Asbestos Exposure, *Manganese Superoxide Dismutase (MnSOD)* Genotype, and Lung Cancer Risk

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To assess whether differences in genetic susceptibility to oxidative stress modify asbestos-related lung cancer risk (caused by lung inflammation, free radical production), we examined possible interactions between manganese superoxide dismutase *(*MnSOD*) genotypes and asbestos in a hospital-based case-control study of 811 white lung cancer cases and 957 friend/spouse controls. Cumulative lifetime asbestos exposure score (AES) was calculated from self-reported duration and intensity of occupational and nonoccupational exposures. A total of 13.5% of cases and 10% of controls had "high" AES (determined by a priori cut point). The homozygous variant* MnSOD *genotype was associated with increased lung cancer risk among individuals with zero or "low" AES (odds ratio [OR], 2.14; 95% confidence interval [CI], 1.52–3.01) and no association (OR = 1.00; 95% CI = 0.36–2.73) among the "high" AES group. We observed no statistically significant interaction between* MnSOD *genotype and asbestos exposure for lung cancer risk.* (J Occup Environ Med. *2004;46:556–564*)

Epidemiologic studies of occupational cohorts[1,2] (ie, asbestos miners,[3–5] insulation,[6,7] construction and factory workers[8–13]) and population based case-control studies[14–18] have consistently observed increased lung cancer risk associated with occupational asbestos exposure. The interaction between asbestos and smoking has also been noted to be approximately multiplicative.[19–23] Asbestosis and mesothelioma are other major asbestos-related diseases.[24] The specific disease outcome appears to depend on factors that contribute to the site of deposition in the lung (ie, fiber type, length, diameter),[24] in addition to exposure intensity and duration.

Inhaled asbestos fibers that are not cleared from the lungs induce an inflammatory response that lead to cytotoxic, genotoxic, and proliferative effects. Fibers (>5 μm in length, <3 μm in diameter) that are incompletely phagocytosed become trapped in the bronchiole and alveolar ducts, causing tissue damage and chronic airway inflammation.[24,25] Activated alveolar macrophages aggregate at the site of injury releasing oxygen-free radicals, which are important in cell signaling but also cause cellular damage.[25] Activated macrophages also release various growth factors (eg, platelet-derived growth factors, insulin-like growth factor-1, fibroblast growth factor) and cytokines (eg, procollagen), leading to fibrosis as a part of chronic inflammation.[25] These processes suggest possible tumor initia-

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tion and promoter mechanisms for asbestos.

Adsorption of tobacco carcinogens (eg, benzo(a)pyrene [BAP]) by asbestos fibers could enhance the carcinogenic potential of the fibers and is a possible mechanism for the observed interaction between asbestos and smoking exposure.[25] The combination of asbestos fibers and BAP, given intratracheally, induced tumor formation in hamsters.[26] At the cellular level, the combination of cigarette smoke and asbestos fiber has been shown to induce hydroxyl radicals and DNA strand breaks in vitro[27] and in vivo.[28]

Manganese superoxide dismutase (MnSOD) is an enzyme that specifically removes superoxide radicals in the mitochondria.[29] The gene contains a common polymorphism (ie, *Ala16Val MnSOD*), and we have previously shown that the *Val* allele was associated with increased lung cancer risk in the current study population.[30] Although the function of the polymorphism is unknown, some studies suggest that the variant *Val* allele could be associated with decreased transport of the protein into its site of action, resulting in decreased enzyme activity.[31,32] Superoxide dismutases or free radical scavengers prevent asbestos-related cell injury in vitro.[33,34] Asbestos fibers have also been observed to induce expression[35] and to increase protein levels of *MnSOD* in rat lungs.[36]

Few studies have examined interactions between past asbestos exposure and polymorphisms in genes involved in response to oxidative stress or metabolism of carcinogens for lung cancer risk. Analysis and interpretation have been hindered by small numbers of occupationally exposed individuals in population-based studies. A recent pooled analysis did not observe an interaction between occupational asbestos exposure and *glutathione S-transferase mu-1* and *theta-1* (*GSTM1, T1*) genotypes in lung carcinogenesis.[37] Earlier smaller studies observed possible but statistically nonsignificant effect modification by *GSTM1*[38] and *CYP2D6*,[39] whereas findings from a more recent study suggested effect modification by *myeloperoxidase (MPO*) among individuals with a history of smoking and/or asbestos exposure.[40] A study in a small Finnish population of asbestos insulation workers reported no significant association between *MnSOD Val* genotype and asbestos-related pulmonary diseases (ie, mesothelioma, asbestosis, and/or pleural plaques),[41] although the direction of risk associated with the *MnSOD Val* allele was increased for mesothelioma and decreased for asbestosis and/or pleural plaques. To date, no published studies have assessed possible effect modification by *MnSOD* genotype on asbestos-associated lung cancer risk.

We evaluated the potential interaction between the *MnSOD* genotype and asbestos exposure in a hospital-based case-control lung cancer study in Boston, Massachusetts. The current study is an extension of our previous report on the *MnSOD* genotype and lung cancer risk in this same population.[30] Because the *MnSOD* enzyme could play an important role in the body's normal response to cigarette smoke and asbestos exposure, less efficient functioning associated with the *MnSOD Val* allele could lead to increased lung cancer risk through superoxide radical accumulation in lung tissue. We hypothesized that individuals with higher levels of total asbestos exposure (ie, from occupational and environmental sources), along with the variant *Val* allele of *MnSOD* and exposure to tobacco smoke, have the greatest lung cancer risk.

Methods

Study Population

Participants in this study were enrolled between December 1992 and September 2000 as part of an ongoing hospital-based case-control study conducted at Massachusetts General Hospital, in Boston, Massachusetts. The study was approved by the Institutional Review Boards at both Massachusetts General Hospital and Harvard School of Public Health. Eligible cases were individuals 18 years or older who were seen at the Massachusetts General Hospital in the Thoracic Surgery, Thoracic Oncology, or Pulmonary Units for newly diagnosed primary lung cancer. Histologic confirmation of all case diagnoses was determined by a lung pathologist. Controls were recruited among the friends and nonblood-related family members of the lung cancer cases with no specific matching characteristics. When such individuals were not available, controls were recruited among friends and nonblood-related family members of nonlung cancer patients admitted to the cardiothoracic wards.

Data Collection

A detailed health questionnaire was completed for each case and control by a trained interviewer at the time of recruitment. For all participants with missing questionnaire information, attempts were made to contact the individual by telephone and obtain more complete data. The questionnaire, a modified version of the standardized American Thoracic Society respiratory questionnaire,[42] was used to obtain information on demographics, medical history, family history of cancer, specific smoking history, and detailed work history, including job titles and tasks. Specific smoking-related information included duration (in years) and intensity (the average cigarettes smoked daily over those years) of smoking and time since quitting (in years) for exsmokers. Pack-years, used as a measure of cumulative smoking exposure, was defined as the average packs smoked per day multiplied by the number of years smoked. Peripheral blood specimens were collected in EDTA and silicone-coated tubes at the time of interview. Details on *MnSOD* genotyping based on polymerase chain

reaction pyrosequencing methods have been reported previously.[30]

Asbestos Exposure Assessment

Participants were first asked if they had "ever" been exposed to asbestos in their lifetime. ("*Have you ever been exposed, on or off the job, to asbestos (even if one time)? Include childhood exposure.*") If they answered "yes," then detailed information on the type (both occupational and environmental sources of asbestos exposure), frequency (year-round, seasonal, occasional), and dates of asbestos exposure was collected.

In this study population, a *cumulative lifetime asbestos exposure score* was previously developed and validated.[43] A high asbestos exposure score (ie, >10) correlated well with the presence of pleural and parenchymal abnormalities on chest x-rays or computed tomography scans in a series of lung cancer cases from our study population.[43] Cumulative asbestos exposure was calculated as the product of the intensity, duration, and frequency of exposure summed over all reported exposure periods. Scores for environmental and occupational sources of asbestos exposure were first calculated separately and then combined to form a total asbestos exposure score. For occupational sources of asbestos exposure, changes in federal regulation of asbestos use and handling over specific time periods (ie, pre-1965, 1965–1972, and post-1972) were also accounted for using predetermined weights (ie, 4, 2, and 1, respectively). These weights were originally developed based on asbestos exposure in the New England building construction trades.[44]

Predetermined weights given for the intensity of exposure were based on a scale of 0 to 2 for environmental sources of exposure and 4 to 6 for occupational sources of exposure. The corresponding weights for the following reported examples of asbestos exposure sources are: "0" for living in a house with asbestos siding, "2" for washing asbestos-covered clothing at home, and "5" for working as a longshoreman. A list of specific industries and jobs or activities, determined a priori to be associated with asbestos exposure, along with their corresponding weights, are listed in Appendix Table 1. Each reported asbestos exposure source was assessed by this prespecified list, and responses not listed were evaluated and assigned a score based on the expertise of the principal investigator of the study (Dr. Christiani), who was blinded to case status. Individuals who reported never being exposed to asbestos were given a total asbestos score of zero. In addition, individuals who answered "yes" to ever being exposed to asbestos, but whose reported source of asbestos exposure was determined to be inconsequential (ie, corresponding to weight = 0 for intensity), could also have a total asbestos score of zero. Weights for frequency of exposure were as follows: year-round exposure = 1, seasonal = 0.5, occasional = 0.125.

Statistical Analysis

From our original study population, only individuals with complete information on age, sex, and smoking variables were included. Because >96% of our study population was white, we restricted our analysis to whites. Finally, we excluded those individuals who reported that they "did not know" if they had ever been exposed to asbestos (n = 363; approximately 14%) or those with missing asbestos scores (n = 203). Analysis of asbestos exposure, smoking, and lung cancer was then conducted on 1950 individuals (966 cases and 984 controls). In our analysis of the interaction between *MnSOD* genotype and asbestos, an additional 182 individuals without *MnSOD* genotype data (as a result of insufficient DNA or unsuccessful *MnSOD* genotyping) were excluded, leaving 1768 individuals (811 cases, 957 controls).

Associations among asbestos exposure, smoking, *MnSOD* genotype, and lung cancer were evaluated using logistic regression to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). *MnSOD* genotype was classified as wild-type *Ala/Ala*, heterozygous *Ala/Val*, or homozygous variant *Val/Val*. Covariates in our adjusted models included age (continuous), sex, smoking status (non-, ex-, current), square root of pack-years, and years since quitting for exsmokers. Previous reports on this study population have determined that this set of variables most appropriately models these potential confounders.[45]

The asbestos exposure score was dichotomized ("high" vs. "no or low") based on cutpoints determined by our previous validation study.[43] Individuals with a score >10 were defined as having a high asbestos exposure score (HAES). Individuals in the "no or low" asbestos exposure score (LAES) category had a total asbestos exposure score <10 including a score of zero and were considered the reference group. To limit the effects of misclassification in our unexposed reference group, we also used a more stringent definition for this group by restricting it to individuals who reported "never" being exposed to asbestos in their lifetime. In analysis based on 3 exposure categories (ie, never, low, high), the low-exposure group was then defined as individuals who reported being exposed at least once during their lifetime and having an asbestos score <10 including zero, whereas the high asbestos exposure group remained the same. The asbestos exposure score was divided into 4 categories to test for trend. The 4 categories consisted of individuals with an asbestos score = 0 and the following tertiles among those with an asbestos scores greater than 0: >0–7, >7–38, >38).

In analyses stratified by both smoking status and asbestos exposure together, the smoking variable was dichotomized into "recent" (ie,

current smoker and exsmokers who had quit smoking for 10 or fewer years) versus "never/remote past" smokers (ie, nonsmokers and exsmokers who had quit for more than 10 years) to allow for sufficient numbers of observations. Formal tests for interaction were conducted using the Wald or the likelihood ratio test (LRT) as appropriate.

Results

In our study population, 13.5% of cases and 10% of controls had a high asbestos exposure score. Individuals with a high asbestos exposure score (HAES) were exposed mainly through occupational sources of asbestos. The total asbestos score correlated strongly with the occupational asbestos score (Spearman correlation coefficient; $r = 0.82$, $P < 0.001$) and moderately with the environmental asbestos score (Spearman correlation coefficient; $r = 0.51$, $P < 0.001$). Asbestos scores ranged from 0 to 852 and among 689 individuals who reported "ever" being exposed to asbestos, 45% had an asbestos score of "0" when determined by self-reported details of the type, frequency, and duration of exposure.

The distribution of case status, demographic and smoking factors among groups of never, low, and high asbestos exposure categories are shown in Table 1. A greater proportion of individuals in the high asbestos exposure group were cases (57% vs. 50% in the low or 48% in the never-exposed groups). Significantly more were men (86% vs. 54% and 43% among the low and never-exposed groups, respectively) and slightly older aged (mean age of 65 vs. 61–62 years in the never and low-exposure groups). The high asbestos exposure group had fewer nonsmokers (8% vs. 22–23% in the other 2 groups). Among ever-smokers, those in the high asbestos exposure group had the greatest cumulative smoking exposure (62 vs. 46–49 pack-years), smoked more intensely and for a greater number of years. Among controls only, older age, smoking (based on pack-years), and being male were positively and significantly associated with having a HAES and therefore were considered as potential confounders for the association between asbestos and lung cancer. *MnSOD* genotype was independent of asbestos exposure among our controls.

Initially, high asbestos exposure was associated with increased lung cancer risk (OR = 1.4); however, this estimate was attenuated after adjustment for age, sex, and additional smoking factors (ie, square root of pack-years, years quit for exsmoker) as confounders (Table 2). After adjustment for smoking, HAES

TABLE 1
Distribution of Demographics, Smoking Characteristics, and Case Status Among Asbestos Exposure Groups

	Never Exposed to Asbestos	Ever Exposed to Asbestos	
		Low*	High†
Total (n = 1950)	n = 1261	n = 461	n = 228
Case (no. [%])	604 (47.9)	232 (50.3)	130 (57.0)
Controls (no. [%])	657 (52.1)	229 (49.7)	98 (43.0)
Gender‖			
Women (no. [%])	714 (56.6)	214 (46.4)	32 (14.0)
Men (no. [%])	547 (43.4)	247 (53.6)	196 (86.0)
Smoking status¶			
Nonsmoker (no. [%])	285 (22.6)	100 (21.7)	19 (8.3)
Exsmoker (no. [%])	590 (46.8)	232 (50.3)	139 (61.0)
Current smoker (no. [%])	386 (30.6)	129 (28.0)	70 (30.7)
Age‡§	62.3 ± 12.3	60.7 ± 11.6	64.7 ± 10.3
	64.3 (18.7–96.3)	62.0 (26.7–90.7)	66.0 (34.8–87.9)
Total asbestos score‡§	0 ± 0	1.1 ± 2.4	113.4 ± 154.2
	0	0 (0–10)	43 (10.25–852)
Occupational asbestos score‡§	0 ± 0	0.6 ± 1.9	108.9 ± 156.8
		0 (0–10)	40 (0–852)
Among ever smokers	N = 976	N = 361	N = 209
Pack-years‡	45.8 ± 34.8	48.7 ± 36.4	62.3 ± 43.5
Cigarettes per day‡	25.2 ± 14.8	28.0 ± 17.0	32.5 ± 17.8
Years smoked‡	34.4 ± 14.6	32.8 ± 14.1	36.8 ± 13.5
Among exsmokers	N = 590	N = 232	N = 139
Years quit‡	16.5 ± 11.9	16.7 ± 11.2	16.8 ± 12.0

* Total asbestos score <10, including score of zero.
† Total asbestos score >10.
‡ Mean ± standard deviation.
§ Median (minimum value-maximum value).
‖ Chi-square, $P < 0.001$ (2 df); MH test for trend $P < 0.001$.
¶ Chi-square $P < 0.001$ (4 df); MH test for trend $P = 0.03$.

was associated with a small but statistically nonsignificant reduction in lung cancer risk (OR = 0.79; 95% CI = 0.56–1.11). The test for linear trend was not significant ($P = 0.36$). We observed similar findings after restricting our analysis to individuals reporting occupational sources of asbestos only or to men only. Increasing the cutpoint used to define the high asbestos group to limit possible misclassification in this group also did not markedly change our findings. For example, the adjusted estimate for a total asbestos score of 38 or greater (based on asbestos divided into 4 categories) versus a score of zero was OR = 0.76.

When we evaluated asbestos exposure according to the categories of never, low, and high (Table 2), the results for the HAES group compared with this new reference group did not change substantially (OR = 0.84; 95% CI = 0.59–1.18). The low asbestos exposure group was associated with a slightly greater, but statistically not significant, risk of lung cancer (OR = 1.20; 95% CI = 0.92–1.55) compared with individuals never exposed to asbestos.

Previous studies have reported a synergistic effect of asbestos and smoking; therefore, we examined possible effect modification of asbestos exposure and lung cancer risk by smoking status (Table 3). In the unadjusted models, HAES compared with the reference group of a "no or low" asbestos score was associated with greater lung cancer risk among current smokers only (OR = 1.52) and a weak or null association among non- or exsmokers (OR = 1.13 and 1.01, respectively). After adjusting for confounders, we observed nonsignificant reductions in lung cancer risk associated with HAES among non-, former, and current smokers. The observed HAES association with lung cancer was not significantly different by smoking status (LRT [2 df]: $P = 0.57$). An additional test for interaction that modeled smoking as a continuous variable (ie, pack-years) was also not statistically significant (Wald test, $P = 0.81$). However, the magnitude of risk associated with a "low" asbestos exposure score was greater

TABLE 2
Association Between Asbestos Exposure and Lung Cancer

Asbestos Exposure	No. of Cases†	No. of Controls†	Unadjusted Odds Ratio	Adjusted Odds Ratio*
Total asbestos score‡				
No/low	836	886	1.0	1.0
High	130	98	1.41 (1.06–1.86)	0.79 (0.56–1.11)
Total asbestos score‡				
Never	604	657	1.0	1.0
Low	232	229	1.10 (0.89–1.36)	1.20 (0.92–1.55)
High	130	98	1.44 (1.09–1.92)	0.84 (0.59–1.18)

* Adjusted for age, sex, smoking status (non-, ex-, current), square root pack-years, years quit.
† Sample size used in adjusted models.
‡ n = 1950 (966 cases, 984 controls).

TABLE 3
Asbestos and Lung Cancer Association Stratified by Smoking Status

Asbestos Exposure Score	Nonsmokers* OR (95% CI) No. of Cases/No. of Controls	Exsmokers† OR (95% CI) No. of Cases/No. of Controls	Current smokers‡ OR (95% CI) No. of Cases/No. of Controls
Total asbestos score			
No/low§	1.0	1.0	1.0
	55/330	435/387	346/169
High	0.87 (0.24–3.24)	0.74 (0.49–1.13)	0.89 (0.46–1.75)
	3/16	74/65	53/17
Likelihood ratio test for interaction (2 df): $P = 0.57$			
Total asbestos score			
Never§	1.0	1.0	1.0
	41/244	310/280	253/133
Low	1.05 (0.54–2.05)	1.20 (0.85–1.71)	1.31 (0.80–2.14)
	14/86	125/107	93/36
High	0.88 (0.24–3.32)	0.78 (0.51–1.21)	0.96 (0.48–1.91)
	3/16	74/65	53/17

* Adjusted for age, sex.
† Adjusted for age, sex, square root pack-years, years since quitting smoking.
‡ Adjusted for age, sex, square root pack-years.
§ Reference group.
OR, odds ratio; CI, confidence interval.

TABLE 4
MnSOD Genotype and Lung Cancer Association Stratified by Asbestos Exposure (no or low vs. high)*

MnSOD Genotype	No/Low Asbestos Exposure OR (95% CI) No. of Case/No. of Controls	High Asbestos Exposure OR (95% CI) No. of Case/No. of Controls
Ala/Ala†	1.0	1.0
	144/236	29/19
Ala/Val	1.71 (1.27–2.31)	0.88 (0.38–2.03)
	363/428	50/54
Val/Val	2.14 (1.52–3.01)	1.00 (0.36–2.73)
	201/197	24/23
Wald test for interaction: $P = 0.13$		
Likelihood ratio test for interaction (2 df): $P = 0.20$		

* Adjusted for age, sex, exsmoker, current smoker, square root pack-years, years since quitting smoking.
† Reference group.
OR, odds ratio; CI, confidence interval.

among former and current smokers (OR = 1.20 and 1.31, respectively) compared with nonsmokers (OR = 1.05), suggesting some evidence for synergy between smoking and asbestos exposure in our study population.

We have reported previously that the *MnSOD Val* allele is positively associated with lung cancer risk in this same study population.[30] Individuals who were heterozygous (*Ala/Val*) or homozygous variant (*Val/Val*) had a greater risk of lung cancer (OR = 1.34 and 1.67, respectively) compared with individuals with the wild-type *MnSOD* genotype (*Ala/Ala*). Similarly, the *Val/Val* genotype was associated with increased lung cancer risk (OR = 2.14; 95% CI = 1.52–3.01) among individuals with "no or low" asbestos exposure (Table 4). Among individuals in the HAES group, we observed no association (OR = 1.00; 95% CI = 0.36–2.73) for the *Val/Val* genotype. An intermediate effect estimate was observed for heterozygotes only among those in the "no or low" asbestos exposure group. The test for interaction between the asbestos score and *MnSOD* genotype was not statistically significant (LRT, $P = 0.20$; or Wald test, $P = 0.13$ assuming the *MnSOD* genotype is modeled as an ordinal variable).

We observed a similar pattern for the associations between the *MnSOD* genotype and lung cancer among individuals stratified by their combined exposure to asbestos (high or no/low) and tobacco (defined as either a recent smoker or never/remote past smoker). Among individuals with both the lowest asbestos and tobacco exposures, having 1 or more *MnSOD Val* allele was associated with a doubling of lung cancer risk (OR = 2.15; 95% CI = 1.40–3.29). Intermediate risks were observed among individuals with intermediate levels of combined asbestos and tobacco exposures (ORs = 1.63 and 1.21). Among individuals with both the greatest asbestos and smoking exposures, having 1 or more *MnSOD Val* allele conferred a reduction in lung cancer risk that was not statistically significant (OR = 0.81; 95% CI = 0.27–2.42).

Discussion

In our study population, high asbestos exposure levels, based on a cumulative lifetime asbestos exposure score, increased lung cancer risk significantly by 40% in our unadjusted analysis. However, after adjusting for smoking, the high asbestos exposure score was no longer statistically associated with lung cancer and instead suggested a reduction in risk of 20%. We did not observe a significant interaction between high asbestos exposure and smoking in our study population. However, low-level asbestos exposure was associated consistently with a proportionally greater lung cancer risk among current smokers and former smokers than among nonsmokers. The *MnSOD Val* allele was associated with an increased lung cancer risk among individuals with "no or low' asbestos exposure score (OR = 2.14) compared with risk among individuals with a HAES (OR = 1.00). However, the confidence intervals around the genotype effect estimate among HAES individuals were wide as a result of the small sample size in this group, making it difficult to determine if the genotype association was truly different by asbestos exposure. The test for interaction between *MnSOD* genotype and asbestos exposure was not statistically significant.

Differential loss of potential cases resulting from differences in survival until time of lung cancer onset could explain the consistent but not significant reduction in risk associated with the high asbestos exposure score in this study population. This bias is also suggested by the lack of an effect of HAES among current smokers, in whom we would expect the greatest increased risk as a result of the interaction between smoking and asbestos. "Survival bias" suggests that individuals with high asbestos and smoking exposures are underrepresented in our study population. Given that the time period when asbestos exposure was the greatest in the Boston area (1930–1970s), those with high asbestos and smoking exposure could have already died of lung cancer or other asbestos-related pulmonary disease (eg, asbestosis, pulmonary fibrosis).

Therefore, they would be underrepresented in our case population because they would have died before they could be recruited into our study in the 1990s. We did observe the expected weak increased risk associated with low asbestos exposure, which suggests that only individuals with high asbestos exposure are underrepresented as lung cancer cases in this population.

The prevalence of high asbestos exposure in our population (13.5% of cases and 10% of controls) was slightly lower than the prevalence of occupational asbestos exposure reported in a population based case-control study of U.S. whites (17.5%) and blacks (23%) in Los Angeles, California[37] where subjects were enrolled in the early 1990s. An earlier study conducted in the mid-1970s[16] enrolled population controls living near a Southern coastal region of the United States, where large shipyards similar to Boston, Massachusetts, were located during World War II. They reported that 33% of their patients with lung cancer and 25% controls had worked at least 6 months in the shipbuilding industry. Because the pattern of asbestos exposure has changed in the last 4 decades in the United States, recent population-based studies of asbestos exposure could be limited in their ability to evaluate the effects of asbestos in this specific group because of the low prevalence of highly exposed individuals.

Over- or underreporting for past asbestos exposure and variation in true exposure for similar industry or job descriptions could have contributed to misclassification in our study. For example, respirator use or a person's proximity to the main source of asbestos exposure at their job site would affect the probability of exposure. Detailed information obtained from our open-ended question on asbestos exposure allowed us to account for some but not all of this variation. Generally, we would expect errors in reporting of exposure to be random, which would bias estimates toward the null. Limiting the extent of possible misclassification, by restricting our analysis to individuals at the extreme ends of the exposure spectrum where misclassification was less likely (ie, occupational asbestos exposure only; using a greater threshold value to define high exposure; using a more stringent definition for the unexposed group), did not change the magnitude and direction of our observed association for asbestos exposure. Recall bias is unlikely because we would expect cases to overreport past asbestos exposure, leading to an overestimate of risk associated with high asbestos exposure, not what we observed in this study.

Smoking is a highly correlated confounder for asbestos. Adjustment for smoking factors altered the direction of the effect estimates for asbestos exposure and could have obscured any increased risk associated with asbestos in our logistic models.

The use of friend or spouse controls could have led to our controls being more similar to our cases with respect to asbestos exposure. For example, friends of cases could have similar occupations (eg, firefighters), whereas wives of husbands who work in asbestos-exposed industries were likely to report some low level of asbestos exposure based on asbestos brought home by their husbands, resulting in an underestimate for the effect of asbestos. However, our estimate for the interaction between asbestos and the *MnSOD* genotype should not be affected assuming the factors are independent of each other (ie, the *MnSOD* genotype distribution for our controls within each level of asbestos exposure represents the distribution in the general population).[46]

We observed a doubling of lung cancer risk associated with the *MnSOD Val* allele compared with the homozygous *Ala* allele as the reference group among the "no or low" asbestos exposure score group and no association for the *MnSOD* genotype among the HAES group. A similar pattern was observed on further stratification by combined asbestos and smoking exposures. It is biologically plausible that the effect of the *MnSOD* genotype on lung carcinogenesis could be more visible at low levels of asbestos and tobacco exposure. The balance of oxidative species in the mitochondria is an important mechanism in the regulation of apoptosis,[47,48] and very high exposure to carcinogens could lead directly to cell death rather than tumor transformation.

Our results could also be explained by lung cancer cases related to both high asbestos and smoking exposure that were not captured in our study as discussed previously. Because individuals with the *MnSOD Val* allele are also more likely to develop lung cancer, there could be a greater proportion of individuals with HAES and the *Val* allele who were not captured in our study. This would lead to an underestimate of the true risk associated with the *MnSOD* genotype among HAES, arguing against an interaction. Further studies in populations in whom asbestos exposure is still prevalent and all developing lung cancer cases can be captured could provide further clarification on our findings.

Given our sample size of 811 cases and approximately an equivalent number of controls, we had 72% power to detect an interaction of magnitude 2.5 assuming an OR = 1.5 for the *MnSOD* genotype (*Val/Val* vs. *Ala/Ala*) and an OR = 1.5 for the HAES versus no/low asbestos exposure group.[49] We used the approximate observed proportion of HAES (15%) and *MnSOD Val/Val* genotype (25%) from our study population for these calculations. This study had fairly good power to detect moderate to large interactions between MnSOD and asbestos; however, larger sample sizes with higher proportions of highly exposed individuals are needed to evaluate potential interactions of smaller magnitude.

In conclusion, in this study of 811 lung cancer cases and 957 controls, we initially observed a 40% increased lung cancer risk for high levels of past asbestos exposure through combined environmental and occupational sources. However, after adjusting for multiple smoking-related factors, high asbestos exposure was associated with a nonsignificant reduction in risk. We observed no significant interaction between asbestos exposure and the *MnSOD* genotype, although having at least 1 *MnSOD Val* allele doubled an individual's lung cancer risk among those with "no or low" levels of asbestos exposure and was associated with a null effect among individuals with high asbestos exposure.

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References

1. Doll R. Mortality from lung cancer in asbestos workers 1955. *Br J Ind Med*. 1993;50:485–490.
2. Selikoff IJ, Churg J, Hammond EC. Asbestos exposure and neoplasia. *JAMA*. 1964;188:22–26.
3. McDonald JC, Liddell FD, Dufresne A, et al. The 1891–1920 birth cohort of Quebec chrysotile miners and millers: mortality 1976–88. *Br J Ind Med*. 1993; 50:1073–1081.
4. Liddell FD, Thomas DC, Gibbs GW, et al. Fibre exposure and mortality from pneumoconiosis, respiratory and abdominal malignancies in chrysotile production in Quebec, 1926–75. *Ann Acad Med Singapore*. 1984;13:340–344.
5. de Klerk NH, Musk AW, Armstrong BK, et al. Smoking, exposure to crocidolite, and the incidence of lung cancer and asbestosis. *Br J Ind Med*. 1991;48:412–417.
6. Selikoff IJ, Hammond EC, Churg J. Asbestos exposure, smoking, and neoplasia. *JAMA*. 1968;204:106–112.
7. Hammond EC, Selikoff IJ, Seidman H. Asbestos exposure, cigarette smoking and death rates. *Ann N Y Acad Sci*. 1979;330:473–490.
8. Berry G, Newhouse ML, Turok M. Combined effect of asbestos exposure and smoking on mortality from lung cancer in factory workers. *Lancet*. 1972;2:476–478.
9. Berry G, Newhouse ML, Antonis P. Combined effect of asbestos and smoking on mortality from lung cancer and mesothelioma in factory workers. *Br J Ind Med*. 1985;42:12–18.
10. Selikoff IJ, Seidman H, Hammond EC. Mortality effects of cigarette smoking among amosite asbestos factory workers. *J Natl Cancer Inst*. 1980;65:507–513.
11. Acheson ED, Gardner MJ, Winter PD, et al. Cancer in a factory using amosite asbestos. *Int J Epidemiol*. 1984;13:3–10.
12. Hilt B, Langard S, Andersen A, et al. Asbestos exposure, smoking habits, and cancer incidence among production and maintenance workers in an electrochemical plant. *Am J Ind Med*. 1985;8:565–577.
13. Cheng WN, Kong J. A retrospective mortality cohort study of chrysotile asbestos products workers in Tianjin 1972–1987. *Environ Res*. 1992;59:271–278.
14. Martischnig KM, Newell DJ, Barnsley WC, et al. Unsuspected exposure to asbestos and bronchogenic carcinoma. *BMJ*. 1977;1:746–749.
15. Blot WJ, Harrington JM, Toledo A, et al. Lung cancer after employment in shipyards during World War II. *N Engl J Med*. 1978;299:620–624.
16. Blot WJ, Morris LE, Stroube R, et al. Lung and laryngeal cancers in relation to shipyard employment in coastal Virginia. *J Natl Cancer Inst*. 1980;65:571–575.
17. Pastorino U, Berrino F, Gervasio A, et al. Proportion of lung cancers due to occupational exposure. *Int J Cancer*. 1984;33: 231–237.
18. Kjuus H, Skjaerven R, Langard S, et al. A case-referent study of lung cancer, occupational exposures and smoking. II. Role of asbestos exposure. *Scand J Work Environ Health*. 1986;12:203–209.
19. Saracci R. The interactions of tobacco smoking and other agents in cancer etiology. *Epidemiol Rev*. 1987;9:175–193.
20. Vainio H, Boffetta P. Mechanisms of the combined effect of asbestos and smoking in the etiology of lung cancer. *Scand J Work Environ Health*. 1994;20:235–242.
21. Erren TC, Jacobsen M, Piekarski C. Synergy between asbestos and smoking on lung cancer risks. *Epidemiology*. 1999; 10:405–411.
22. Lee PN. Relation between exposure to asbestos and smoking jointly and the risk of lung cancer. *Occup Environ Med*. 2001;58:145–153.
23. Liddell FD. The interaction of asbestos and smoking in lung cancer. *Ann Occup Hyg*. 2001;45:341–356.
24. Witschi HR, Last JA. Toxic responses of the respiratory system. In: Klaassen CD, ed. *Casarett and Doull's Toxicology: The Basic Science of Poisons*. New York: McGraw-Hill Health Professions Division; 1996:443–462.
25. Begin R. Asbestos-related lung diseases. In: Banks DE, Parker JE, eds. *Occupational Lung Disease*. Chapman & Hall; 1998:219–238.
26. Kimizuka G, Azuma M, Ishibashi M, et al. Co-carcinogenic effect of chrysotile and amosite asbestos with benzo-(a)pyrene in the lung of hamsters. *Acta Pathol Jpn*. 1993;43:149–153.
27. Jackson JH, Schraufstatter IU, Hyslop PA, et al. Role of oxidants in DNA damage. Hydroxyl radical mediates the synergistic DNA damaging effects of asbestos and cigarette smoke. *J Clin Invest*. 1987;80:1090–1095.
28. Jung M, Davis WP, Taatjes DJ, et al. Asbestos and cigarette smoke cause increased DNA strand breaks and necrosis in bronchiolar epithelial cells in vivo. *Free Radic Biol Med*. 2000;28:1295–1299.
29. Robinson BH. The role of manganese superoxide dismutase in health and disease. *J Inherit Metab Dis*. 1998;21:598–603.
30. Wang LI, Miller DP, Sai Y, et al. Manganese superoxide dismutase alanine-to-valine polymorphism at codon 16 and lung cancer risk. *J Natl Cancer Inst*. 2001;93:1818–1821.
31. Shimoda-Matsubayashi S, Matsumine H, Kobayashi T, et al. Structural dimorphism in the mitochondrial targeting sequence in the human manganese superoxide dismutase gene. *Biochem Biophys Res Commun*. 1996;226:561–565.
32. Hiroi S, Harada H, Nishi H, et al. Polymorphisms in the SOD2 and HLA-DRB1 genes are associated with nonfamilial idiopathic dilated cardiomyopathy in Japanese. *Biochem Biophys Res Commun*. 1999;261:332–339.
33. Mossman BT, Marsh JP, Shatos MA. Alteration of superoxide dismutase activity in tracheal epithelial cells by asbestos and inhibition of cytotoxicity by antioxidants. *Lab Invest*. 1986;54:204–212.
34. Mossman BT, Surinrut P, Brinton BT, et al. Transfection of a manganese-containing superoxide dismutase gene into hamster tracheal epithelial cells ameliorates asbestos-mediated cytotoxicity. *Free Radic Biol Med*. 1996;21:125–131.
35. Janssen YM, Marsh JP, Driscoll KE, et al. Increased expression of manganese-containing superoxide dismutase in rat lungs after inhalation of inflammatory and fibrogenic minerals. *Free Radic Biol Med*. 1994;16:315–322.

36. Holley JA, Janssen YM, Mossman BT, et al. Increased manganese superoxide dismutase protein in type II epithelial cells of rat lungs after inhalation of crocidolite asbestos or cristobalite silica. *Am J Pathol*. 1992;141:475–485.
37. Stucker I, Boffetta P, Antilla S, et al. Lack of interaction between asbestos exposure and glutathione S-transferase M1 and T1 genotypes in lung carcinogenesis. *Cancer Epidemiol Biomarkers Prev*. 2001;10:1253–1258.
38. London SJ, Daly AK, Cooper J, et al. Polymorphism of glutathione S-transferase M1 and lung cancer risk among African-Americans and Caucasians in Los Angeles County, California. *J Natl Cancer Inst*. 1995;87:1246–1253.
39. Caporaso N, Hayes RB, Dosemeci M, et al. Lung cancer risk, occupational exposure, and the debrisoquine metabolic phenotype. *Cancer Res*. 1989;49:3675–3679.
40. Schabath MB, Spitz MR, Delclos GL, et al. Association between asbestos exposure, cigarette smoking, myeloperoxidase (MPO) genotypes, and lung cancer risk. *Am J Ind Med*. 2002;42:29–37.
41. Hirvonen A, Tuimala J, Ollikainen T, et al. Manganese superoxide dismutase genotypes and asbestos-associated pulmonary disorders. *Cancer Lett*. 2002;178:71–74.
42. Ferris BG. Epidemiology Standardization Project (American Thoracic Society). *Am Rev Respir Dis*. 1978;118:1–120.
43. Schaeffner ES, Miller DP, Wain JC, et al. Use of an asbestos exposure score and the presence of pleural and parenchymal abnormalities in a lung cancer case series. *Int J Occup Environ Health*. 2001;7:14–18.
44. Sprince NL, Oliver LC, McLoud TC, et al. Asbestos exposure and asbestos-related pleural and parenchymal disease. Associations with immune imbalance. *Am Rev Respir Dis*. 1991;143:822–828.
45. Zhou W, Thurston SW, Liu G, et al. The interaction between microsomal epoxide hydrolase polymorphisms and cumulative cigarette smoking in different histological subtypes of lung cancer. *Cancer Epidemiol Biomarkers Prev*. 2001;10:461–466.
46. Garcia-Closas M, Thompson WD, Robins JM. Differential misclassification and the assessment of gene–environment interactions in case-control studies. *Am J Epidemiol*. 1998;147:426–433.
47. Jacobson MD. Reactive oxygen species and programmed cell death. *Trends Biochem Sci*. 1996;21:83–86.
48. Pani G, Bedogni B, Anzevino R, et al. Deregulated manganese superoxide dismutase expression and resistance to oxidative injury in p53-deficient cells. *Cancer Res*. 2000;60:4654–4660.
49. Garcia-Closas M, Lubin JH. Power and sample size calculations in case-control studies of gene-environment interactions: comments on different approaches. *Am J Epidemiol*. 1999;149:689–692.

APPENDIX I.

List of Specific Asbestos-Related Industries, Jobs, or Activities and the Corresponding Intensity Factor Used in Calculation of Asbestos Scores

Industries	Intensity Factor
Construction	5
Job or activities	
Boilermaking	5
Building maintenance	4
Carpentry	4
Demolition of buildings	4
Drywall hanging	4
Foundry work	5
Insulation installation	6
Iron/steel manufacturing	5
Pipefitting	5
Pipe covering/insulating	6
Shipbuilding/repair	6
Smelting	4
Tunnel construction	4
Welding	4