Glutathione S-Transferase and N-Acetyltransferase Genotypes and Asbestos-Associated Pulmonary Disorders

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Background: Humans vary in their ability to metabolize endogenous and exogenous compounds. Glutathione S-transferases (GSTs) and N-acetyltransferases (NATs) are enzymes involved in the detoxification of hazardous agents. The GSTM1 and GSTT1 genes exhibit null (i.e., deletion) polymorphisms; in specific individuals, homozygous deletion (i.e., both copies lost) of these genes can be detected. Polymorphism of the NAT2 gene results in slow and fast acetylators of potentially toxic substances. The GSTM1-null and the NAT2 slow-acetylator genotypes have been associated with increased risks for the development of environmentally induced cancers. Purpose: We assessed whether homozygous GSTM1-null or GSTT1-null genotypes or the NAT2 slow-acetylator genotype were associated with increased risks for the development of malignant and nonmalignant pulmonary disorders in a cohort of Finnish construction workers. Methods: The study population consisted of 145 asbestos insulators who were classified as having been exposed to high levels of asbestos; 69 of these individuals had no pulmonary disorders (control subjects), and 76 had either malignant mesothelioma (n = 24) or nonmalignant pulmonary disorders, such as asbestosis and/or pleural plaques (n = 52). Lymphocyte DNA and the polymerase chain reaction were used to determine the GSTM1, GSTT1, and NAT2 genotypes of the study subjects. Odds ratios (ORs) and 95% confidence intervals (CIs) estimating the relative risks of disease associated with specific genotypes were calculated from 2 × 2 tables by use of Fisher's exact method. Results: Risks for the development of asbestos-related pulmonary disorders were not affected significantly by homozygous deletion of the GSTM1 or GSTT1 genes. However, the risk of developing both malignant and nonmalignant pulmonary disorders for individuals with a NAT2 slow-acetylator genotype was more than twice that observed for those with a NAT2 fast-acetylator genotype (OR = 2.3; 95% CI = 1.1-4.7); the risk of developing malignant mesothelioma for NAT2 slow acetylators was increased almost fourfold (OR = 3.8; 95% CI = 1.2-14.3). Individuals who lacked the GSTM1 gene and possessed a NAT2 slow-acetylator genotype had a risk of developing malignant and nonmalignant pulmonary disorders that was approximately fivefold greater than that observed for those who had the GSTM1 gene and a NAT2 fast-acetylator genotype (OR = 5.1; 95% CI = 1.6-17.6); these individuals had a fourfold increased risk of developing nonmalignant pulmonary disorders (OR = 4.1; 95% CI = 1.1-17.2) and an eightfold increased risk of developing malignant mesothelioma (OR = 7.8; 95% CI = 1.4-78.7) when compared with the same reference group. Conclusions: Individuals with homozygous deletion of the GSTM1 gene and a NAT2 slow-acetylator genotype who are exposed to high levels of asbestos appear to have enhanced susceptibility to asbestos-related pulmonary disorders. [J Natl Cancer Inst 1996;88:1853-6]

Inhalation of asbestos fibers has been shown to cause lung cancer, diffuse interstitial pulmonary fibrosis (asbestosis),

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Notes

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malignant mesothelioma of the pleura and peritoneum, and several other pleural diseases such as pleural plaques (1). Since the causal relationship between asbestos exposure and pulmonary diseases was established, much work has been undertaken to understand the mechanisms of fiber-induced pathogenesis. According to present knowledge, both direct and indirect cellular effects of fibers may contribute to disease outcome. These effects include the generation of reactive free radicals, which result from interactions of fibers and target cells or are generated following the phagocytosis of fibers by inflammatory cells (2). The other factors (e.g., individual susceptibility) that could contribute to the development of asbestos-related diseases have not yet been identified.

Humans vary in their ability to metabolize exogenous and endogenous compounds, and individuals with a genetically determined reduced capacity to detoxify hazardous compounds may be at increased risk of adverse health effects compared with those with unaltered metabolic capacity (3). In this respect, conjugation of electrophilic compounds to glutathione, mediated by glutathione S-transferases (GSTs), may be of great importance (4). The GST M1 and T1 genes are polymorphic in humans, and a phenotypic absence of enzyme activity results from the homozygous deletion of the respective genes (i.e., the null genotypes (5,6). About 50% of Caucasians have the GSTM1-null genotype, which has been suggested in several studies (7-10) to pose an increased risk for the development of environmentally induced cancers. To date, there are no studies on the association between the GSTT1-null genotype, which putatively has a frequency of 10%-25% in Caucasians, and individual cancer risk. However, lack of the GSTT1 gene was recently associated with an enhanced susceptibility to myelodysplastic syndromes, which are clonal proliferative disorders of bone marrow that often progress to acute myeloid leukemia (11). In addition to glutathione conjugation, acetylation by N-acetyltransferase 2 (NAT2) is an important detoxification pathway in humans (3). Consequently, the slow-acetylation-associated NAT2 genotypes, which have a frequency of about 50% among Caucasians, have been suggested to modulate individual risk for the development of, for example, environmentally induced bladder and colon cancers (3).

We previously found an association between the occurrence of malignant mesothelioma and GSTM1 and NAT2 genotypes among workers who were highly exposed to asbestos (10). However, only a population-based control group without occupational exposure data was available, and mesothelioma was the sole asbestos-related pulmonary disorder studied. We therefore substituted the population-based control subjects with a carefully characterized group of workers who had a history of high asbestos exposure but no pulmonary disorders, and we extended the study to cover workers with two other asbestos-caused disorders, asbestosis and pleural plaques. We also assessed the recently described GSTT1 gene polymorphism (6) to clarify further the potential role of GST genes in asbestos-associated pulmonary disorders.

**Subjects and Methods**

**Study Subjects**

The study subjects were selected from a cohort of approximately 1500 construction workers who were recruited for a health-screening survey carried out at the Finnish Institute of Occupational Health. All of the workers were interviewed for detailed occupational and smoking histories by a physician who was specialized in occupational medicine. The degree of asbestos exposure, on the basis of all available data (e.g., personal interview and measurements of lung fiber burden from some of the patients with mesothelioma), was classified as 1) definite or probable, 2) possible, and 3) unlikely/unknown. On the basis of the data, individuals were classified to high, moderate, or low asbestos-exposure groups. A subcohort of asbestos insulators who were classified to the high asbestos-exposure group comprised the present study population consisting of 145 workers, 69 of whom had no pulmonary disorders detectable in thorax x-rays (mean subject age, 52.8 years [standard deviation, 9.3 years]). The distribution of years of known asbestos exposure was as follows: 1-9 years (n = 29); 10-19 years (n = 17); 20-29 years (n = 54); and 30 years or more (n = 45). A minority of the study subjects were never smokers, both among the healthy workers (25 of 76 [33%]) and among the workers with pulmonary disorders (16 of 69 [23%]). The rest were either ex-smokers (23 of 76 [30%] and 33 of 69 [48%], respectively) or current smokers (28 of 76 [37%] and 20 of 69 [29%], respectively), with mean cumulative tobacco smoke doses of 28 (standard deviation, 20) pack-years and 27 (standard deviation, 18) pack-years in the two groups, respectively. The more specific tobacco smoke dose distribution for the study population was as follows: 0 pack-years (n = 41); 1-19 pack-years (n = 36); 20-39 pack-years (n = 48); and 40 or more pack-years (n = 20).

All of the patients with mesothelioma had been admitted to the Department of Pulmonary Medicine at the Helsinki University Central Hospital between 1985 and 1993 and were subsequently diagnosed as having malignant mesothelioma. The tumors were classified as having epithelial, mixed, or fibromatous histology. The diagnoses were confirmed by both the Finnish National Mesothelioma Panel and the European Organization for Research and Treatment of Cancer Mesothelioma Panel. The diagnoses of asbestosis and pleural plaques were determined first by conventional chest x-rays, using the International Labour Office classification of Radiographs of Pneumoconiosis. The diagnoses of asbestosis were subsequently confirmed by the Finnish National Panel of Pneumoconiosis by use of high-resolution central tomography.

**Determination of Genetic Polymorphisms**

Genomic DNA was extracted from lymphocytes by the use of standard techniques. A multiplex polymerase chain reaction (PCR) method was used to detect the presence or absence of the GSTM1 and GSTT1 genes as described by Chen et al. (11). This modified PCR method (5,6,9,12) had both GST-specific primer pairs in the same amplification mixture and included a third primer pair for β-globin. The PCR mixture was composed of 100 ng genomic DNA template, 30 pmol of each GST primer (GS5'-GAA CTC CCT GAA AAG CTA AAG C3' and GS6-GTT GGG CTC AAA TAT ACG GTG G3'), for amplifying GSTM1; gatf-5'TTC CTG CAC ATC TC3' and gatR-5'TCA CCG ACG CAG CAG CAC CAC CAC TCC3' for amplifying GSTT1 [primers synthesized by the Institute of Biotechnology, Helsinki, Finland], 10 pmol β-globin gene primers (BGI-5'GCA CTT CCT CCA GTG TCA CC3' and BG2-5'GAA GAG CCA AGG ACA GGT AC3' [primers synthesized by the Institute of Biotechnology]), 200 μmol deoxynucleoside triphosphates (dNTPs) (Boehringer Mannheim GmbH, Federal Republic of Germany), 1 U Taq polymerase (Promega Corp., Madison, WI), and 3.3 mM MgCl₂ (stock solution, 25 mM; Promega Corp.) in a final volume of 30 μL with the PCR buffer (final concentrations: 16.6 mM [NH₄]₂SO₄, 50 mM β-mercaptoethanol, 6.8 μM EDTA, 67 mM Tris [pH 8.8], and 80 μg/mL bovine serum albumin). The reaction mixture was assembled at 85 °C, placed in a Perkin-Elmer 9600 thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) for 3 minutes at 94 °C, and then subjected to 30 cycles of 94 °C for 10 seconds, 60 °C for 20 seconds, and 72 °C for 45 seconds. The thermal cycling program was followed by a final step at 72 °C for 5 minutes. The GSTT1 (480 base pair [bp]), β-globin (268 bp), and GSTM1 (215 bp) amplification products were resolved in an ethidium bromide-stained 4% 3:1 NuSieveagarose gel (FMC Corp., Rockland, ME). The absence of the GSTM1-
or the GSTT1-specific fragment indicated the corresponding null genotype, whereas the β-globin-specific fragment confirmed the presence of amplifiable DNA in the reaction mixture (11). To verify the GSTT1-null genotype further and to ensure that DNA degradation did not influence the results, a second PCR was performed that yielded a 112-bp fragment (data not shown).

The NAT2 allele corresponding to the fast-acetylator phenotype and the four slow-acetylator phenotype-associated alleles were distinguished by use of primers specific for the NAT2 gene in a PCR reaction and restriction enzyme digestion of the resulting amplification product as described by Bell et al. (13). A single PCR was carried out using 50 pmol of the primers (N5-5'GGA ACA AAT TGG ACT TGG3' and N4-5TCT AGC ATG AAT CAC TCT GC3' [synthesized by Institute of Biotechnology]), 200 pmol dNTPs (Boehringer Mannheim GmbH), 1 U Taq polymerase (Promega Corp.), and 2.0 mM MgCl2 (Promega Corp.) in a volume of 50 μL with the above-described PCR buffer. After PCR, which was performed as indicated for the GST analyses, except that an annealing temperature of 57 °C was used, 7.5-μL aliquots were removed and subjected to restriction digestion with Kpn I (for the M1 allele), Taq I (for the M2 allele), BamHI (for the M3 allele), or Msp I/Afl I (for the M4 allele). (The restriction enzymes were from New England Biolabs, Inc., Beverly, MA.) The digests were subjected to electrophoresis in 4% agarose gels. The absence of a restriction site indicated the presence of a defective NAT2 allele, and the presence of two reverse sites indicated the presence of a defective alleles identified the slow acetylators (13).

#### Statistical Methods

The odds ratio (OR) is an estimate of the relative risk of disease associated with specific genotypes and is defined as the odds of a case patient having the at-risk genotype divided by the odds of a control subject having the same genotype. ORs and 95% confidence intervals (CIs) were calculated from 2 x 2 tables with the Fisher's exact model, and Pearson coefficients of correlation were calculated between determinants and potential confounders (14).

### Results and Discussion

When the three genes were studied separately (Table 1), no statistically significant differences were observed for the GST genotypes, although the frequency of the GSTM1-null genotype was clearly higher among the patients with malignant mesothelioma (67%) and somewhat higher among the asbestos workers with nonmalignant pulmonary disorders (58%) than among workers who lacked evidence of these disorders (46%). In contrast, the NAT2 slow-acetylator genotypes were significantly more prevalent among the workers with pulmonary disorders (69%) than among those lacking them (50%).

The frequency of NAT2 slow-acetylator genotypes was highest among the patients with malignant mesothelioma (79%) (OR = 3.8; 95% CI = 1.2-14.3). Moreover, the slow acetylators lacking the GSTM1 gene had nearly an eightfold higher risk of mesothelioma (OR = 7.8; 95% CI = 1.4-78.7) compared with those who had the gene and the NAT2 fast-acetylator genotype (Table 2). They were also at a fourfold higher risk of nonmalignant pulmonary disorders (OR = 4.1; 95% CI = 1.1-17.2) and, consequently, at about a fivefold higher risk overall (OR = 5.1; 95% CI = 1.6-17.6) of developing one of the pulmonary disorders studied. As expected, correlates of the genotypes and age, pack-years of cigarette smoking, duration of smoking, and duration of asbestos exposure were negligible, with correlation coefficients ranging from -0.05 to 0.12. Therefore, no adjustment of the ORs by these factors was needed. In contrast to the effects shown by combinations of GSTM1 and NAT2 genotypes, other combinations of the studied genotypes showed no additive effects.

### Table 1. Distribution of glutathione S-transferase (GST) and N-acetyltransferase (NAT) genotypes in workers exposed to asbestos in relation to pulmonary disorders

<table>
<thead>
<tr>
<th>End point</th>
<th>GSTM1 genotype</th>
<th>GSTT1 genotype</th>
<th>NAT2 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of patients</td>
<td>% null OR (95% CI)</td>
<td>No. of patients</td>
</tr>
<tr>
<td>No pulmonary disorders</td>
<td>69</td>
<td>46</td>
<td>1.0</td>
</tr>
<tr>
<td>Pulmonary disorders (all)</td>
<td>76</td>
<td>61</td>
<td>1.8 (0.3-3.4)</td>
</tr>
<tr>
<td>Nonmalignant</td>
<td>52</td>
<td>58</td>
<td>1.5 (0.8-3.3)</td>
</tr>
<tr>
<td>Malignant§§</td>
<td>24</td>
<td>67</td>
<td>2.3 (0.8-7.1)</td>
</tr>
</tbody>
</table>

*OR = odds ratio; CI = confidence interval; null = absence of the gene; slow = slow acetylator.

†Malignant mesothelioma.

### Table 2. Combinations of GSTM1 and NAT2 genotypes in workers exposed to asbestos in relation to pulmonary disorders

<table>
<thead>
<tr>
<th>NAT2 slow acetylators</th>
<th>GSTM1-null</th>
<th>GSTM1-positive</th>
<th>OR†</th>
<th>95% CI‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>End point</td>
<td>No. of patients</td>
<td>%</td>
<td>No. of patients</td>
<td>%</td>
</tr>
<tr>
<td>No pulmonary disorders</td>
<td>15</td>
<td>22</td>
<td>19</td>
<td>28</td>
</tr>
<tr>
<td>Pulmonary disorders (all)</td>
<td>30</td>
<td>40</td>
<td>22</td>
<td>29</td>
</tr>
<tr>
<td>Nonmalignant</td>
<td>17</td>
<td>33</td>
<td>16</td>
<td>31</td>
</tr>
<tr>
<td>Malignant§§</td>
<td>13</td>
<td>54</td>
<td>25</td>
<td>5</td>
</tr>
</tbody>
</table>

*Null = absence of the gene; positive = presence of the gene.
†OR = odds ratio. OR shown is for the NAT2 slow-acetylator and GSTM1-null genotype combination compared with the NAT2 fast-acetylator and GSTM1-positive genotype combination; reference group consists of subjects with no pulmonary disorders.
‡CI = confidence interval.
§Malignant mesothelioma.
The lack of effect of smoking in this study stands in contrast to previous analyses of the GSTM1 gene and cancer susceptibility, where the risk associated with the GSTM1-null genotype was mostly attributable to smoking (7-9). In the previous analyses, the associations appeared, however, to be heavily influenced by the asbestos exposure levels, in agreement with two more recent studies. An association was reported between the GSTM1-null genotype and the risk of developing asbestosis among highly exposed asbestos workers (15), whereas no association was found between radiographic or lung function changes and GSTM1 genotype in a group of asbestos workers with mostly low or moderate exposure levels (16).

Certain GST isoenzymes are considered to be part of a system required for the repair of free-radical-induced lipid peroxidation (4), which is considered to be one possible mechanism in the multistep process of asbestos carcinogenesis (2). Therefore, the observed association between the GSTM1-null genotype and asbestos-associated pulmonary disorders was not surprising, whereas the remarkable impact of the NAT2 genotype observed both in this study and in our previous study (10) was rather unexpected. However, asbestos fibers are able to induce ornithine decarboxylase enzyme activity, resulting in increased cell proliferation as a consequence of enhanced polyamine synthesis (17). Since an acetylation step is known to be involved in the catalysis of polyamines (18), the slow NAT2 acetylators may accumulate greater amounts of polyamines than the fast acetylators. This could explain, in part, the modulating effect of NAT2 polymorphism in individual responses to asbestos exposure.

In conclusion, we found that, among workers with high levels of asbestos exposure, individuals concurrently having a NAT2 slow-acetylator genotype and homozygous deletion of the GSTM1 gene showed a marked excess of asbestos-associated pulmonary disorders. Even if the present study size is relatively small, the high prevalence of these potential at-risk genotypes indicates that our observations may have important implications for public health.

References


Notes

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