Inherited Glutathione-*S*-Transferase Deficiency Is a Risk Factor for Pulmonary Asbestosis[1]

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Abstract

Pulmonary diseases attributable to asbestos exposure constitute a significant public health burden, yet few studies have investigated potential genetic determinants of susceptibility to asbestos-related diseases. The glutathione-*S*-transferases are a family of conjugating enzymes that both catalyze the detoxification of a variety of potentially cytotoxic electrophilic agents and act in the generation of sulfadipeptide leukotriene inflammatory mediators. The gene encoding glutathione-*S*-transferase class μ (GSTM-1) is polymorphic; approximately 50% of Caucasian individuals have a homozygous deletion of this gene and do not produce functional enzyme. Glutathione-*S*-transferase μ (GST-μ) deficiency has been previously reported to be associated with smoking-induced lung cancer. We conducted a cross-sectional study to examine the prevalence of the homozygous deletion for the *GSTM-1* gene in members of the carpentry trade occupationally exposed to asbestos. Members of the United Brotherhood of Carpenters and Joiners of America attending their 1991 National Union conference were invited to participate. Each participant was offered a chest X-ray and was asked to complete a comprehensive questionnaire and have their blood drawn. All radiographs were assessed for the presence of pneumoconiosis in a blinded fashion by a National Institute for Occupational Safety and Health-certified International Labor Office "B" reader. Individual GSTM-1 status was determined using polymerase chain reaction methods. Six hundred fifty-eight workers were studied. Of these, 80 (12.2%) had X-ray abnormalities associated with asbestos exposure. Individuals genetically deficient in GST-μ were significantly more likely to have radiographic evidence of nonmalignant asbestos-related disease than those who were not deficient ($\chi^2 = 5.0$; $P < 0.03$). Logistic regression analysis showed that susceptibility was most strongly associated with individuals with radiographic evidence of parenchymal disease (odds ratio, 2.1; $P < 0.05$). Further, no significant association between GSTM-1 deletion and X-ray abnormalities was noted in current smokers, while ex-smokers and never-smokers with the deleted genotype were significantly more likely to have radiographic evidence of asbestos-induced parenchymal fibrosis ($\chi^2 = 6.5$; $P < 0.01$). Inherited GST-μ deficiency appears to contribute to individual susceptibility to asbestos-induced pulmonary disease. This may be due to a reduced ability of GST-μ deficient cells in the lung to detoxify reactive electrophiles associated with asbestos exposure. Alternatively, an altered inflammatory response to asbestos fibers resident in the lung may be present in GST-μ-deficient individuals that results in more pronounced pulmonary fibrogenesis.

Introduction

Asbestos-related diseases, including lung cancer, mesothelioma, interstitial fibrosis, and pleural fibrosis, are a significant public health problem. As many as 14 million people in the United States have been exposed to asbestos (1) and as many as 100,000 deaths may ultimately be attributable to diseases associated with this exposure (2, 3). Many more will experience significant functional impairment due to past exposures to asbestos fibers.

While asbestos-related disorders are among the most well studied occupational diseases, relatively little is known about host factors that may affect individual susceptibility to these conditions. Although a significant health risk, exposure to even large amounts of asbestos does not invariably lead to discernible disease. Conversely, some individuals develop serious pulmonary disease following modest asbestos exposure. As is the case with many toxic agents, host factors are important in the development of the pulmonary disorders caused by this agent. A better understanding of the factors that determine these differences may lead to improved screening programs for identifying individuals at risk of developing asbestos-related diseases and shed light on the mechanisms of asbestos toxicity.

The biological mechanism by which asbestos leads to pulmonary fibrosis is the subject of considerable ongoing research. Current hypotheses implicate the production of oxygen-free radicals with the induction of inflammatory mediators in the pathogenesis of this disease (4–7). Oxygen-free radicals may be produced as a result of the ability of asbestos to catalyze a Fenton-type reaction in the lung (8–10), leading to the production of cytotoxic and poten-

Received 3/3/94; revised 5/12/94; accepted 5/12/94.
[1] Supported in part by Public Health Service Grants ES-00002, CA-09078, K01-OH-00110, P42-ES04705 and PO1-ES06409.
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tially genotoxic electrophilic compounds. Pulmonary deposition of asbestos fibers is also known to induce the production of cytokines by activated alveolar macrophages and other immune response cells (5–7, 11, 12). Thus, pulmonary asbestosis is the end result of a specific pattern of cellular inflammatory response that induces cell death and subsequent fibrogenesis.

In addition to their roles in the detoxification of a variety of metabolically activated organic compounds, including polyaromatic hydrocarbons, free radical-associated electrophiles produced *in vivo* may also be substrates for glutathione-mediated detoxification reactions (13, 14). Further, the glutathione transferases are also thought to be involved in the 5-lipoxygenase pathway of arachidonic acid metabolism and, thereby, to participate in the mediation of the inflammatory response through the production of leukotrienes (15). One GST[4] isozyme, GST-μ, is known to be polymorphic in humans (16). GST-μ is deficient in a high percentage of Caucasians; this deficiency has previously been reported to be associated with increased susceptibility to smoking-induced lung cancers as well as smoking-related bladder cancer (17–24). Here, we report our results of an investigation done to determine whether glutathione-*S*-transferase class μ activity is associated with asbestos-induced nonmalignant lung disease.

Materials and Methods

Study Population. The study group consisted of members of the United Brotherhood of Carpenters and Joiners of America attending the October, 1991 Union Conference in Atlantic City, NJ. At this meeting, union members were offered the opportunity to participate in a health-screening effort organized by the Mount Sinai School of Medicine in collaboration with the union. Study participation was voluntary. All participants were asked to complete a detailed questionnaire covering medical, occupational, and smoking histories. The questionnaire was a modified version of that derived from the American Thoracic Society and included standardized questions on current and past cigarette smoking habits (25). Participants received chest X-rays and were asked to provide blood samples for additional medical analysis and genotyping. Blood samples were collected into heparinized Vacutainer tubes, dispensed into coded 1.5-ml polypropylene tubes, and stored at −20°C until analysis.

Diagnostic Protocol and Criteria. Asbestos-induced disease status was evaluated by physicians with specialties in occupational and pulmonary medicine. Primary disease classifications included asbestos-induced pleural disease and asbestosis, the later indicating the presence of irregular interstitial opacities on chest X-ray. All diagnoses of asbestos-related conditions were based exclusively on chest radiography. All radiographs were examined by a National Institute for Occupational Safety and Health-certified "B" reader blinded to GST-μ status, job classification, and smoking status. Criteria for the diagnosis of asbestosis in this study included only the presence of interstitial changes on chest radiography; using the 1980 revised ILO radiographic classification of pneumoconioses (26), this included radiographs rated 1/0 or higher. Criteria for the diagnosis of asbestos-related pleural disease were again those of the ILO classification of chest radiographs.

Asbestos Exposure. As no air or personal sampling data relating to the asbestos exposures of the study group were available, it was not possible to construct quantitative estimates of asbestos exposures. Participants, however, were asked about their employment in the carpentry trade, including the age at which they began in the trade and the total number of years that they were employed both as a carpenter and as a union member. Information concerning prior employment in seven job classifications involving potentially significant exposure to asbestos was also collected. These included employment in shipyards, in the construction industry, in building or industrial maintenance, as an asbestos insulator, in a powerhouse, or in brake repair. A history of employment in these jobs prior to work as a carpenter was also included as a surrogate indicator of asbestos exposure in subsequent analyses. Workers were not asked whether they had previously been diagnosed with asbesto-related diseases.

GST-μ PCR-based Assay. A PCR-based assay has been developed that allows for the rapid detection of GSTM-1. The assay used here is a modification of that described by Comstock *et al.* (27). Briefly, primers hybridizing to the 5′ region of exon 4 (5′-CTGCCCTACTTGATTGATGGG-3′) and the 3′ region of exon 5 (5′-CTGGATTGTAGCAGATCATGC-3′) of GSTM-1 were used to amplify a 273-base pair fragment of GSTM-1. PCR analysis of DNA from individuals carrying the homozygous deletion for the *GSTM-1* gene yields no amplified product.

Amplifications were performed using 0.5–1 μl of whole blood. Reaction mixtures were preincubated for 3 cycles of 3 min at 55°C and 3 min at 94°C. Taq DNA polymerase (2.5 units; Perkin Elmer Cetus) was then added and the reactions were heated to 94°C for 1 min, cooled to 60°C for 1 min, and heated to 72°C for 2 min for 40 cycles. Amplifications were performed on an Ericomp Thermal Cycler. PCR products were electrophoresed through 2% agarose and the diagnostic 273-base pair fragment was visualized via ethidium bromide staining. This method does not distinguish heterozygous deleted individuals from homozygous nondeleted individuals.

Controls for the PCR method consist of known GST-μ-positive and GST-μ-negative individuals (previously determined by enzymatic analysis), and blanks were included in each run. Control primers that amplify a 493-base pair fragment of human actin (indicative of the presence of the light chain coding sequence) were also included in each reaction as an internal standard (28). The actin primers hybridize to the 5′ region of base pairs 1612–1632 (5′-GGGCACGAAGGCTCATCATT-3′) and the 3′ region of base pairs 1139–1159 (5′-GGCCCCTCCATCGTCCACCG-3′). Amplification of the ubiquitous actin gene serves to confirm the presence of amplifiable DNA in the sample.

Statistical Analysis. GSTM-1 genotype was analyzed as a dichotomous variable; individuals who were heterozygous or homozygous for the presence of the gene were considered "GSTM-1 positive" while individuals who were homozygous deleted were considered "GSTM-1 null". Data were analyzed using the χ^2 test to compare the proportions of the GSTM-1 null genotype between groups of workers with asbestos-related radiographic abnormalities and workers without discernible chest X-ray changes. Multivariate

[4] The abbreviations used are: GST, glutathione-*S*-transferase; GST-μ, glutathione-*S*-transferase μ; GSTM-1, glutathione-*S*-transferase class μ; ILO, International Labor Office; PCR, polymerase chain reaction; CI, confidence interval; LT, leukotriene.

Table 1 Demographic characteristics and occupational history of the study population

	Total study population	Distribution by genotype	
		GSTM-1 null	GSTM-1 positive
No. of subjects (%)	658	351 (53.3)	307 (46.7)
Age (mean ± SD)	49.4 ± 8.8	49.3 ± 8.7	49.5 ± 8.9
Cigarette smoking			
Current (%)	157 (23.8)	78 (49.7)	79 (50.3)
Former	304 (46.2)	162 (52.7)	142 (46.3)
Years of nonsmoking (mean ± SD)	15.9 ± 10.1	16.6 ± 9.9	14.9 ± 9.7
Never	197 (30.0)	111 (56.3)	86 (43.7)
Prior employment			
Insulation (%)	328 (49.9)	169 (48.1)	159 (51.8)
Construction	229 (34.8)	120 (34.2)	109 (35.5)
Power plant	190 (28.8)	93 (26.5)	97 (31.6)
Shipyard	47 (7.1)	24 (6.8)	23 (7.5)
Building maintenance	183 (27.8)	101 (28.8)	82 (26.7)
Industrial maintenance	175 (26.6)	91 (25.9)	84 (27.4)
Brake work	22 (3.3)	13 (3.7)	9 (2.9)
Years since beginning in the trade (mean ± SD)	26.1 ± 9.2	26.1 ± 8.9	26.1 ± 9.5
Race			
Caucasian	622 (94.5)	339 (54.5)	283 (45.5)
African-American	15 (2.3)	2 (13.3)	13 (86.7)
Hispanic	21 (3.2)	10 (47.6)	11 (52.4)

analysis included logistic regression that modeled the relationship between radiographic evidence of asbestos-related disease and genotype adjusting for the effects of other variables potentially related to the development of asbestos-related disease. These included smoking status, age, tenure in the trade, and occupational history prior to entry into the carpentry trade.

Results

Study Population. A demographic summary of the study population is presented in Table 1. A total of 757 individuals participated in the health-screening effort. Chest X-rays were refused by 29 workers and blood samples were not provided by 69 workers. Genotype was not determinable in one subject. Thus, a total of 658 workers were included in this analysis. Within this group, 80 (12.2%) workers had radiographic evidence of asbestos-related disease. These included 23 with pleural disease only, 31 with both pleural and parenchymal changes on chest X-ray, and 26 who had solely parenchymal radiographic findings. The remaining 568 members of the cohort (87.8%) had no discernible asbestos-related X-ray abnormalities. The cohort ranged in age from 24 to 71, with a mean age of 49.4 ± 8.8 (SD) years. There were 157 current cigarette smokers, 304 ex-smokers, and 197 workers who reported never-smoking cigarettes. Mean consumption of cigarettes among current smokers was 38.9 ± 22.1 pack-years. The ex-smokers reported a mean of 24.3 ± 21.5 pack-years of smoking.

GSTM-1 Status and Asbestos-induced Radiographic Abnormalities. Overall, 351 of the 658 workers (53.3%) studied were deleted for GSTM-1 (the null genotype), a frequency approximately equal to that observed in populations previously studied (17–24). In the small number of African Americans studied, the frequency of the null genotype appeared to be lower than that in Caucasians, consistent with previous reports (24). Among those with X-ray evidence of asbestos-related pulmonary disease, 52 (65%) were found to be GST-μ negative (Table 2). This prevalence of the null genotype was significantly different from that observed in workers without radiographic evidence of asbestos-related disease ($\chi^2 = 5.0$; $P < 0.03$). Thus, the GST-μ-negative genotype was overrepresented among individuals with X-ray abnormalities within this cohort.

In order to ensure that the higher prevalence of asbestos-induced X-ray abnormalities in the workers with the GSTM-1 null genotype was not a result of systematic differences in the asbestos exposure, we examined surrogate measures of exposure by genotype. There were no significant differences in age, time since initial employment in the trade, smoking habits, or other occupational exposure to asbestos in groups of workers with different genotypes (Table 1).

To investigate whether cigarette smoking affected the observed association of the GSTM-1 trait with radiographic abnormalities, the data was stratified into current smokers, ex-smokers, and nonsmokers and the prevalence of the GSTM-1 genotypes in each group was examined. In the current smokers there was no significant association between the GSTM-1-deleted genotype and radiographic lung abnormalities (Table 2). However, in the nonsmoking carpenters the prevalence of the GSTM-1 deletion in workers with X-ray changes was significantly different than that in the workers without disease (Table 2). In the ex-smokers alone, the excess was of borderline significance ($\chi^2 = 3.6$; $P = 0.06$), and the calculated odds ratios for ex-smokers and never-smokers were twice that for the current smokers (Table 2). When the data from the ex-smokers and never-smokers were combined, the prevalence of the GSTM-1-deleted genotype was significantly greater than expected ($\chi^2 = 6.5$; $P < 0.01$) and the calculated odds ratio was 2.4.

Multivariate Analysis. In order to investigate the potential effects of other variables likely to be associated with the development of asbestos-induced X-ray abnormalities, multivariate logistic models were constructed. In these models radiographic end points were the dependent variables and genotype, age, race, time since beginning in the carpentry trade, smoking history, and a history of work in any of the previously identified non-carpentry job classifications potentially involving asbestos exposures were entered as independent variables.

When all ILO-rated lung abnormalities were used as the dependent variable in this analysis, the null GSTM-1 genotype was twice as frequent in workers with asbestos-induced X-ray abnormalities than in those without ($P < 0.009$; Table 3). This analysis was conducted stepwise to determine which of the surrogate exposure variables might significantly contribute to the prevalence of X-ray abnormalities from asbestos exposure. The following contributed significantly to the prevalence of ILO-rated abnormalities: smoking ($P < 0.005$); time since beginning in the carpentry trade ($P < 0.0001$); and prior employment in the construction trades ($P < 0.05$). Prior employment in a power plant was borderline significant in its association with all asbestos-related X-ray changes ($P < 0.07$). Controlling for these factors, the odds ratio for the null genotye was similar to that calculated from the unadjusted data at 2.0, with a 95% confidence interval of 1.2–3.3. This point estimate of the odds ratio exceeded that for the other significant measures of asbestos exposure (time since beginning in the trade and

Table 2 Chest X-ray abnormalities according to the ILO system in union carpenters stratified by GSTM-1 genotype and smoking status

	All Subjects		Current Smokers		Ex- and Never-Smokers	
	Present	Absent	Present	Absent	Present	Absent
GSTM-1 Null	52	299	17	61	35	238
GSTM-1 Positive	28	279	15	64	13	215
	$\chi^2 = 5.0$, $P = 0.03$		$\chi^2 = 0.1$, $P = 0.8$		$\chi^2 = 6.5$, $P = 0.01$	
	Odds ratio = 1.8		Odds ratio = 1.2		Odds ratio = 2.4	

Table 3 Results of stepwise logistic regression analysis for significant predictors of asbestos-induced X-ray abnormalities[a]

Variables	Odds ratio	*P* value
Pleural and parenchymal disease		
GSTM-1 null	2.0	0.009
Smoking	1.9	0.005
Construction work	1.7	0.05
Tenure in the trade	1.1	0.0001
Pleural disease only		
Tenure in the trade	1.1	0.0001
Parenchymal disease only		
GSTM-1 null	2.1	0.02
Construction work	2.0	0.02
Smoking	2.0	0.007

[a] Additional variables considered in the model included pack-years of smoking, insulation work, power plant work, shipyard work, building and construction maintenance, and brake work.

prior work in construction), as well as being larger than the point estimate for the effect of smoking (Table 2).

When the stepwise logistic regression analysis was done by smoking status, the results were similar to those observed in examining the unadjusted data. In current smokers alone, all asbestos-associated X-ray findings were significantly associated only with tenure in the carpentry trade (odds ratio, 1.07; 95% CI, 1.01–1.13). In ex-smokers, logistic analysis showed both years worked as a carpenter (odds ratio, 1.07; 95% CI, 1.02–1.12), and genotype (odds ratio, 2.3; 95% CI, 1.0–5.5) to be significantly associated with X-ray abnormalities. In the never-smokers, only genotype was significantly associated with abnormalities on X-ray (odds ratio, 3.6; 95% CI, 1.0–12.7). When the data was modeled using age at initiation of smoking, no change in the significant findings was observed. Previous employment involving brake repair ($P = 0.06$), years as a carpenter ($P = 0.14$), and previous work in a power plant ($P = 0.11$) were not significantly associated with radiographic chest abnormalities in the never-smokers. As was seen in the unadjusted analysis, the point estimate for the odds ratio was largest for the never-smokers.

The ILO system for classifying radiographic changes attributable to asbestos exposure includes distinct categories for parenchymal abnormalities (interstitial lung disease commonly termed asbestosis) and pleural changes (pleural fibrosis characterized by hyaline plaques in the parietal pleura). Since the mechanism responsible for the generation of these distinct pathological entities may differ, we examined the association between the GSTM-1 null genotype and pleural and parenchymal disease separately using logistic models. When logistic analysis was restricted to the 31 individuals with pleural abnormalities, only tenure in the carpentry trade was significantly associated with the presence of X-ray findings (Table 3; odds ratio, 1.11; $P = 0.0001$). In this analysis, genotype, cigarette smoking, and the previous job history variables were not associated with the presence of pleural changes on X-ray. When this analysis was restricted to the 23 workers who had only pleural findings on chest X-ray, again only tenure in the trade was associated with an abnormal radiograph ($P = 0.001$).

Interestingly, when logistic analysis was done for the 57 individuals with parenchymal findings, genotype was again the most predictive variable significantly associated with positive X-ray changes (Table 3). Genotype, smoking status, and previous employment in the construction trades were significantly associated with parenchymal findings. The odds ratio for genotype was 2.1 (95% CI, 1.1–3.7), for a history of construction work was 2.0 (95% CI, 1.1–3.6), and for smoking was 2.0 (95% CI, 1.2–3.3).

Discussion

We have shown that union carpenters who carry the homozygous deletion for the *GSTM-1* gene are significantly more likely to have radiographic evidence of asbestos disease. This association is not likely the result of systematic differences in asbestos exposure, since job history prior to joining the union, time since beginning in the trade, and age and race were similar among genotypes.

When the effect of cigarette smoking was examined, we noted that the GSTM-1 trait was strongly associated with asbestos-related radiographic findings in the workers who did not smoke. In the current smokers the GSTM-1 null genotype was not associated with asbestos-related X-ray changes, while the point estimate for the adjusted odds ratio was significantly elevated in the ex-smokers and was highest overall in the never-smokers. This finding suggests that the *GSTM-1* gene deletion exerts its effects specifically in the production of radiographically detectable asbestos-induced disease rather than in the production of cigarette smoke associated X-ray changes. Radiographically, detectable small irregular opacities are found with greater frequency in current smokers who are exposed to asbestos (29–35). At autopsy, however, smoking was not found to be associated or interactive with exposure in producing asbestosis (36). Hence, the underlying processes responsible for the generation of irregular opacities on X-ray by smoking and asbestos may not be pathologically identical. The observation that the absence of the gene was most important in the never-smokers suggests that the trait does not affect the induction of opacities associated with current smoking. The absence of an association in the smokers may, then, be due to the misclassification of asbestos-induced disease; smoking-induced small opacities are misinterpreted as asbestos-related abnormalities in current smokers, but because the GSTM-1 gene deletion does not affect these lesions, no overall association is observed.

As we have noted, radiographic changes induced by asbestos include both parenchymal and pleural fibrosis. We

found that the GSTM-1 deletion was significantly associated only with parenchymal X-ray changes and not X-ray evidence of pleural disease. This suggests that this trait confers susceptibility to interstitial disease via a mechanism that is less significant or not operative in the generation of asbestos-induced pleural changes. While the precise mechanism responsible for each of these radiographic lesions is not known, substantial research has demonstrated that there are important pathological differences in these conditions.

The pleural changes consist chiefly of fibrotic hyaline membranes that can occur throughout the chest. Asbestos-induced pleural fibrosis is thought to be a submesothelial process that occurs through an interaction of the lymphatic system with asbestos fibers (36, 37). Histologically, adhesions and mesothelial cell proliferation are uniformly absent in asbestos-induced pleural fibrosis (37). Pleural changes are most commonly observed asymmetrically on the diaphragm and at the costal margins (38). The mechanism of pleural plaque formation and their peculiar localization to to the parietal rather than the visceral pleura is poorly understood. For example, it is not known whether there are specific types or dimensions of fibers that can preferentially gain access to the pleural compartment (39). In addition, unlike the generation of interstitial fibrosis, pleural fibrosis occurs as a relatively acellular process, typically lacking histological evidence of an active cell-mediated inflammatory response (6, 40). In contrast, parenchymal disease induced by asbestos exposure (asbestosis) is currently believed to be induced by the asbestos fiber-catalyzed generation of oxygen-free radicals with the concomitant induction of cytokine-mediated inflammation and, ultimately, the production of potentially fatal pulmonary fibrosis (5–7).

In light of this, several mechanisms that might underlie the association of GST-μ deficiency and pulmonary asbestosis should be considered. As noted, the iron content of asbestos fibers has been demonstrated to catalyze the generation of oxygen radicals via a Fenton-type reaction, resulting in the generation of lipid peroxidation products (5–7). The glutathione-*S*-transferases, including the μ class, are known to be capable of mitigating the cytotoxic effects of oxidative lipid-associated free radicals, including those associated with mineral fiber exposure (41, 42). Thus, genetic deficiency in GST-μ may lead to an increase in oxidative tissue damage by fiber-induced radicals that are substrates for this isoenzyme.

Another possible mechanism that could account for our observations arises from the observation that glutathione transferases, and in particular the μ isoform, are thought to be involved in the 5-lipoxygenase pathway of arachidonic acid metabolism (43–46). Sulfadipeptide LTs, (LTC4, LTD4, and LTE4) are formed through conjugation with glutathione. Peptidyl LTs are potent mediators of inflammatory responses within the lung; these agents increase vascular permeability, mucosal edema, and bronchoconstriction. At the step involving glutathione conjugation the leukotriene pathway diverges into the synthesis of another inflammatory mediator, LTB4, a powerful chemotactic agent (47). Although the peptidyl leukotrienes have been associated with acute pulmonary inflammation (*e.g.*, bronchial asthma), LTB4 has been implicated more directly in pulmonary fibrosis in both animal models and studies of occupationally exposed workers. Fibrogenic mineral fibers stimulate the production of LTB4 by pulmonary macrophages, which in turn regulate other cytokines such as tumor necrosis factor (48). Alveolar inflammatory cells from asbestos-exposed workers with asbestosis produce increased amounts of LTB4 compared to workers without asbestosis or smoking controls whose cells actually produce lower levels than nonsmoking controls (49). LTB4 is also known to induce suppressor T-cells (50); this may be one reason for the increase in their number that has been found in the lung in experimental models of asbestosis (51). Given that the pathways for both peptidyl LTs and LTB4 include a common precursor (*i.e.*, LTA4), it is possible that genetic deficiency in GST-μ may lead to a shift in the balance of the two pathways towards increased LTB4 production and its associated profibrogenic effects. Shunting from one pathway in lipoxygenase metabolism to another has been described previously (15). In addition, in the guinea pig, which is a well known animal model of asbestosis, a deficiency in glutathione-*S*-transferase is associated with increased LTB4 production and undetectable levels of LTC4, LTD4, and LTE4 (52).

Our findings may also have implications for the interpretation of the previously reported association between the GSTM-1 null genotype and smoking-induced lung cancer. This association has been proposed to be mediated by enhanced genetic toxicity in GST-μ-deficient individuals (53). There have been numerous groups that have sought to replicate the original report of Seidegard *et al.* (17), showing an approximate 2-fold elevation in the risk of lung cancer in smokers who had the GSTM-1 null genotype. These studies have produced conflicting results (17–23). Importantly, none of these studies have controlled for exposure to asbestos. Our results indicate that the association between the GSTM-1 null genotype and susceptibility to smoking-induced lung cancer may be confounded by a history of exposure to asbestos or the presence of asbestosis. It is also conceivable that the *GSTM-1* gene deletion could be involved in the production of the known synergy between asbestos exposure and smoking in the genesis of lung cancer. Previous epidemiological studies of associations between polymorphisms in cytochrome P-450 enzymes and lung cancer have demonstrated interactions with asbestos exposure (54). However, without any knowledge of asbestos exposure or disease status in these studies, this issue cannot be addressed.

Several recent studies illustrate the possible importance of asbestos-induced disease in the studies of the GSTM-1 trait and lung cancer. In cement workers an excess of lung cancer was observed only among those workers with radiographic opacities, even after controlling for exposure (55). A Finnish study also recently showed that approximately one-third of individuals with lung cancer in that country have pathologically confirmed evidence of significant asbestos exposure (56). Consequently, studies of an association between the GSTM-1 deletion and lung cancer must control for asbestos exposure and asbestos-induced pulmonary disease in order to fully evaluate the association between this trait and smoking-induced malignancy.

Finally, it has been shown that the relative risk of acquiring an exposure-related disease that is mediated by a genetic trait is a function of the frequency of exposure, the strength of the interaction between exposure and the trait, and the specificity of the cellular effects of exposure in the presence and absence of the trait (57). Elevated risks associated with the trait imply a frequent environmental exposure or a strong pattern of interaction. In this study, we

attempted to control for exposure using years in the trade and previous occupation as surrogate measures. However, cumulative asbestos exposure is difficult to estimate. We found that 12% of the carpenters had asbestos-related X-ray changes. Previous work is consistent with this, showing that carpenters have a relatively low prevalence (approximately 11% of those screened) of asbestos-induced X-ray changes (58). The prevalence of X-ray findings has been reported to be markedly higher in recent, similar studies of sheet metal workers (47%), ironworkers (38%), and millwrights and machinery erectors (44.5%) (59–61). In addition, we studied carpenters who were all union officials and some may have had primarily administrative positions with minimal contact with asbestos. Therefore, our study group may include workers with a relatively low frequency of exposure to asbestos. This argues that the gene-environment interaction that we have observed is relatively strong and that the relative risk is possibly underestimated.

In summary, the null genotype of GSTM-1 is overrepresented in carpenters who have radiographic evidence of asbestos-related disease. The GST-μ trait may mediate this susceptibility by free radical scavenging or via its participation in the lipoxygenase pathway. Regardless, our work suggests that other inflammatory diseases with characteristic patterns of tissue fibrosis should be investigated for any similar association with the GST-μ trait. In fact, recent reports suggest that the GSTM-1 deletion is associated with alcohol and other exposure-induced fibrosis in the liver (62, 63).

Our results, examined in combination with the knowledge of the high prevalence of this polymorphism, also suggest that the GST-μ status is a significant population risk factor for the development of asbestos-related pulmonary disease. The previously observed relationships between smoking, asbestos exposure, and lung cancer further suggest that GST-μ status needs to be carefully investigated as a potential risk factor for malignant disease in smokers exposed to asbestos. Hence, our results have important implications for public health; however, only if they can be replicated and confirmed by others is there any indication for use of GSTM-1 genotyping in the clinical evaluation of patients with asbestos exposure or asbestos-related diseases.

Acknowledgments

The authors thank the members of the United Brotherhood of Carpenters and Joiners of America for their participation and their interest. We also acknowledge the central role of the Carpenters Health and Safety Fund of North America and, in particular, the role of Edward J. Gorman, III, J.D., in organizing the medical screenings. We also thank Dr. Ruth Lilis for her expert assistance in reading the chest radiographs, Dr. Howard Hu for his help with this health screening, Lucille Pothier for computer programing, and Dr. Richard Monson for helpful suggestions. Finally, we are indebted to Dr. I. J. Selikoff for his original research on asbestos-related diseases and for his assistance in organizing this health screening.

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Inherited glutathione-S-transferase deficiency is a risk factor for pulmonary asbestosis.

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Cancer Epidemiol Biomarkers Prev 1994;3:471-477.