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Inherited *GSTM1* and *NAT2* Defects as Concurrent Risk Modifiers in Asbestos-related Human Malignant Mesothelioma

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Abstract

Besides asbestos exposure, the factors that determine susceptibility to malignant mesothelioma are unknown. We evaluated the risk of *GSTM1* null genotype and slow acetylation-associated *NAT2* genotype for malignant mesothelioma in relation to asbestos exposure. Both the *GSTM1* null genotype and the *NAT2* slow acetylator genotype placed individuals at about 2-fold increased risk of developing malignant mesothelioma [odds ratio (OR) = 1.8, 95% confidence interval (CI) = 1.0–3.5 and OR = 2.1, 95% CI = 1.1–4.1, for the *GSTM1* and *NAT2* genes, respectively]. When the patients were divided into low/moderate and high exposure groups according to their asbestos exposure histories, the effect of the at-risk genotypes was mostly attributable to the high exposure groups (OR = 2.3, 95% CI = 1.0–5.6 and OR = 3.7, 95% CI = 1.3–10.2, for the *GSTM1* and *NAT2* genes, respectively). The individuals with combined *GSTM1* and *NAT2* defects had about a 4-fold risk of developing malignant mesothelioma compared to those with the *GSTM1* gene and *NAT2* fast acetylator genotype (OR = 3.6; 95% CI = 1.3–9.6). Moreover, the risk among subjects highly exposed to asbestos with the double at-risk genotype was more than 7-fold greater compared to those with the more beneficial genotypes of both *GSTM1* and *NAT2* genes (OR = 7.4; 95% CI = 1.6–34.0).

Introduction

Diffuse malignant mesothelioma is a rapidly fatal tumor of the pleural or peritoneal mesothelial cell lining. Smoking, which has been shown to be the major cause of lung cancer, does not enhance the prevalence of malignant mesothelioma in humans (1). In contrast, approximately 80% of all mesothelioma patients have a history of asbestos exposure, and the onset of illness is usually 35–40 years after first exposure (2). The association between asbestos exposure and malignant mesothelioma was established in 1960 (3). Animal studies have further confirmed that asbestos can act as a complete carcinogen (4). In Finland, more than 200,000 currently active or retired workers have been exposed to asbestos, and the incidence of asbestos-related diseases is estimated to reach its peak after the year 2000. About 50 cases (10/million inhabitants) of pleural mesotheliomas are diagnosed annually in Finland (5). Because the causal relationship has been determined, much work has been undertaken to understand the mechanisms of fiber-induced carcinogenesis. According to present understanding, both direct and indirect cellular effects of fibers may contribute to cell transformation, including generation of reactive free radicals from the fiber surface in target cells or after the interaction of fibers with inflammatory cells (6). Apart from mesothelioma, exposure to asbestos can result in the development of lung cancer or benign diseases [*e.g.*, pulmonary fibrosis (asbestosis) and pleural disorders].

Possible genetic traits that predispose individuals to asbestos-associated diseases are mainly unclear. To date, only a couple of studies have addressed this issue; recently, Smith *et al.* (7) reported that individuals occupationally exposed to asbestos were significantly more likely to have radiological evidence of nonmalignant asbestos-related disease if they lacked the glutathione *S*-transferase *M1* gene (*i.e.*, they were *GSTM1*[2] null) compared to those who were not deficient. Another recent study failed, however, to show any such association (8). GSTM1 is important in the detoxification of several carcinogens, including polycyclic aromatic hydrocarbons. Studies have indeed implicated that the genetically determined variation in the expression of *GSTM1*, as well as several other metabolic enzymes, including the *N*-acetyltransferase 2 (NAT2), which is associated with the biotransformation of aromatic amines, may be associated with individual cancer susceptibility (9, 10). In the present study, we explored the possible role of *GSTM1* and *NAT2* polymorphisms in asbestos-related malignant mesothelioma.

Patients and Methods

Study Population. The present study involved 44 Finnish mesothelioma patients (37 males and 7 females) hospitalized at the Helsinki University Central Hospital and 270 consecutive blood donors (all males) at the Finnish Red Cross Blood Transfusion Service as a healthy genetic control group. The mean age for the mesothelioma patients was 56.6 ± 9.6 years and for the controls, 41.1 ± 11.1 years. Most of the patients were either exsmokers (15 of 44) or never-smokers (11 of 44). Similarly, only 59 of the 270 control subjects were current smokers; the rest were either ex- or never-smokers. All the patients had been admitted to Helsinki University Central Hospital between 1985 and 1993 and diagnosed subsequently 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 degree of asbestos exposure, based on all available data and interview of the patients, was classified as: (*a*) definite or probable, including patients employed in the manufacture of asbestos products, the asbestos cement industry, asbestos insulation work, demolition of old buildings, shipyards, the construction industry, or metal workshop; (*b*) possible, including patients employed in trades liable to dust exposure, such as mining, power generation, transportation, and the paper and pulp industry; and (*c*) unlikely/unknown, including patients employed in occupations having no apparent liability to asbestos exposure. Only patients with established asbestos exposure history were included in this study. On the basis of the interview, the individuals were classified to high, moderate, or low asbestos exposure groups.

Laboratory Methods. The genotyping analyses were performed on leukocyte DNA from the healthy controls or on peripheral tissue DNA from the mesothelioma patients with the use of PCR-based methods as described in detail elsewhere (11, 12). Briefly, of the three primers used in the *GSTM1* analysis, two could also anneal to another class μ gene (*GSTM4*; Ref. 13), whereas the third primer was specific for the *GSTM1* gene. The *GSTM1* null genotype was identified on the basis of the absence of the *GSTM1*-specific fragment, whereas the consistent presence of the other fragment was used as an internal standard to detect failure of the amplification reaction. The *NAT2*

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[2] The abbreviations used are: GSTM1, glutathione *S*-transferase M1; NAT2, *N*-acetyltransferase 2; OR, odds ratio; CI, confidence interval.

allele, corresponding to the fast acetylator phenotype, and the three slow acetylator phenotype-associated alleles were distinguished with the use of primers specific for the *NAT2* gene followed by restriction enzyme digestion of the amplified product. Two defective alleles identified the slow acetylators.

Results and Discussion

The frequency of the *GSTM1* null genotype was clearly higher among the mesothelioma patient group (61%) compared to controls (46%), although the difference was only of marginal statistical significance (OR = 1.8; 95% CI = 1.0–3.5; $P = 0.06$; Table 1). Similarly, the *NAT2* slow acetylation-associated genotypes were more prevalent among the patients (68%) than among the controls (51%; OR = 2.1; 95% CI = 1.1–4.1; $P = 0.03$). The risk associated with the *GSTM1* null genotype and the *NAT2* slow acetylator genotype was mostly attributable to the high asbestos exposure; genotype frequencies were 67% (OR = 2.3; 95% CI = 1.0–5.6; $P = 0.05$) and 79% (OR = 3.7; 95% CI = 1.3–10.2; $P = 0.005$) for the patients in the high exposure group, respectively.

When the study populations were divided according to combined genotypes, the patients with the double at-risk genotype had about a 3-fold increased risk of mesothelioma compared to all other genotypes (OR = 2.7; 95% CI = 1.4–5.2; $P = 0.006$) and about a 4-fold risk compared to those having *GSTM1* gene and *NAT2* fast acetylator genotype (OR = 3.6; 95% CI = 1.3–9.6; $P = 0.006$); Table 2). Again, the most remarkable risk was found for the people with high asbestos exposure; patients highly exposed to asbestos and with the double at-risk genotype were at about a 4-fold elevated risk of mesothelioma compared to all other combinations of the genotypes (OR = 4.1; 95% CI = 1.8–9.7; $P = 0.002$) and at more than a 7-fold risk compared to those having *GSTM1* gene and *NAT2* fast acetylator genotype (OR = 7.4; 95% CI = 1.6–34.0; $P = 0.002$). Since there were only 7 females included in the study, the possible association of the polymorphisms with gender could not be properly addressed. However, exclusion of the females did not affect the outcome of the analyses. Similarly, stratification by age had little effect on odds ratio estimates (data not shown), suggesting that differential mortality by genotype is unlikely.

In contrast to the previous observations where the association between *GSTM1* null genotype and cancer risk has been mostly attributable to smokers (14–17), cumulative tobacco smoke dose did not appear to affect the risk for developing malignant mesothelioma, regardless of the *GSTM1* and *NAT2* genotype (data not shown). This is, however, in agreement with the previous observations on tobacco smoking and risk of mesothelioma (1).

Given that certain glutathione *S*-transferase isoenzymes are considered as a part of a system for the repair of free-radical-induced lipid peroxidation (18), which is considered as one possible mechanism in the multistep process of asbestos carcinogenesis (6), the observed association between *GSTM1* null genotype and malignant mesothelioma was not surprising. Moreover, our observation supports the finding of overrepresentation of *GSTM1* null genotype in a sample of people with asbestos-related disease (7). Because this association was mainly attributable to the high asbestos-exposure group, it is also in agreement with a recent report where no association between radiographical or lung function changes was found in a group of asbestos workers with mostly low or moderate exposure levels (8). However, the remarkable association between the *NAT2* slow acetylation-related genotypes and mesothelioma risk was rather unexpected. Theoretically, the biological significance of the slow acetylator genotype in the development of asbestos-induced mesothelioma might be mediated via acetylation step in the interconversion of polyamines (19); it has been shown that asbestos fibers are able to induce ornithine decarboxylase enzyme activity resulting in stimulated polyamine synthesis and increased cell proliferation (20). It is not certain, though, whether NAT2 is involved in the acetylation step of polyamines.

To conclude, we have found for the first time an association between genetic polymorphisms in the metabolic genes *GSTM1* and *NAT2* and asbestos-associated mesothelioma. Because our patient study group was of relatively small size, additional studies are needed to confirm the role of the *GSTM1* null genotype and the *NAT2* slow acetylator genotype in individual risk of malignant mesothelioma. However, knowing the high prevalence of these at-risk genotypes, if true differences between the *GSTM1* and *NAT2* groups exist, our results may have important implications for public health.

Table 1 *Distribution of the GSTM1 and NAT2 genotypes in relation to asbestos exposure*[a]

	GSTM1−[b]		*GSTM1*+		*NAT2*−		*NAT2*+	
	n	%	*n*	%	*n*	%	*n*	%
Control subjects ($n = 270$)	125	46	145	54	137	51	133	49
Mesothelioma patients ($n = 44$)	27[c]	61	17	39	30[d]	68	14	32
Asbestos exposure								
Low/moderate ($n = 20$)	11	55	9	45	11	55	9	45
High ($n = 24$)	16[e]	67	8	33	19[f]	79	5	21

[a] Data were analyzed using the logit model. *P* values were two-sided $P(\chi^2)$.
[b] *GSTM1*−, *GSTM1* gene absent; *GSTM1*+, *GSTM1* gene detected; *NAT2*−, slow acetylator genotype; *NAT2*+, fast acetylator genotype.
[c] OR = 1.8 (95% CI = 1.0–3.5; $P = 0.06$).
[d] OR = 2.1 (95% CI = 1.1–4.1; $P = 0.03$).
[e] OR = 2.3 (95% CI = 1.0–5.6; $P = 0.05$).
[f] OR = 3.7 (95% CI = 1.3–10.2; $P = 0.005$).

Table 2 *Distribution of combinations of the GSTM1 and NAT2 genotypes in relation to asbestos exposure*[a]

	GSTM1−				*GSTM1*+			
	NAT2−		*NAT2*+		*NAT2*−		*NAT2*+	
	n	%	*n*	%	*n*	%	*n*	%
Control population ($n = 270$)	60	22	65	24	77	29	68	25
Mesothelioma patients ($n = 44$)	19[b,c]	43	8	18	11	25	6	14
Asbestos exposure								
Moderate/low ($n = 20$)	6	30	5	25	5	25	4	20
High ($n = 24$)	13[d,e]	54	3	13	6	25	2	8

[a] For genotype definitions and statistical analyses see Table 1.
[b] *GSTM1*−/*NAT2*− *versus* all other combined genotypes; OR = 2.7 (95% CI = 1.4–5.2; $P = 0.006$).
[c] *GSTM1*−/*NAT2*− *versus* *GSTM1*+/*NAT2*+; OR = 3.6 (95% CI = 1.3–9.6; $P = 0.006$).
[d] *GSTM1*−/*NAT2*− *versus* all other combined genotypes; OR = 4.1 (95% CI = 1.8–9.7; $P = 0.002$).
[e] *GSTM1*−/*NAT2*− *versus* *GSTM1*+/*NAT2*+; OR = 7.4 (95% CI = 1.6–34.0; $P = 0.002$).

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