the study participants were briefed on the study and signed an informed consent, a 45-min structured personal interview was conducted by The University of Texas M. D. Anderson Cancer Center research interviewers, during which they obtained information on sociodemographic characteristics, smoking and SHS exposure history, prior exposures and certain respiratory conditions, and family history of cancer in first-degree relatives. Family history included cancer histories of all first-degree relatives (parents, siblings, and offspring), their year of birth, age at the time of study or at death, and smoking status (yes or no), collected for each relative, as well as type of cancer and age at diagnosis for affected relatives. The degree of missing data on cancer family history was reported previously (18). The participants were asked about the SHS exposure in their lifetime. Specifically, SHS exposure was defined as having been around someone else's cigarette smoke on a regular basis, daily or weekly, as identified by the embedded probes within the questionnaire. SHS exposures at home and at work were reported separately. Exposed individuals also reported the number of years of exposure. Exposure to SHS was analyzed as a dichotomous (presence/absence of the exposure) variable. Those who reported being exposed every day or few times a week, either at home or at work, were considered exposed, whereas those reporting no exposure or being exposed only few times a month or rarely were considered not exposed (very few individuals reported exposure few times a month or rarely).

Host-Cell Reactivation Assay.

DRC was measured in cultured peripheral lymphocytes using the host-cell reactivation assay with a reporter gene damaged by an activated tobacco carcinogen, BPDE. Details of the assay have been reported previously (14). Briefly, the assay uses a BPDE-damaged nonreplicating recombinant plasmid (pCMV*cat*) harboring a chloramphenicol acetyltransferase (CAT) reporter gene that is transfected into T-lymphocytes. Because even a single unrepaired BPDE DNA adduct blocks CAT transcription, any measurable CAT activity will reflect the ability of the transfected cells to remove BPDE-induced DNA adducts from the plasmids. The cells are stimulated by phytohemagglutinin so that they can uptake the plasmids. Duplicate transfections with either untreated plasmids or BPDE-treated plasmids are always done. CAT activity is assayed by adding chloramphenicol and [^{3}H]acetyl-CoA and measuring the production of [^{3}H]monoacetylated and [^{3}H]diacetylated chloramphenicols with a scintillation counter. DRC is reported as the ratio of the radioactivity of cells transfected with treated plasmids to the radioactivity of cells transfected with untreated plasmids. Assuming that the transfection efficiencies of BPDE-treated and untreated plasmids are equal, this ratio reflects the percentage of damaged CAT reporter genes repaired in lymphocytes transfected with BPDE-treated plasmids.

Because certain laboratory characteristics can potentially affect DRC values, we adjusted DRC for sample storage time (the difference between the date the DRC assay was done and date of blood collection), baseline CAT expression levels, and blastogenic rates. DRC also showed a substantial temporal variation that was controlled for by including the registration year in the analysis as a grouping variable (see Statistical Analysis). Optimal DRC was defined as storage time–, blastogenic rate–, and baseline CAT expression-adjusted DRC above the control median, suboptimal DRC—as that below the control median.

Statistical Analysis.

Descriptive statistical analyses were done to compare the characteristics of lung cancer cases and controls. To evaluate the effect of DRC on lung cancer risk in never smokers, we used the generalized estimating equations approach, whereby registration year was treated as a repeated variable, to account for the observed nonlinear temporal variation in DRC. We obtained odds ratios (OR) and 95% confidence intervals (95% CI) by stratifying DRC at the control median or by control quartiles. Family history of any cancer, smoking-related cancer, lung cancer, and cancer not related to smoking was analyzed as a binary variable (present or absent). Here, smoking-related cancers included lung, head and neck, kidney, bladder, pancreatic, stomach, esophageal, cervical, and liver cancers (22).

To evaluate aggregation of cancer in families of probands with optimal versus suboptimal DRC, we applied unconditional logistic regression using generalized estimating equations to allow for the relatedness within families, treating presence of lung cancer among the relatives as the outcome and DRC status as the predictor. In this analysis, we combined relatives of cases and controls, adjusting for ethnicity of the proband, age, gender of the proband and of the relatives, smoking status, type of relationship to proband, the case-control status of the proband, and birth cohort of the relative, where the birth cohorts were defined as born (*a*) in or before 1900, (*b*) between 1901 and 1940, (*c*) between 1941 and 1960, and (*d*) in or after 1961. To make sure there was no heterogeneity between case and control relatives, separate analyses of case and control relatives are desirable. Unfortunately, such analyses could not be done when keeping all the adjustments due to the absence of lung cancer outcomes among relatives in certain ethnic groups. However, when we limited the analysis to Whites only, we were able to run the separate analyses.

Missing values for DRC and for SHS were treated as a separate category.

The median age at cancer diagnosis in relatives of probands with the optimal versus suboptimal DRC was compared by Wilcoxon sum-rank test and the mean age by Student's *t* test. We used unconditional logistic regression to assess interaction between SHS exposure and DRC.

All statistical tests were two-sided. All analyses were done using the SAS 9.1 statistical software package (SAS System for Windows Release V9.1, SAS Institute, 2002–2003).

Results

Characteristics of the study participants are summarized in Table 1. Overall, there were 385 cases and 536 controls. As a result of the frequency matching, there were no statistically significant case-control differences in age, gender, and ethnicity. About two-third of both cases and controls were women and about 80% were Caucasian. The mean ages of the cases and controls were 60.6 and 59.1 years, respectively. Because The University of Texas M. D. Anderson Cancer Center is a tertiary cancer center, a substantial number of patients have been diagnosed and treated elsewhere and thus not eligible for the study. We compared the demographic characteristics of the study participants with those of the patients who were diagnosed and treated elsewhere (Supplementary Table S1). There were no significant

differences in gender and ethnicity between the study participants and other never smokers seen at our institution, and the difference in age was less than 5 years.

Because the host-cell reactivation assay is done in batches on frozen cultured samples, DRC data were available on only a subset of cases and controls (14). We compared participants (separately cases and controls) with and without DRC measurements. There were no differences in gender, and the age difference did not exceed the matching criterion in both cases and controls, but there was a difference in ethnicity in controls; the proportion of Hispanic and African American participants was higher in controls with than without DRC measurements (Supplementary Table S2).

The mean DRC in controls (8.70%; $n = 309$) was significantly higher than in cases (7.97%; $n = 219$; $P = 0.0019$). Self-reported SHS exposure was significantly more frequent in the cases than in the controls (81% versus 70%; $P = 0.0014$). There was no case-control difference in the blastogenic rate or baseline CAT activity (Table 1). There was less than 2 months difference between cases and controls in the cell storage time because, by necessity, control accrual lags behind case recruitment (14). There also was a difference in the registration year (such that in some years more cases than controls were enrolled and in other years control recruitment exceeded case accrual) related to the recruitment strategy of cases and controls (Table 1). Previously, it has been shown that these variables do not affect DRC (14), whereas in our analysis there was only a weak negative correlation between the DRC and storage time (Pearson's $\rho = -0.102$; $P = 0.02$).

Table 2 summarizes distribution of DRC by select variables. DRC did not show any association with age. Women exhibited lower DRC than men, but the difference was only significant in cases [7.72 (SD, 2.41) versus 8.45 (SD, 2.82); $P = 0.0033$; Table 2]. African American controls had lower DRC (8.03%; SD, 2.68) compared with Whites (8.89; SD, 2.86; $P = 0.0005$), although based on only 80 African American subjects (Table 2). DRC was significantly lower in controls exposed to SHS ($P = 0.007$; Table 2), whereas the pattern was reversed in the cases. Among cases, there was no difference in DRC by stage at diagnosis (7.83; SD, 2.30 for stages I and II versus 7.94; SD, 2.65 for stages III and IV; $P = 0.93$). After applying the Bonferroni correction for nine independent tests (five among cases and four among controls), only the difference by ethnicity in controls remained significant.

DRC was a significant risk factor for lung cancer in never smokers (Table 3): DRC below the control median was associated with a 1.92-fold increased risk (95% CI, 1.26–2.93). When DRC was stratified by control quartiles, there was a linear increase in risk with decreasing DRC [OR, 1.99, 2.38, and 3.28 for the third, second, and first quartiles, treating the fourth (highest) quartile as a reference]. The dose-response relationship was significant ($P = 0.002$ for the test for trend).

Family history of any cancer was also associated with risk (OR, 1.38; 95% CI, 1.01–1.87), as was family history of non-smoking-related cancer (OR, 1.45; 95% CI, 1.04–2.01), whereas family history of smoking-related cancer (OR, 1.38; 95% CI, 0.95–2.02) or lung cancer (OR, 1.28; 95% CI, 0.79–2.05) did not reach statistical significance (Table 3). SHS was a significant risk factor in this group (OR, 1.84; 95% CI, 1.26–2.69; Table 3),

confirming our previous finding (17). After the conservative Bonferroni adjustment for seven tests, however, only main effects of DRC and SHS remained statistically significant.

We also examined joint effects of DRC and SHS (Table 4) and noted that SHS-exposed individuals with suboptimal DRC had a 3.81-fold risk for lung cancer. However, the interaction term between DRC and SHS exposure did not reach statistical significance (OR, 0.39; 95% CI, 0.14–1.08; $P = 0.07$).

Our previous analyses have shown a significant role of cancer family history as a lung cancer risk factor in never smokers (17). Therefore, we did an analysis of joint effects of suboptimal DRC and cancer family history (Table 4). We noted that individuals with optimal DRC did not have an increased lung cancer risk regardless of their family history. However, in the presence of suboptimal DRC, individuals with a lung cancer family history were at a more than 2-fold increased lung cancer risk (OR, 2.49; 95% CI, 1.11–5.60). The results were qualitatively similar and significant for a family history of smoking-related cancer and any cancer (details not shown). For the family history of non-smoking-related cancer, the risk in individuals with suboptimal DRC was somewhat higher (OR, 2.88; 95% CI, 1.32–6.28). After Bonferroni adjustment for five independent tests for the joint effects, the results remained significant for the joint effects of DRC with SHS and with non-smoking-related cancer family history but not with other types of cancer family history (lung, smoking related, or overall).

Cancer history data were reported for 1,647 first-degree relatives of 212 individuals with suboptimal DRC and 2,046 first-degree relatives of 257 individuals with optimal DRC (Supplementary Table S3). There were no differences in the number of relatives per proband, age, gender, and percent of smoking relatives, although there was a difference in ethnicity, in that among relatives of probands with suboptimal DRC there was a higher percent of African Americans (23.7%) than among relatives of probands with proficient DRC (18.0%). Likewise, no differences in demographic characteristics other than ethnicity were observed among relatives of probands stratified by DRC control quartiles (Supplementary Table S3).

Table 5 shows the effect of proband's (cases and controls combined) DRC status as a predictor of lung cancer in the first-degree relatives. The limited number of lung cancer outcomes in the relatives did not allow performing separate analysis by case-control status. However, because the case-control status of study participants is a potential confounder in such an analysis, it was also included in the model. We did not observe risk elevation in the first-degree relatives of participants with DRC as a dichotomous variable. However, first-degree relatives of the study participants with DRC below the first quartile did show a 2.69-fold risk (1.08–6.72) of lung cancer (Table 5), although the dose-response relationship was neither obvious nor statistically significant. The absence of any significant effect of the case-control status (data not shown) implied that there was no heterogeneity in the effect of DRC on lung cancer risk by the proband's case-control status. To further rule out heterogeneity, we did analyses stratified by the proband's case-control status in Whites only to ensure convergence of the models (due to the absence of lung cancer outcomes within certain strata for minorities). We observed qualitatively similar effects in the two groups, based on rather

low numbers, albeit the significant effect (the same as in the combined sample) was seen only in control relatives (Supplementary Table S4).

There was no difference in age at lung cancer diagnosis by DRC stratified at the median (mean age at diagnosis was 59.5 for proficient versus 58.7 for suboptimal DRC; $P = 0.66$; Table 6) or by quartiles (data not shown). On the other hand, relatives (most of them smokers) of study participants with suboptimal DRC tended to be diagnosed with lung cancer at earlier ages than relatives of participants with optimal DRC (60.5 versus 65.4 years; $P = 0.07$; Table 6). The difference in age at diagnosis by DRC was observed for virtually all types of relatives but was most obvious in female relatives (55.4 versus 67.7 years; $P = 0.03$). A similar pattern was observed for age at diagnosis with any type of cancer. In particular, fathers of study participants with suboptimal DRC had a significantly earlier age at diagnosis with all cancers (64.7 versus 71.3 years; $P = 0.003$; details not shown).

Discussion

In this analysis, we have shown the importance of DRC as a lung cancer risk factor in never smokers. Suboptimal DRC jointly with SHS exposure was especially strongly associated with risk (OR, 3.81). Moreover, DRC status of study participants was predictive of lung cancer risk in their first-degree relatives, consistent with the hypothesis that DRC is constitutive and can serve as a marker of genetic susceptibility to lung cancer.

Wei et al. (14) were the first to show a significant association of suboptimal DRC with lung cancer risk, later confirmed in larger studies, in which it was shown that smokers tended to have more proficient DRC than never smokers (refs. 15, 16; although the numbers did not reach statistical significance) and that heavier smokers had more efficient DRC than lighter smokers (15). It was suggested that active smoking up-regulates DRC (15). In this study, we did not observe higher DRC levels in never smoking controls exposed to SHS probably due to the much lower level of exposure. In fact, SHS-exposed controls exhibited lower DRC, although the difference from nonexposed controls was not statistically significant after adjustment for multiple testing. Another possible explanation for the lack of upregulation of DRC by SHS may be the difference in the mainstream versus sidestream smoke composition (23, 24). Sidestream smoke, which rises from the tip of the burning cigarette between puffs, constitutes approximately 85% of SHS (25), and the rest of SHS consists of mainstream smoke, drawn through the cigarette and inhaled/exhaled by he smoker. Recent reviews of previously unpublished *in vivo* animal research on sidestream cigarette smoke conducted by Philip Morris Tobacco Company during the 1980s (23, 24) showed higher toxicity of fresh sidestream smoke (up to 4-fold) and especially of sidestream smoke aged for 30 min (up to 12-fold) compared with mainstream smoke.

Patients not exposed to SHS had a nonsignificantly lower DRC than SHS-exposed cases, as reported previously (15), suggesting that never smokers who develop lung cancer without exposure to SHS constitute an especially susceptible subgroup. The magnitude of the association of SHS with DRC in controls was relatively low (Spearman’s nonparametric correlation coefficient of 0.087), not leading to high collinearity.

It has been reported that smokers with suboptimal DRC had an earlier age at diagnosis compared with smokers with optimal DRC (14). However, we did not observe this effect in never smokers, implying that active smoking together with suboptimal DRC influence the age at diagnosis. Indeed, relatives (mostly smokers) of subjects with suboptimal DRC tended to be diagnosed with lung cancer earlier than relatives of subjects with proficient DRC.

Our analysis of joint effects of cancer family history and suboptimal DRC on risk highlights the importance of both suboptimal DRC and family history of cancer in conferring risk. Our finding that DRC status of the study participants was predictive of lung cancer risk among their first-degree relatives is consistent with DRC being a constitutive marker. Relatives of individuals with the lowest DRC (below the first quartile) were at a significantly higher lung cancer risk compared with relatives of individuals with the highest DRC (above the third quartile). Importantly, lung cancer familial aggregation studies in relatives of never smokers have an advantage of less likelihood of confounding by aggregation of smoking habits within families (20, 26).

Strengths and Limitations.

The strength of this study is its sample size, one of the largest to include both genders, and availability of a comprehensive array of epidemiologic variables and functional repair data for the study participants. Also, the demographics of our study population reflects well the demographics of never smoker lung cancer patients seen at The University of Texas M. D. Anderson Cancer Center in general.

DRC, as a phenotypic marker, represents a direct measure of DNA repair kinetics. However, because this assay is relatively costly and time-consuming, DRC has not been prospectively validated as a risk predictor, and the possibility that presence of cancer can affect DRC level could not be completely ruled out. Our analysis showed no association of disease stage with DRC, suggesting that DRC is a true susceptibility factor rather than a tumor marker.

Among study limitations is also absence of direct DRC measurements in the relatives. SHS exposure data were derived from personal interviews, which did not include assessment of the intensity of exposure. Also, cancer history in the relatives was not validated through medical records. However, it is unlikely that participants with optimal versus suboptimal DRC reported family history differentially. Based on population incidence of cancers, Pinsky et al. (27) estimated a 30% underreporting of cancer family history, with male probands underreporting more often compared with female probands. In our study, most of the probands were women, which in part alleviates underreporting. Besides, gender of the proband was adjusted in all analyses, which may have reduced the effect of gender bias in reporting. Another limitation of our study is case-control differences in the laboratory variables, such as sample storage time, which potentially could affect DRC. The difference, however, was less than 2 months on average. Because the correlation of DRC with the storage time is weak and the difference between cases and controls in the cell storage time is small, the effect of storage time on DRC and therefore on the case-control difference in DRC is likely to be very small. Moreover, because in our analyses DRC was always adjusted for the storage time, this effect is unlikely to affect our results.

Conclusion.

Our analysis shows that DRC is an important predictor of lung cancer risk in never smokers. The association of probands' DRC with lung cancer risk in their first-degree relatives also suggests that DRC might be a factor underlying familial aggregation of lung cancer. Suboptimal DRC jointly with SHS exposure is a very strong lung cancer risk factor in lifetime never smokers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1.

Characteristics of the study participants

A comparison of case and control subjects				
Variables	**Cases (*n* = 385)**	**Controls (*n* = 536)**	***P***	**OR* (95% CI)**
Age, mean (SD)	60.6 (13.1)	59.1 (12.5)	0.065	
Gender, *n* (%)				
Men	131 (34.0)	182 (34.0)		
Women	254 (66.0)	354 (66.0)	0.982	
Ethnicity, *n* (%)				
White	308 (80.0)	404 (75.4)		
Hispanic	34 (8.8)	47 (8.8)		
African American	40 (10.4)	280 (14.9)		
Others	3 (0.8)	5 (0.9)	0.239	
Cell storage time, mo†	14.26 (6.5)	12.42 (6.1)	0.001	
Blastogenic rate, %†,‡	24.55 (18.7)	22.50 (16.2)	0.190	
Baseline CAT activity, scintillation counts/min†	32,756 (20,258)	33,096 (24,163)	0.861	
Registration year (1995–2006)†				
1995	2	0		
1996	8	15		
1997	9	16		
1998	15	9		
1999	22	18		
2000	33	20		
2001	47	120		
2002	47	70		
2003	66	70		
2004	57	56		
2005	57	78		
2006	22	64	<0.001	

A comparison of case and control subjects

Variables	Cases (n = 385)	Controls (n = 536)	P	OR* (95% CI)
DRC, mean (SD)[†]	7.97 (2.57)	8.70 (2.82)	0.002	0.90 (0.84–0.96)
SHS exposure, n (%)[§]	217 (81.0)	298 (69.6)	0.001	

*Adjusted by gender, ethnicity, and age.

†Based on 219 cases and 309 controls.

‡Percentage of cells that were stimulated by phytohemagglutinin.

§Based on 268 cases and 428 controls.

Table 2.

DRC (%) for cases and controls by selected subgroups

Variable	Cases			Controls		
	n (%)	DRC, % (SD)	*P**	*n* (%)	DRC, % (SD)	*P**
Age						
<50	52 (23.7)	7.91 (2.46)	Reference	60 (19.5)	8.12 (2.87)	Reference
≥50	167 (76.3)	7.98 (2.61)	0.5173	247 (80.5)	8.84 (2.57)	0.0852
Gender						
Men	74 (33.8)	8.45 (2.82)	Reference	96 (31.3)	9.06 (3.06)	Reference
Women	145 (66.2)	7.72 (2.41)	0.0033	211 (68.7)	8.54 (2.70)	0.1189
Ethnicity						
White	308 (80.6)	7.84 (2.62)	Reference	404 (76.1)	8.89 (2.86)	Reference
Hispanic	34 (8.9)	8.21 (2.34)	0.6881	47 (8.9)	8.87 (2.78)	0.7392
African American	40 (10.5)	8.52 (2.38)	0.0879	80 (15.0)	8.03 (2.68)	0.0005
SHS exposure						
No	30 (21.6)	7.76 (2.34)	Reference	77 (34.1)	9.23 (2.52)	Reference
Yes	109 (78.4)	8.39 (2.62)	0.1334	149 (65.9)	8.60 (2.56)	0.0066
Stage						
I and II	34 (16.6)	7.83 (2.30)	Reference	—	—	—
III and IV	171 (83.4)	7.94 (2.65)	0.9264	—	—	

*Adjusted for the sample storage time, baseline CAT expression levels, and blastogenic rates, and registration year by generalized estimating equations.

Table 3.

Main effects of DRC, cancer family history, SHS exposure, and lung cancer risk in never smokers

	Controls, *n* (%)	Cases, *n* (%)	OR* (95% CI*)	*P*
DRC stratified at the median†				
>Median	153 (49.8)	88 (40.2)		
<Median	154 (50.2)	131 (59.8)	1.92 (1.26–2.93)	0.0024
DRC stratified by quartiles†,‡				
Highest	75 (24.4)	29 (13.2)		
Third	78 (25.4)	59 (26.9)	1.99 (1.09-3.64)	0.0258
Second	77 (25.1)	60 (27.4)	2.38 (1.21–4.70)	0.0123
First	77 (25.1)	71 (32.4)	3.38 (1.81–6.32)	0.0001
Family history of any cancer§				
No	189 (40.2)	103 (30.5)		
Yes	281 (59.8)	235 (69.5)	1.38 (1.01–1.87)	0.043
Family history of smoking-related cancer§				
No	346 (74.2)	231 (69.8)		
Yes	120(25.8)	100 (30.2)	1.38 (0.95–2.02)	0.092
Family history of non-smoking-related cancer§				
No	262 (56.2)	154 (46.5)		
Yes	204 (43.8)	177 (53.5)	1.45 (1.04–2.01)	0.027
Family history of lung cancer§				
No	407 (87.3)	284 (85.8)		
Yes	59 (12.7)	47 (14.2)	1.28 (0.79–2.05)	0.314
SHS exposure				
No	130 (30.4)	51 (19.0)		
Yes	298(69.6)	217 (81.0)	1.84 (1.26–2.69)	0.002

*Adjusted for age, gender, ethnicity, dust exposure, and presence of asthma and hay fever.

†Generalized estimating equation model with grouping by year of assay was used; DRC adjusted for the laboratory variables.

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‡ $P = 0.002$ for the test for trend.

§ The reference group is negative family history of cancer.

Table 4.

Joint effects of DRC with SHS exposure and with lung cancer family history

		Controls, *n* (%)	Cases, *n* (%)	OR* (95% CI*)	*P*
DRC	SHS exposure				
Optimal	No	45 (19.9)	10 (7.2)	1	
Optimal	Yes	70 (31.0)	49 (35.3)	2.68 (1.29–5.56)	0.0081
Suboptimal	No	32 (14.2)	20 (14.4)	2.94 (1.58–5.48)	0.0007
Suboptimal	Yes	79 (35.0)	60 (43.2)	3.81 (2.26–6.41)	<0.0001
	Family history of lung cancer				
Optimal	No	44 (30.2)	23 (25.8)	1	
Optimal	Yes	17 (11.6)	7 (7.9)	0.59 (0.24–1.48)	0.2610
Suboptimal	No	68 (46.6)	40 (44.9)	1.40 (0.86–2.29)	0.1780
Suboptimal	Yes	17 (11.6)	19 (21.4)	2.49 (1.11–5.60)	0.0276
	Family history of non-smoking-related cancer				
Optimal	No	44 (19.6)	23 (13.7)	1	
Optimal	Yes	59 (26.2)	37 (22.0)	1.15 (0.46–2.91)	0.7627
Suboptimal	No	68 (30.2)	40 (23.8)	1.31 (0.78–2.19)	0.3042
Suboptimal	Yes	54 (24.0)	68 (40.5)	2.88 (1.32–6.28)	0.0081

*Adjusted for age, gender, ethnicity, dust exposure, and presence of asthma and hay fever and grouped by year of assay using generalized estimating equation model; DRC adjusted for the laboratory variables.

Table 5.

DRC of case and control probands as a predictor of lung cancer in first-degree relatives

	Affected *n* (%)	Not affected *n* (%)	OR* (95% CI*)	*P*
DRC				
>Median	28 (38.4)	914 (38.9)	1.0	
<Median	45 (61.6)	1,436 (61.2)	1.15 (0.69–1.93)	0.589
DRC by quartiles				
Highest (fourth)	6 (8.2)	401 (17.1)	1.0	
Third	22 (30.2)	513 (21.8)	2.62 (0.99–6.86)	0.051
Second	16 (21.9)	650 (27.7)	1.73 (0.64–4.68)	0.279
Lowest (first)	29 (39.7)	786 (33.5)	2.69 (1.08–6.72)	0.034

*Adjusted for age and gender of the proband and of the relatives, ethnicity of the proband, type of relationship to the proband, smoking status and birth cohort of the relative, and the case-control status of the proband; data on the case and control relatives separately (Whites only) are provided in Supplementary Table S4.

Table 6.

Age at lung cancer diagnosis (cases and relatives) by DRC status

Type of relationship	DRC above the median			DRC below the median			*P* for median/mean
	n	Median	Mean (SD)	*n*	Median	Mean (SD)	
Probands (cases)	88	59	59.5 (12.1)	131	57	58.7 (13.6)	0.77/0.66
All relatives	29	65	65.4 (9.1)	44	60	60.5 (13.6)	0.18/0.07
Case relatives	9	60	60.6 (7.5)	23	62	59.0 (15.0)	1.00/0.78
Control relatives	20	67.5	67.6 (9.1)	21	60	62.1 (12.1)	0.16/0.11
Smoking relatives	23	62	64.2 (9.0)	36	60	60.5 (12.9)	0.41/0.24
Nonsmoking relatives	6	71.5	69.8 (8.8)	8	63.5	60.3 (17.3)	0.24/0.24
Female relatives	12	71.5	67.7 (10.2)	13	60	55.4 (15.7)	0.06/0.03
Male relatives	17	62	63.7 (8.2)	31	61	62.6 (12.3)	0.94/0.73

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