Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2009 December ; 18(12): 3375–3383. doi: 10.1158/1055-9965.EPI-09-0986.

Childhood Exposure to Secondhand Smoke and Functional Mannose Binding Lectin Polymorphisms Are Associated with Increased Lung Cancer Risk

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Abstract

Background—Exposure to secondhand smoke during adulthood has detrimental health effects, including increased lung cancer risk. Compared with adults, children may be more susceptible to secondhand smoke. This susceptibility may be exacerbated by alterations in inherited genetic variants of innate immunity genes. We hypothesized a positive association between childhood secondhand smoke exposure and lung cancer risk that would be modified by genetic polymorphisms in the mannose binding lectin-2 (*MBL2*) gene resulting in well-known functional changes in innate immunity.

Methods—Childhood secondhand smoke exposure and lung cancer risk was assessed among men and women in the ongoing National Cancer Institute-Maryland Lung Cancer (NCI-MD) study, which included 624 cases and 348 controls. Secondhand smoke history was collected via in-person interviews. DNA was used for genotyping the *MBL2* gene. To replicate, we used an independent case-control study from Mayo Clinic consisting of 461 never smokers, made up of 172 cases and 289 controls. All statistical tests were two-sided.

Results—In the NCI-MD study, secondhand smoke exposure during childhood was associated with increased lung cancer risk among never smokers [odds ratio (OR), 2.25; 95% confidence interval (95% CI), 1.04-4.90]. This was confirmed in the Mayo study (OR, 1.47; 95% CI, 1.00-2.15). A functional MBL2 haplotype associated with high circulating levels of MBL and increased *MBL2* activity was associated with increased lung cancer risk among those exposed to childhood

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Disclosure of Potential Conflicts of Interest: No potential conflicts of interest were disclosed.

secondhand smoke in both the NCI-MD and Mayo studies (OR, 2.52; 95% CI, 1.13-5.60, and OR, 2.78; 95% CI, 1.18-3.85, respectively).

Conclusions—Secondhand smoke exposure during childhood is associated with increased lung cancer risk among never smokers, particularly among those possessing a haplotype corresponding to a known overactive complement pathway of the innate immune system.

Introduction

In 2009, an estimated 219,440 Americans will be diagnosed with lung cancer and 159,390 people will die from it (1). Although tobacco smoke is a major determinant of lung cancer risk among smokers, it is unclear why some never smokers develop lung cancer. Several risk factors have been shown to increase lung cancer risk among never smokers, including radon, asbestos, and coal and cooking fumes as well as others that are still being researched (2). One major risk factor for never smokers is secondhand smoke exposure (3-5). Study results linking adult secondhand smoke exposure and increased lung cancer risk are consistent (3,6,7), but the association between lung cancer risk and childhood secondhand smoke exposure is uncertain (3,4,8). Recently, it has been shown that there is still a significant percentage of American children exposed to secondhand smoke in the home (9). Specifically, approximately one fourth of all children ages 4 to 11 years and one fifth of all children ages 12 to 19 years are exposed to secondhand smoke in the home (9). In addition, the most recent Surgeon General's Report (8) concludes that children exposed to parental smoke have an increased risk of upper and lower respiratory illnesses, recurrent otitis media, and childhood asthma (8), but a meta-analysis of 24 studies that examined childhood secondhand smoke and lung cancer risk did not find a significant association (8). Furthermore, a pooled estimate showed a relative risk of 1.11 [95% confidence interval (95% CI), 0.94-1.31] from smoking by either parent (8). A nested case-control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) reported a modest increased risk of lung cancer among those exposed to secondhand smoke daily during childhood (10). A study of never-smoking Taiwanese women observed increased lung cancer risk as the duration of childhood secondhand smoke exposure increased (11), which has not been consistently observed in other studies (3,4).

Compared with adults, children may be more susceptible to the carcinogens in tobacco smoke because of increased air intake proportional to lung size (12) and less efficient carcinogen detoxification (13). It is also plausible that some children may be more vulnerable to specific environmental carcinogens due to differences in genetic background (12-14). Children with varying genetic backgrounds affecting immunity, inflammation, tobacco metabolism, or DNA repair may be more susceptible to the carcinogenic effects of secondhand smoke exposure.

Genetic variation of an innate immunity gene, mannosebinding lectin-2 (*MBL2*), has been shown to affect susceptibility to respiratory diseases both during childhood and adulthood (15-19). A recent study observed that high MBL levels in cord blood were associated with an increase in respiratory symptoms in infants (20). However, the data examining the association between MBL levels and childhood asthma are conflicting. Some studies observe no association (21,22), whereas others illustrate higher levels of MBL in asthmatic children (23, 24).

The *MBL2* gene is a critical component of the innate immune system. MBL binds to microorganisms promoting phagocytosis by macrophages and monocytes (19). Six single nucleotide polymorphisms (SNP) in the *MBL2* gene are associated with well-characterized haplotypes correlating with varying circulating levels and functional activity of MBL protein (15,19,25,26). Specifically, seven common haplotypes have been well established to be associated with high, intermediate, and low MBL serum levels that have been shown to have functional consequences in innate immunity (15). Further studies show that these haplotypes

can also be associated with high, intermediate, and low promoter activity correlating with the serum levels (27). We have recently shown an association between *MBL2* haplotypes and lung cancer survival where the *MBL2* haplotype associated with high MBL levels was associated with poorer lung cancer survival (28). Previous research showed an association between low MBL levels and increased gastric cancer risk (25), which is supported by the fact that low MBL levels increase susceptibility to infection, and a main risk factor for gastric cancer is *Helicobacter pylori* infection. However, high levels of MBL may increase susceptibility to tobacco smoke because of MBL's ability to activate the complement system, releasing proinflammatory cytokines and free radicals, thereby creating a chronic inflammatory environment (29-31). Therefore, we hypothesized that childhood exposure to secondhand smoke is a risk factor for lung cancer, and this risk would increased in the presence of specific functional *MBL2* haplotypes associated with high serum MBL levels.

Materials and Methods

Study Design

National Cancer Institute-Maryland Lung Cancer Study—Lung cancer patients were recruited from hospitals in the greater Baltimore metropolitan area as previously described (32). Population controls were recruited from the same greater Baltimore metropolitan area as cases (32) and were frequency-matched to cases by age and gender. Hospital controls were also recruited for this study, but were not included in the analysis for this study because we focused on the effect of secondhand smoke and extrapolated to the general population. The population controls effectively reflect the general population of Maryland in terms of smoking status (33), but the hospital controls were matched to the cases on smoking, and therefore do not reflect the general population.

Additionally, because the hospital controls were more likely to be smokers, it is difficult to examine the effects of secondhand smoke exposure that will be overwhelmed by the effects of active smoking. As the majority of cases and controls were non-Hispanic Caucasians, and our validation cohort was limited to non-Hispanic Caucasians, we limited cases and controls to this ethnic group. Never smokers smoked <100 cigarettes in their lifetime. Exsmokers reported quitting >1 y before the date of diagnosis. Current smokers continued to smoke or quit smoking <1 y since the lung cancer diagnosis. Light exposure during childhood was categorized as exposure to <0.5 pack per day smoked by members of the household, moderate exposure was 0.5 to 1 pack of exposure per day, and heavy exposure was categorized as exposure to >1 pack per day. The study was approved by the Institutional Review Boards of the National Cancer Institute, the University of Maryland Medical System, the Baltimore VA Medical Center, the Johns Hopkins University School of Medicine, Sinai Hospital, MedStar Research Institute, and the Research Ethics Committee of Bon Secours Baltimore Health System.

Mayo Clinic Study—Lung cancer patients were recruited at Mayo Clinic as described previously (34,35). All patients with newly diagnosed primary lung cancer were enrolled into the study from 1997 to 2001. The majority of cases were diagnosed with non–small cell lung cancer (98%). Controls were population controls drawn from Olmsted County residents enrolled in the same time period and were matched to cases on age, sex, and race/ethnicity (36). The majority of cases (93%) and controls (96%) were non-Hispanic Caucasians, so we limited cases and controls to this ethnic group to minimize population heterogeneity (or stratification). The Mayo Clinic study consisted of only never smokers that were defined the same way as the National Cancer Institute-Maryland Lung Cancer (NCI-MD) study. This study was approved by the Mayo Clinic Institutional Review Board.

Eligibility Criteria

NCI-MD Study—Eligibility criteria had been previously described (32). Briefly, eligibility criteria included being free of known diagnosis of HIV, hepatitis C virus, and hepatitis B virus; a United States citizen, a resident of Baltimore City or adjacent counties of Maryland or the Maryland Eastern Shore; English-speaking; noninstitutionalized; not currently taking antibiotics or steroid medications; and for cases, not having been interviewed as a control for the study. As of June 2008, 6,851 lung cancer cases were screened with 1,091 (16%) eligible for the study. The majority of the cases (34%) were ineligible due to living outside the collection area. Other major reasons included time since diagnosis was >2 y (10%); tumor type was not a non-small cell lung cancer (17%), or death (12%). Of the 16% eligible cases, 88% agreed to participate in the study. Population controls were recruited via the Maryland Department of Motor Vehicles where 24,236 records were imported. From these 24,236 records, telephone numbers were found for 7,732 people (32%). This may be partly due to the fact that many people no longer are using residential phones. Of the 7,732 people, 773 (10%) were eligible for the study. Of the 7,732 people, we were unable to make contact with almost 40% and many of the others were ineligible due to a previous diagnosis of cancer, language issues, or death. From those eligible, 91% agreed to participate. Eligible controls have been shown to be very similar to the general Maryland population in terms of education level, smoking status, and body mass index (33).

Interviews were conducted in person with the study participant during which the trained interviewer also noted the overall quality of the interview and the participant's cooperation. Because death of the study participant was cause for ineligibility for the study, next-of-kin interviews were not conducted. After informed consent was obtained, a structured instrument including information on prior medical and cancer history; tobacco use; occupational, spousal, and childhood secondhand smoke exposure; family medical history; and socioeconomic history was administered by a trained interviewer (Supplementary Text 1). Blood was obtained by the interviewers, and frozen blood components were sent to the Laboratory of Human Carcinogenesis at the National Cancer Institute (NCI) within 24 h of collection. Samples were stored at –80°C until use.

Mayo Clinic Study—Eligibility criteria were described previously (35). Eligible controls were administered a questionnaire and a request for permission to use their blood. Of the controls that responded, 78% gave permission to use their blood sample and of these 78%, 84% completed the questionnaire. Questionnaires were self-administered and included data on family history of cancer, family medical history, smoking history, and secondhand smoke exposure.

Secondhand Smoke Exposure

NCI-MD Study—Information on childhood secondhand smoke exposure was collected via questionnaire data (Supplementary Text 1). Specific information was collected on whether anyone smoked in the childhood home; how many people smoked in the childhood home; what the relationship to the study participant was; whether they smoked lightly, moderately, or heavily; the average number of cigarettes that were smoked; and how many years they smoked in the childhood home. Additional information was collected on secondhand smoke exposure during adulthood. Specifically, information on who smoked in the home; how many people smoked in the home; whether they smoked lightly, moderately, or heavily; the average number of cigarettes smoked per day; the number of years they smoked; whether they stopped smoking while the study participant was in the house; if they quit, how long ago they quit; and how many cigarettes were smoked in the home during the past 30 days. Further data were also gathered on occupational exposure to secondhand smoke that included if they were exposed to cigarette smoke in the workplace during the last 48 h, if they were employed at a job for >5

y where coworkers smoked in their immediate area, how many years they worked at a job where people smoked in their immediate work area, how long it has been since they worked at job where people smoked in their immediate area, and if they were exposed to cigarette smoke lightly, moderately, or heavily.

Exposure to father's smoke was determined if the study participant reported that the father smoked in the childhood home. Exposure to mother's smoke was determined in a similar manner, but no data were collected on exposure to mother's smoke *in utero*. Exposure amounts were calculated in the following manner: light exposure during childhood was categorized as exposure to <0.5 pack per day smoked by members of the household, moderate exposure was 0.5 to 1 pack of exposure per day, and heavy exposure was categorized as exposure to >1 pack per day. One hundred twelve cases (18%) and 33 controls (9%) were missing specific data on the number of cigarettes smoked per day by their mother or father, and therefore exposure level was not calculated in these individuals.

Mayo Clinic Study—Secondhand smoke exposure was collected via questionnaire. Specific information was gathered on whether exposure occurred during childhood, during adulthood, or across the lifetime; whether exposure was from a parent, spouse, or coworker; the number of cigarettes per day they were exposed to; and the number of years they were exposed.

DNA Isolation and Genotyping

NCI-MD Study—Genomic DNA was isolated from buffy coats using the Qiagen Flexigene kit according to the manufacturer's instructions. Genotyping was done both at the NCI Core Genotyping Facility and at BioServe (Beltsville, MD). A total of 26 *MBL2* SNPs were genotyped using Taqman assays (Applied Biosystems, Inc.; Supplementary Table S1). Assays were validated as described on the SNP500 website[8] (37). All genotyping assays contained positive and negative controls as well as at least 10% blinded and randomized duplicates. The overall concordance among sample duplicates was 99.4% (discordance range per genotype, 99-100%). Hardy Weinberg equilibrium was conducted for all 26 *MBL2* SNPs among the controls. MBL2-06 and MBL2-11 were in Hardy Weinberg violation at a level of $P = 0.01$. To validate that the Hardy Weinberg violation was not due to genotyping errors, 40 controls were randomly selected for sequencing for both MBL2-06 and MBL2-11. The concordance rate was 100% for all 80 genotypes.

Mayo Clinic Study—Blood samples were collected from all cases and controls as previously described (34). All genotyping was done at the Mayo Clinic Cancer Center's Genotyping Laboratory. The six *MBL2* secretor SNPs were genotyped (MBL2_01, MBL2_02, MBL2_03, MBL2_11, MBL2_12, MBL2_25) using the same Taqman assays described above in the NCI-MD study. All genotyping assays contained positive and negative controls as well as 10% blinded and randomized duplicates. Hardy Weinberg equilibrium was conducted for the six *MBL2* secretor SNPs among the controls and there were no violations.

Statistical Analyses

The characteristics of the lung cancer patients were compared with the controls to determine differences using a χ^2 test for categorical variables and Student's *t*-test for continuous measurements as indicated. All statistical tests were two-sided.

Unconditional logistic regression models were used to calculate adjusted odds ratios (OR) for the risk of lung cancer due to childhood secondhand smoke exposure and were adjusted for potential confounding variables, including age, gender, smoking status, adult secondhand

[8]http://snp500cancer.nci.nih.gov

smoke exposure, first-degree relative with lung cancer, education (categorized as less than high school, high school degree, college degree, and graduate degree), number of cigarettes per day (for ex- and current smokers only), number of years smoked (for ex- and current smokers only), age at initiation (for ex- and current smokers only), and time since quitting (for ex-smokers only). All of the above analysis was conducted using Stata Statistical Software (38).

Haplotype and Genotype Analysis

Haplotype analysis was conducted using Haplo.stat software written for R 2.3.1 (39). OR and 95% CI, adjusted for gender, age, smoking status, adult exposure to secondhand smoke, firstdegree relative with lung cancer, and education were calculated for each haplotype using a generalized linear model (haplo.glm). The *MBL2* secretor haplotypes, HYPA, LYQA, LYPA, LXPA, LYPB, HYPD, and LYQC, were generated using the six MBL2 secretor SNPs, MBL2_01, MBL2_02, MBL2_03, MBL2_11, MBL2_12, and MBL2_25. In the NCI-MD study, some of the genotyping for the MBL2 secretor SNPs were conducted in a multiplex platform that did not allow for the incorporation of MBL2_01. Therefore, some of the study participants (36%) have incomplete genotype data for MBL2_01. In addition, to account for incomplete genotyping, anyone with <90% complete genotyping was removed from the haplotype analysis, which resulted in a lower number of study participants from the NCI-MD study with complete MBL2 genotyping data (32%). Next, the seven haplotypes were grouped into categories correlating with low, medium, or high levels of circulating MBL protein based on previous reports (15,40,41), and subsequently used in unconditional logistic regression models to determine their association with lung cancer risk. Furthermore, we investigated the possibility that MBL levels modify the effect of childhood secondhand smoke on lung cancer risk in two ways.

First, an interaction term was generated between MBL levels and childhood secondhand smoke exposure. When incorporated into the logistic regression model, the interaction term was significant ($P < 0.001$). Second, the logistic regression models were stratified by exposure to childhood secondhand smoke. Unconditional logistic regression models were also used to examine the effect of *MBL2* genotypes on lung cancer risk and were adjusted for the above confounders. To control for the effect of multiple comparison testing, we applied the false discovery rate (FDR) to the *P* values of the individual *MBL2* haplotypes. Specifically, we used the Benjamini-Hochberg method in the “multtest” package in R which calculates the expected proportion of type I errors (rejecting a true hypothesis) among the rejected hypotheses (42). The FDR for the HYPA haplotype which correlates with high MBL levels was 0.16.

Results

Study Population

The mean age was 67 years for the NCI-MD cases and controls (Table 1). Reflecting that younger ages were oversampled in the Mayo Clinic study, the mean ages for the cases and controls were 61 and 62 years, respectively. There were approximately equal numbers of male and female participants among the cases and controls in the NCI-MD study. In the Mayo Clinic study, however, there was a higher percentage of females among both cases and controls. Confirming previous studies, lung cancer cases tended to be less educated than controls ($P < 0.001$) in the NCI-MD study. This did not reach statistical significance in the Mayo Clinic study ($P = 0.06$), and cases and controls from this study were more educated than in the NCI-MD study.

Smoking History and Exposure

As expected from what is observed in the general Maryland population (33), among the NCI-MD participants, a personal history of cigarette smoking was more prevalent among cases than

controls (Table 1). Smoking intensity ($P < 0.001$), duration ($P < 0.001$), and pack-years ($P < 0.001$) were all associated with lung cancer (Supplementary Table S2). In both studies, secondhand smoke exposure during childhood was significantly higher in cases than in controls (NCI-MD, 82% versus 66%, $P < 0.001$; Mayo, 57% versus 47%, $P = 0.04$; data not shown). Secondhand smoke exposure during adulthood was also assessed through home and occupational settings (Supplementary Table S2). Household exposure was higher among cases than among controls in both the NCI-MD and Mayo Clinic studies, but it was associated with a significant increase in lung cancer risk only in the NCI-MD study (OR, 1.78; 95% CI, 1.31-2.42). In addition, secondhand smoke exposure in the workplace was associated with lung cancer risk only in the NCI-MD study (OR, 1.46; 95% CI, 1.07-1.99).

Adult Lung Cancer Risk

In analyses stratified by smoking history in the NCI-MD study, childhood secondhand smoke exposure was associated with a significant increase in lung cancer risk only among never smokers (OR, 2.25; 95% CI, 1.04-4.90; Table 2). The association between childhood secondhand smoke exposure and adult lung cancer risk remained robust even in analyses that included all study participants with adjustments for smoking status (OR, 2.33; 95% CI, 1.66-3.25). The association between childhood exposure to secondhand smoke was confirmed in the Mayo Clinic study (OR, 1.47; 95% CI, 1.00-2.15).

In the NCI-MD study, lung cancer risk in association with parental exposure was examined in all cases with an adjustment for smoking. Light exposure during childhood was categorized as exposure to <0.5 pack per day smoked by members of the household, moderate exposure was 0.5 to 1 pack of exposure per day, and heavy exposure was categorized as exposure to >1 pack per day. Exposure to secondhand smoke increased risk of lung cancer regardless of paternal or maternal exposures. Lung cancer risk seemed to increase in a dose-dependent fashion according to the degree of exposure from the father or mother (Table 2). However, when examining overall association between exposure levels and lung cancer risk, the moderate and heavy exposures were both associated with a similar significant increase in lung cancer risk compared with no and light exposure. The association between paternal exposure and lung cancer risk was validated using the Mayo Clinic study. However, we did not observe the same association between lung cancer risk and maternal exposure among the Mayo Clinic study participants (Table 2).

Differences in the age of diagnosis among lung cancer cases by smoking status and childhood secondhand smoke exposure were examined (Table 3). In the NCI-MD study, among never smokers, the age of diagnosis was 14.9 years younger ($P < 0.001$) in those exposed versus nonexposed. In the Mayo Clinic study lung cancer cases were on average 6.2 years younger ($P = 0.035$) in those exposed than in those not exposed to childhood secondhand smoke.

There was no significant difference in histologic subtypes between those exposed to childhood secondhand smoke and those that were not exposed ($P = 0.33$; data not shown). This remained true even after stratification by smoking status in the NCI-MD study.

Risk in Association with *MBL2* Genetic Variants

We hypothesized that the increased risk of lung cancer observed in those exposed to secondhand smoke during childhood may be increased among those with genetic alterations in *MBL2*. Of the 26 *MBL2* SNPs assayed in the NCI-MD study (Supplementary Table S1), 4 were associated with increased lung cancer risk among those who reported exposure to childhood secondhand smoke and 2 with decreased risk (Supplementary Table S3). We further investigated the *MBL2* secretor haplotype that is made up of six *MBL2* SNPs, and we focused only on these six SNPs in the Mayo Clinic study (Supplementary Table S4). Two haplotypes

were significantly associated with risk in the NCI-MD study and two were also significantly associated with risk in the Mayo Clinic study (Supplementary Table S5). We examined *MBL2* haplotypes in terms of their association with MBL levels because these haplotypes have been well established to have functional consequences on innate immunity.

Furthermore, to examine a possible interaction, we stratified by exposure to childhood secondhand smoke (Table 4). Among all of the NCI-MD participants, adjusting for smoking, we found a significant increase in lung cancer risk among people who were exposed to childhood secondhand smoke and had the *MBL2* haplotype associated with high levels of circulating MBL (OR, 2.52; 95% CI, 1.13-5.60). The same results were observed among the Mayo Clinic participants who were all never smokers and adjustment for smoking was not necessary (OR, 2.78; 95% CI, 1.18-3.85). Furthermore, analysis in a logistic regression model using an interaction term between MBL levels and childhood secondhand smoke exposure without stratification showed a significant interaction between MBL levels and childhood secondhand smoke exposure ($P < 0.001$).

Discussion

The primary purpose of this report is to characterize the association between childhood secondhand smoke exposure and lung cancer risk among smokers and never smokers. Our ongoing NCI-MD study indicated a 2-fold excess risk among those exposed to childhood secondhand smoke compared with those not exposed. This risk was higher than that observed in the meta-analysis conducted in the Surgeon General's report (OR, 0.93; 95% CI, 0.81-1.07, in US studies), but it was lower than the risk observed in the EPIC study (OR, 3.63; 95% CI, 1.19-11.11). Among ex- and current smokers it is difficult to tease apart the independent effect of childhood secondhand smoke exposure because children of smokers are more likely to become smokers themselves (43,44), making it a challenge to separate the effects of secondhand versus active smoke. In our study, lung cancer patients who smoked were more likely to have a parent who smoked (OR, 1.74; 95% CI, 1.07-2.85) and an earlier age at initiation ($P < 0.001$); however, these associations with smoking behavior were not observed among the controls.

Because the NCI-MD case-control study was not designed as a never-smoker investigation, we decided to further test the hypothesis and initial results in an independent case-control study consisting only of never smokers. In this second study, a 1.5-fold excess risk among never smokers exposed to secondhand smoke was found. Among never smokers of both studies, the age of lung cancer diagnosis was significantly younger in those exposed versus nonexposed, providing further evidence that this association is genuine.

A correlative finding with the effect we observed of childhood passive smoke exposure on lung cancer risk was the association of paternal and maternal exposure and lung cancer. Although paternal exposure was significantly associated in both the Maryland and the Mayo Clinic studies, maternal exposure was associated in the Maryland study only. This may be explained in part by the fact that there were fewer smoking mothers in the Mayo Clinic study. Our Maryland study confirmed previous studies illustrating an inverse association between education and smoking status ($P < 0.001$). The Mayo Clinic participants were more highly educated, which may explain the lower number of smoking mothers in this population.

The second central finding of our study was that haplotypes associated with high circulating MBL levels and increased known functional activity (15,19,25,26) were associated with increased lung cancer risk among people exposed to childhood secondhand smoke. Gazdar and Thun (45) recently suggested that genetic predisposition may play a role in lung cancer risk among never smokers. Our results support this hypothesis, in that the risk associated with

exposure to secondhand smoke during childhood was considerably stronger among those with the putative high-risk *MBL2* haplotypes and there was a significant statistical interaction between MBL levels and exposure to childhood secondhand smoke.

The adverse impact of high levels of MBL in early life development of the lung is evidenced by its link with asthma (21,46). This makes it plausible that high levels of circulating MBL, particularly during childhood, may also increase susceptibility to lung cancer. High levels may activate both the complement system and macrophages (29,30) to release proinflammatory cytokines and free radicals that may cooperate in cancer development (29,31). Therefore, increased MBL levels may increase susceptibility to lung tumorigenesis caused by secondhand smoke exposure by inducing a chronic inflammatory environment. This notion is supported by the observations that (*a*) MBL-deficient mice had lower levels of proinflammatory cytokines in the lung (47), and (*b*) increased MBL levels in humans enhanced inflammation and increased risk of ischemic heart disease (48). Our data are opposite from a recent study that observed an association of gastric cancer with haplotypes correlating with low MBL levels (25). This may be explained because the main risk factor for gastric cancer is *H. pylori*, and low MBL levels have been associated with increased risk of infection (49,50). Therefore, people with low MBL levels may be more susceptible to *H. pylori* infection putting them at increased risk of gastric cancer.

Our current studies did not have data of childhood asthma to investigate the association with MBL levels. Although we had information on other respiratory diseases such as adult asthma, chronic bronchitis, emphysema, asbestosis, and tuberculosis, no association was observed between MBL levels and these diseases, which may be explained by the low number of participants with these diseases, so that further studies to address this issue are warranted. Additionally, there were no differences observed in the age of lung cancer diagnosis based on MBL levels ($P = 0.57$).

The major strength of our study is consistent results found in two independently conducted case-controls studies. However, our study does have limitations. Because of the logistical difficulties in tracking a cohort from childhood through adulthood to monitor cancer incidence, the alternative case-control design relies on personal recall. Thus, one limitation in our study is that it can be prone to differential recall bias. It is possible that cases recalled exposure to secondhand smoke during childhood differently, especially among the never smokers, which would make our odds ratios seem higher than they truly are. Additionally, this type of recall bias can be further amplified when interviews are conducted with next of kin, but due to our eligibility requirements, all interviews were conducted in person with the study participant, further lowering this chance of recall bias. The 2006 Surgeon General's report confirms this bias by stating that the weaker association they observed between childhood secondhand smoke and lung cancer risk may be due to high rates of misclassification when assessing childhood exposure (8). This would attenuate the odds ratios observed. Finally, any recall bias occurring with secondhand smoke exposure for the genetic analysis of *MBL2* would be nondifferential because study participants were unaware of their genotypes. A second limitation of our study is that a subset of cases and controls was investigated to confirm the association of the *MBL2* secretor haplotype correlating with high MBL levels although the association between *MBL2* haplotypes and serum MBL levels is well established (15,19,25,26).

Although our sample size was not large, our results were statistically significant, suggesting a strong and genuine association in two independent cohorts that will need to be replicated in other studies. Because of the small number of never smokers in the NCI-MD study, the majority of analyses were done in all study participants with an adjustment for smoking, leaving the possibility of residual confounding due to smoking. The possibility of residual confounding also exists in terms of adult exposure to secondhand smoke. Although there was no association

between adult exposure to secondhand smoke and lung cancer risk in the Mayo Clinic study, there was in the NCI-MD study. We attempted to control for adult secondhand smoke exposure, but there is a possibility that residual confounding exists. To examine this possibility, it would be ideal to examine never smokers that have been exposed to secondhand smoke during childhood, but not in adulthood. Unfortunately, due to the small number of study participants in these strata, this analysis was not possible, thus warranting a larger future study. Finally, it would be ideal to examine the effect of childhood secondhand smoke exposure in those that were only exposed during childhood. Unfortunately, the number of study participants exposed only during childhood was small, therefore we adjusted for adult exposure.

In summary, our study supports the hypothesis that among never smokers, childhood secondhand smoke exposure increases adult lung cancer risk. Further, our data suggest that genetic background in *MBL2* with well-known functional consequences in innate immunity is an important modifier of the association between childhood secondhand smoke exposure and lung cancer risk. These findings are bolstered both by the fact they were replicated in two independent case-control studies and warrant additional studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Stefan Ambs and Krista Zanetti for the valuable feedback, discussion, and comments; Dorothea Dudek-Creaven for editorial assistance; Donna Perlmutter, Raymond Jones, Leoni Leondaridis, Glenwood Trivers, John Cottrell, and the Surgery and Pathology Departments from participating hospitals for their contributions; and Audrey Salabes, John Cottrell, and Rex Yung and Mark Krasna for their contributions to patient accrual and tissue collection. We also acknowledge Zhifu Sun for facilitating the Mayo Clinic sample testing and data assembly.

Grant support: Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research to [C. Harris], NIH grants [R03-77118, R01-80127 to P. Yang, R. Jiang, and J. Cunningham], and the Cancer Prevention Fellowship Program, Office of Preventive Oncology, National Cancer Institute to [S. Olivo-Marston].

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Table 1

Demographic characteristics of study participants

	NCI-MD study		*P**	Mayo Clinic study		*P**
	Cases (*n* = 624)	**Controls (*n* = 348)**		**Cases (*n* = 172)**	**Controls (*n* = 289)**	
Age (y)			0.99			0.64
Mean ± SD	66.6 ± 10.3	66.6 ± 9.5		61.3 ± 12.7	61.9 ± 14.6	
Gender (%)			0.70†			0.50†
Male	320 (51)	183 (53)		49 (28)	91 (31)	
Female	304 (49)	165 (47)		123 (72)	198 (69)	
Highest levels of education (%)‡			<0.001†			0.06†
Less than high school	139 (24)	27 (9)		12 (7)	10 (4)	
High school degree	340 (58)	180 (58)		80 (48)	140 (49)	
College degree	87 (15)	76 (25)		39 (23)	49 (17)	
Graduate degree	20 (3)	26 (8)		37 (22)	85 (30)	
Smoking history (%)§			<0.001†			
Never smoker	45 (7)	134 (39)		172 (100)	289 (100)	
Ex-smoker	305 (49)	176 (51)		0 (0)	0 (0)	
Current smoker	274 (44)	38 (11)		0 (0)	0 (0)	

*P values are calculated from a Pearson χ^2 test, except for age, which is calculated using Student's t-test.

†P value is based on an overall comparison, rather than individual strata, of the characteristic in cases versus controls.

‡Education data missing from 38 cases and 39 controls from the NCI-MD study and 4 cases and 5 controls from the Mayo Clinic study.

§The Mayo Clinic study consisted only of never smokers.

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Table 2

Lung cancer risk of study participants exposed to childhood secondhand smoke

	NCI-MD study			Mayo Clinic study		
	Cases (*n* = 624)	**Controls (*n* = 348)**	**Adjusted OR (95% CI)**	**Cases (*n* = 172)**	**Controls (*n* = 289)**	**Adjusted OR (95% CI)**
Subjects exposed during childhood to secondhand smoke (%)						
Never smoker*	28 (62)	61 (46)	2.25 (1.04-4.90)	98 (57)	135 (47)	1.47 (1.00-2.15)
Ex-smoker†	250 (82)	140 (80)	1.32 (0.79-2.19)			
Current smoker‡	236 (86)	29 (76)	1.91 (0.77-4.73)			
No exposure in childhood home§,//,¶	109 (21)	118 (37)	1.00 (reference)	76 (44)	163 (56)	1.00 (reference)
Light exposure in childhood home	33 (6)	33 (10)	1.02 (0.56-1.87)	27 (16)	34 (12)	1.78 (0.99-3.19)
Moderate exposure in childhood home	31 (6)	17 (5)	2.19 (1.08-4.42)	30 (17)	33 (11)	1.84 (1.04-3.26)
Heavy exposure in childhood home	339 (66)	147 (47)	2.23 (1.55-3.20)	39 (23)	59 (20)	2.37 (1.83-4.28)
P trend			<0.001			<0.001
Exposed to father's smoke §	437 (80)	192 (62)	2.30 (1.62-3.25)	91 (53)	117 (41)	1.22 (1.04-1.45)
No exposure in childhood home	109 (23)	118 (41)	1.00 (reference)	74 (45)	153 (57)	1.00 (reference)
Light exposure from father	145 (31)	79 (27)	1.85 (1.21-2.83)	30 (17)	41 (14)	1.51 (0.88-2.61)
Moderate exposure from father	153 (33)	66 (23)	2.04 (1.31-3.17)	27 (16)	37 (13)	1.51 (0.85-2.66)
Heavy exposure from father	58 (12)	25 (9)	2.89 (1.59-5.26)	34 (20)	39 (14)	1.80 (1.05-3.08)
P trend			0.001			0.02
Exposed to mother's smoke §	253 (70)	107 (48)	2.46 (1.63-3.73)	26 (15)	44 (15)	0.92 (0.72-1.16)
No exposure in childhood home	109 (34)	118 (56)	1.00 (reference)	74 (74)	153 (78)	1.00 (reference)
Light exposure from mother	27 (8)	17 (8)	1.83 (0.87-3.84)	9 (5)	12 (4)	1.55 (0.63-3.84)
Moderate exposure from mother	108 (34)	53 (25)	1.93 (1.17-3.16)	6 (4)	8 (3)	1.55 (0.52-4.63)
Heavy exposure from mother	74 (23)	23 (11)	2.92 (1.55-5.48)	11 (6)	24 (8)	0.95 (0.44-2.04)
P trend			<0.001			0.47

*OR is adjusted for age, gender, exposure to secondhand smoke during adulthood, education, and first-degree relative with lung cancer.

†OR is adjusted for same as never smokers along with number of cigarettes/day, number of years smoked, age at initiation, and time since quitting.

‡OR is adjusted for same as never smokers along with number of cigarettes/day, number of years smoked, and age at initiation.

[§] OR is adjusted for age, gender, smoking status, exposure to secondhand smoke during adulthood, education level, first-degree relative with lung cancer, number of cigarettes/day, number of years smoked, age at initiation, and time since quitting.

[//] Light exposure during childhood was <0.5 half pack per day smoked by members of the household, moderate exposure was 0.5 to 1 pack of exposure per day, and heavy exposure was >1 pack per day.

[¶] One hundred twelve cases (18%) and 33 controls in the NCI-MD study (9%) were missing data on number of cigarettes smoked by their mother or father so exposure level could not be calculated.

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Table 3

Age at lung cancer diagnosis among those unexposed or exposed to childhood secondhand smoke

	NCI-MD study				*P*	Mayo Clinic study				*P*
	Unexposed during childhood		Exposed during childhood			Unexposed during childhood		Exposed during childhood		
	n	Age in y (mean ± SD)	*n*	Age in y (mean ± SD)		*n*	Age in y (mean ± SD)	*n*	Age in y (mean ± SD)	
Never smokers	17	67.0 ± 9.8	28	52.1 ± 9.6	<0.001	74	63.0 ± 13.3	98	56.8 ± 12.9	0.035
Former smokers	55	71.5 ± 8.4	250	70.0 ± 9.4	0.167					
Current smokers	37	65.4 ± 9.6	236	62.5 ± 8.3	0.054					

Table 4

The modification of MBL2 haplotypes on risk of lung cancer due to childhood secondhand smoke

MBL2 Haplotype	NCI-MD study*			Mayo Clinic study		
	Cases *n* (%)	Controls *n* (%)	OR (95% CI)†	Cases *n* (%)	Controls *n* (%)	OR (95% CI)†
Exposed to childhood secondhand smoke						
Medium MBL levels	181 (63)	89 (68)	1.00 (reference)	79 (81)	104 (77)	1.00 (reference)
LYQA						
LYPA						
Low MBL levels	65 (23)	33 (25)	0.97 (0.59-1.58)	8 (8)	28 (21)	1.06 (0.68-1.98)
LXPA						
LYPB						
HYPD						
LYQC						
High MBL levels	41 (14)	8 (6)	2.52 (1.13-5.60)	11 (11)	3 (2)	2.78 (1.18-3.85)
HYPA						
No exposure to childhood secondhand smoke						
Medium MBL levels	38 (63)	47 (73)	1.00 (reference)	56 (76)	129 (84)	1.00 (reference)
LYQA						
LYPA						
Low MBL levels	15 (25)	14 (22)	0.57 (0.18-1.83)	12 (16)	20 (13)	0.75 (0.40-1.66)
LXPA						
LYPB						
HYPD						
LYQC						
High MBL levels	7 (12)	3 (5)	1.19 (0.18-7.79)	6 (8)	4 (3)	1.05 (0.61-11.53)
HYPA						

*Only 285 cases and 130 cases had complete MBL2 genotyping information available because of DNA degradation and since the study is ongoing some study participants were accrued after samples were sent for genotyping.

†ORs are adjusted for age, gender, smoking status, exposure to adult secondhand smoke, education level, first-degree relative with lung cancer number of cigarettes/day (NCI-MD), number of years smoked (NCI-MD), age at initiation (NCI-MD), and time since quitting (NCI-MD).