Genetic polymorphism for glutathione-S-transferase mu in asbestos cement workers

K Jakobsson, A Rannug, A-K Alexandrie, L Rylander, M Albin, L Hagmar

Abstract

Objective—To investigate whether a lack of glutathione-S-transferase mu (GSTM1) activity was related to an increased risk for adverse outcome after asbestos exposure.

Methods—A study was made of 78 male former asbestos cement workers, with retrospective cohort data on exposure, radiographical findings, and lung function. Venous blood samples were obtained for the analysis of GSTM1 polymorphism by the polymerase chain reaction technique. Chest x ray films were classified according to the International Labour Organisation (ILO) 1980 classification. Vital capacity (VC) and forced expiratory volume during 1 s (FEV₁) were determined. Individual estimates of asbestos exposure were calculated, and expressed as duration of exposure, average exposure intensity, and cumulative dose. Data on smoking were obtained from interviews.

Results—The lung function in the study group was reduced, compared with reference equations. 23% of the workers had small opacities $\geq 1/0$, 29% circumscribed pleural thickenings, 14% diffuse thickenings, and 12% obliterated costophrenic angles. 54% of the workers were GSTM1 deficient. They were comparable with the other workers in age, follow up time (median 30 years), and duration of exposure (median 18 years), but had a slightly higher cumulated dose (median 18 v 10 fibre-years) than the others. Neither in radiographical changes nor lung function variables were there any differences between the different GSTM1 groups. The findings were similar when smoking habits and estimated asbestos exposure were taken into account.

Conclusions—We could not show that lack of GSTM1 activity was related to an increased risk for radiographical or lung function changes in a group of asbestos cement workers, followed up for a long period after the end of exposure.

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Keywords: glutathione-S-transferase mu, asbestos cement, lung disease

Although asbestos related disorders are among the most extensively studied occupational diseases, little is still known about fac-

tors that determine individual susceptibility to these conditions. As exposure levels decrease, individual characteristics may well assume a greater importance in determining not only the actual amount of fibre delivered to the lung tissue and retained there, but also the nature of the response. Peribronchiolar fibrosis is the characteristic early lesion after asbestos exposure, found in both animal and human studies.¹ Based on experimental evidence, it has been suggested that this is a dose-related response, whereas individual susceptibility determines the degree of macrophagic alveolitis, resulting in subsequent fibrosis.^{2,3}

Experimental data indicate that active oxygen species may be causally involved in the development of asbestosis.^{1,4} Data from studies of cell free systems show that active oxygen species may be generated by asbestos fibres themselves. Also, alveolar macrophages that are activated during the phagocytosis of fibres release active oxygen species. At high concentrations active oxygen species are directly cytotoxic to cells of the respiratory tract; also they may cause lipid peroxidation of membrane components. Hence, individual differences in activity of enzymes that are scavengers for active oxygen species may be determinants of the risk for adverse pulmonary effects.

The aim of our study was to investigate whether the activity of one of these scavengers, the enzyme glutathione-S-transferase mu (GSTM1), which is polymorphically distributed in humans, was related to individual differences in adverse outcome of asbestos exposure. The cytosolic glutathione-S-transferases (GSTs) catalyse glutathione conjugation of electrophilic compounds that can be produced from exogenous xenobiotics or arise endogenously during oxidative stress. Rat GSTs 3-3 and 4-4, which are homologous to human GSTM1 (one of the mu class isoenzymes of glutathione transferase), are induced when incubated with xanthine and xanthine oxidase in a superoxide producing system.⁵ These enzymes also have glutathione peroxidase activity, particularly with DNA hydroperoxides.⁶ Furthermore, other electrophilic substrates of biological importance like benzo(a)pyrene-7,8-diol-9,10-epoxide and 4-hydroxyalkenals are also conjugated with glutathione by the rat 3-3 and especially the 4-4 isoenzymes.⁶ Allelic variants of GSTM1 were assessed in a group of asbestos cement workers with well characterised individual asbestos exposure, who had been followed up for a long period after the end of exposure.

Department of
Occupational and
Environmental
Medicine, University
Hospital, S-221 85
Lund, Sweden

K Jakobsson
L Rylander
M Albin
L Hagmar

Department of
Toxicology, National
Institute of
Occupational Health,
S-171 84 Solna,
Sweden

A Rannug
A-K Alexandrie

Correspondence to:
Dr Kristina Jakobsson,
Department of Occupational
and Environmental
Medicine, University
Hospital, S-221 85 Lund,
Sweden.

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Table 1 Descriptive data for asbestos cement workers with genotype GSTM1(−) and GSTM1(+), and non-participants

	Participants		Non-participants
	GSTM1(−)	GSTM1(+)	
Subjects (n)	42	36	17
Age (in 1993)	72 (60, 81)	74 (64, 79)	70 (61, 82)
Start of employment*	1954 (1938, 1959)	1954 (1945, 1961)	1954 (1945, 1962)
End of employment*	1976 (1972, 1976)	1976 (1970, 1977)	1977 (1975, 1977)
Employment time (y)*	19 (13, 28)	17 (12, 23)	19 (14, 26)
Cumulated dose (f-y)*	18 (8.8, 35)	10 (6.1, 21)	24 (10, 28)
Average intensity (f/ml)*	1.0 (0.6, 1.3)	0.6 (0.3, 1.2)	0.9 (0.7, 1.3)
Age:			
At chest x ray*	62 (51, 73)	66 (55, 70)	61 (50, 70)
At lung function test*	69 (57, 79)	68 (60, 75)	62 (46, 72)
Years since start of employment:			
At chest x ray*	30 (24, 46)	30 (23, 39)	27 (21, 38)
At lung function test*	37 (30, 53)	36 (27, 39)	26 (19, 37)
Smoking habits at lung function test:			
Non-smoker (n (%))	10 (24)	4 (11)	3 (18)
Ex-smoker (n (%))	21 (50)	17 (47)	8 (47)
Smoker (n (%))	11 (26)	15 (42)	6 (35)
Tobacco consumption among smokers and ex-smokers (pack-y)*	14 (8, 30)	18 (11, 34)	21 (7, 27)

*Median (25, 75 percentiles).

Material and methods

EXPOSED WORKERS

All present and former workers at an asbestos cement plant that had been operating since 1907 were invited to a health examination at the Department of Occupational and Environmental Medicine, Lund when the plant closed down in 1977. In 1984 and 1991 all employees with at least 10 years of employment were invited to other examinations. The examinations included a chest x ray film, spirometry, a work history, and a medical history. We calculated individual cumulative asbestos doses expressed in fibre-years (f-y).⁷ The average intensity of exposure (f/ml) was also estimated, simply by dividing cumulative dose by employment time.

On 15 March 1993 there were 95 male workers with more than 10 years of employment at the plant still alive, who had participated in at least one of the above mentioned health examinations and had had chest x ray films classified according to the International Labour Organisation (ILO) 1980 classification.⁸ These men formed the study base. Blood samples for determination of genetic polymorphisms for GSTM1 were obtained from 78 (92%) of them. Table 1 shows demographic data and exposure data for participants and non-participants.

CHEST X RAY FILM FINDINGS

The chest x ray films from these workers had been classified according to the ILO 1980 classification by a panel of five readers.⁹ The median reading from the last film classified was used in our study. The profusion of small opacities regardless of size and shape was considered, as nearly all opacities noted were of irregular shape. Circumscribed and diffuse pleural thickening were noted, regardless of width or extent. Costophrenic angle obliteration was defined as in the ILO standard film.

LUNG FUNCTION

The results from the last available spirometry were compared with the reference values most widely used in Sweden.¹⁰ As these reference equations do not take smoking habits into

consideration, we also used reference equations for non-smokers, ex-smokers, and smokers, obtained from active Swedish construction workers (Engholm G, *et al*, personal communication). As our study consists of a large proportion of old workers, and the expected values for ages ≥ 70 are based on linear extrapolation only, we used the spirometry results at age ≤ 70 in additional analyses.

GENOTYPE DETERMINATION

Venous blood samples were collected in tubes containing sodium citrate and were stored at -70°C . The samples were subjected to lysis and proteinase digestion before extraction with phenol and chloroform.¹¹ DNA was isolated after ethanol precipitation.

The analysis of the GSTM1 polymorphism was performed essentially as described by Brockmöller and collaborators.¹² The polymerase chain reactions (PCRs) were carried out in a total volume of 25 μl in the presence of 10 mM Tris-hydrochloric acid, pH 8.3; 50 mM KCl; 0.2 mM of each deoxynucleotide triphosphate; 1 μM of each primer; 0.8 units Taq polymerase; 90 ng genomic DNA as template; and 1 mM MgCl_2 . Amplifications were, after an initial denaturation at 94°C for 2 min, performed during 35 cycles with denaturation at 94°C for 24 s, annealing at 53°C for 46 s and extension at 73°C for 60 s. Detection of the fragments amplified by the PCR was made by electrophoresis on a 1.5% agarose gel. All PCRs were carried out in a PCR instrument (Thermal Cycler 9600) from Perkin Elmer Cetus.

Deficiency in GSTM1 activity, GSTM1(−), has been ascribed to a homozygous deletion of the gene. The PCR assay used in this work detects the presence or absence of the intact gene, but does not differentiate between heterozygous and homozygous carriers. Two separate PCRs were performed. The first primer set generates an amplification product exceeding exon 4 to 5 if one or both of the GSTM1 alleles are intact. To confirm the results all samples were further tested with a second primer set. This amplification yields a

fragment overlapping the first fragment but which also includes intron 3. Both fragments are absent in GSTM1(−) people.

STATISTICS

Differences between subgroups were examined by Fisher's exact test or the Mann-Whitney U test. For radiographic outcome variables logistic regression was used to estimate age (continuous variable), smoking (smoking status at the time of the examination), and exposure adjusted odds ratios (ORs). Duration of employment, average intensity of employment, and cumulative dose (all variables dichotomised at the median values for the combined group) were examined in separate models, one at a time. For the lung function variables (expressed as a percentage of the predicted values, thus adjusted for age and height) we performed linear regressions, adjusted similarly for exposure. The different statistical methods were performed as generally described.¹³ P values given are two-tailed. The term significant refers to $P < 0.05$ or to a 95% confidence interval (95% CI) not including 1.00.

Results

The prevalence of genotype GSTM1(−) was 54%. As expected, there were no significant differences between the GSTM1(−) and GSTM1(+) groups in age distribution (table 1). In the group of men aged ≥ 70 , the distribution of GSTM1 genotype was similar to that in younger men (data not shown). The proportion of lifelong non-smokers was slightly higher in the GSTM1(−) group, 24%, compared with 11% in the GSTM1(+) group, but the difference in distribution of smoking habits (as reported at the time of the lung function test) was not significant (Fisher, $P = 0.21$). Further, the cumulative tobacco consumption among smokers and ex-smokers did not differ significantly between the GSTM1 groups.

The time lapse between start of employ-

ment and time of examination did not differ between the groups. The calendar years for the start and end of employment, and thus also the duration of employment, were also comparable (table 1). The average intensity of exposure had been somewhat higher in the GSTM1(−) group, median 1.0 f/ml, compared with 0.6 f/ml in the GSTM1(+) group (Mann-Whitney, $P = 0.05$), and thus also the cumulative dose, median 18 v 10 f-y (Mann-Whitney, $P = 0.02$). In the GSTM1(−) group the proportion of men with an estimated cumulative dose ≥ 20 f-y was 45%, compared with 21% in the GSTM1(+) group (Fisher, $P = 0.24$).

The distribution of radiographic abnormalities was remarkably similar between the different GSTM1 groups (table 2). Neither for parenchymal abnormalities nor for pleural abnormalities (circumscribed and diffuse pleural thickening, obliteration of costophrenic angles) were there any differences. The differences in exposure levels between the GSTM1 groups were accounted for in the logistic regression model, but still showed no significant differences between the GSTM1 groups for any of the radiographic changes. The odds ratio (OR) for small opacities of profusion $\geq 1/10$ in men with GSTM1(−) was 0.8 (95% CI 0.3–2.1), with cumulative dose as the exposure variable.

The two different reference equations used gave somewhat different estimates of vital capacity (VC) (expressed as a percentage of the predicted value). There were no consistent differences between the GSTM1 groups regardless of which reference equation was used, neither when we analysed VC obtained from the last examination (table 2), nor when we used lung function data obtained at age ≤ 70 (data not shown).

The forced expiratory volume in one second (FEV₁) was slightly higher in the GSTM1(−) group than in the GSTM1(+) group, but the differences were not significant (table 3), regardless of whether we used reference equations not corrected for smoking (Mann-Whitney, $P = 0.21$) or corrected for smoking (Mann-Whitney, $P = 0.41$). The results for analyses with age limits were similar. The differences in exposure levels were accounted for in the multiple linear regression model, but showed no significant differences between the GSTM1 groups for either VC or FEV₁.

Non-participants had a slightly, but not significantly, higher cumulative dose than participants (table 1). Radiographic and lung function changes were no more severe than in the participants (table 2).

Discussion

The question whether the biological response to environmental exposure is modulated by host polymorphism is currently under extensive investigation. The enzymes of interest in the context of asbestos exposure include the GSTs, which may be involved in the inactivation of active oxygen species. Preliminary results have been reported that suggested an

Table 2 Radiographic changes and lung function among asbestos cement workers with genotype GSTM1(−) and GSTM1(+), and among non-participants

	Participants		Non-participants
	GSTM1(−)	GSTM1(+)	
Small opacities (n (%)):			
≤ 0/1	30 (71)	24 (67)	14 (82)
1/0	5 (12)	5 (14)	2 (12)
1/1–1/2	7 (17)	7 (19)	1 (6)
≥ 2/1	0 (0)	0 (0)	0 (0)
Circumscribed pleural thickening (n (%)):			
Absent	28 (67)	27 (75)	14 (82)
Present	14 (33)	9 (25)	3 (18)
Diffuse pleural thickening (n (%)):			
Absent	37 (88)	30 (83)	13 (76)
Present	5 (12)	6 (17)	4 (24)
Costophrenic angle obliteration (n (%)):			
Absent	38 (90)	31 (86)	16 (94)
Present	4 (10)	5 (14)	1 (6)
VC (% predicted)*	79 (70, 89)	78 (69, 83)	79 (72, 86)
VC (% predicted, corrected for smoking habits)*	92 (78, 98)	86 (79, 96)	92 (80, 96)
FEV ₁ (% predicted)*	95 (81, 102)	86 (78, 101)	92 (80, 96)
FEV ₁ (% predicted, corrected for smoking habits)*	92 (84, 99)	88 (73, 98)	86 (78, 96)

*Median (25, 75 percentiles)

over-representation of GSTM1(−) genotype in a sample of people with asbestos related disease, compared with controls.¹⁴ Studies in populations defined by their asbestos exposure are to our knowledge not previously reported.

Our population was defined on the basis of exposure. The population studied—that is, those with available data on outcome—comprised 78% of all living workers with ≥ 10 years of former employment at the plant. Further, the non-participants did not constitute a group with more severe asbestos related disease than the participants. Thus we can assume that the participants are fairly representative of the surviving exposed population. The outcome was studied after the end of exposure, and for all workers ≥ 20 years after the start of employment. Thus, we also assume that sufficient time had elapsed for the development of asbestos related disease in our study group.

The prevalence of genotype GSTM1(−) was 54%, which is somewhat higher than an earlier study on 70 chimney sweeps, where GSTM1(−) people comprised 49%.^{15,16} In a previous estimate of the proportion of GSTM1(−) people in Sweden, 54% in a group of 248 examined had a phenotype lacking activity towards trans-stilbene oxide.¹⁷

We could not show any radiographical and lung function differences between men with the genotypes GSTM1(−) and GSTM1(+). The investigated subjects were survivors from a population exposed to asbestos cement. In this population we have found increased risks of respiratory cancer (including mesotheliomas), non-malignant respiratory mortality,⁷ and a somewhat shorter life span compared with a reference cohort of industrial workers.¹⁸ The population investigated might thus theoretically display an under-representation of people with GSTM1(−), were this genotype strongly associated with premature deaths (related or not with asbestos exposure). The overall prevalence of GSTM1(−) was not lower than expected. Further, we did not find any significantly different distribution of genotypes in older than younger workers, and no overall correlation between age and genotype. Thus, a selective survival bias is not a likely explanation of the present lack of an association between genotype and adverse pulmonary and pleural outcome.

Of the human cytosolic GSTs the GSTA, GSTM and GSTP forms have all been identified in lung tissue.¹⁹ The GSTM1 enzyme probably contributes to only a minor part of the total GST activity in the lung but it may still be crucial for the detoxification of potentially toxic substrates in certain cell populations in the lung. Increased levels of aromatic DNA adducts found in lung tissue of GSTM1(−) people²⁰ and the increased risk of lung cancer of GSTM1(−) smokers^{21–23} support the theory that GSTM1 has an important protective role in the lung.²⁰ The susceptibility to lung cancer in smokers of the GSTM1(−) genotype has been most firmly established in smokers with a low tobacco consumption²⁴

and also in lung cancer patients diagnosed at a young age.²⁵

The relative importance of GST activity in parenchymal and bronchial lung tissue is not yet clarified. The results from lung cancer studies are conflicting; some studies correlate GSTM1(−) with an increased risk primarily for adenocarcinomas,^{22,25} whereas other studies report an association with squamous cell carcinomas only.^{26,27} It has been found that parenchymal lung preparations have higher concentrations of reduced glutathione than bronchial preparations.²⁸ Thus, there is reason to assume that individual differences in GST activity may influence the risk of development of a mainly parenchymal disease like asbestosis.

Despite the fact that the GSTM1(−) men had a somewhat higher asbestos exposure than the GSTM1(+) men, no difference in outcome was found. From the results it can be calculated^{29,30} that there is a probability of 0.10 for a $\geq 50\%$ increase in the relative risk of small opacities $\geq 1/0$ in the GSTM1(−) group. Thus, although our study group was of a moderate size only, the power was reasonable. The corresponding probability estimates for circumscribed pleural thickenings and obliterated costophrenic angles were 0.50 and 0.13. In our study, however, the exposure levels for most workers were low or moderate, and only a small fraction of the men had radiographic changes of ILO grade $\geq 1/1$. True differences between GSTM1 groups for more severe parenchymal disease may well exist, but this cannot be evaluated within our study group.

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- 2 Soter NA, Wasserman SI, Austen KF. Cold urticaria: release into the circulation of histamine and eosinophil chemotactic factor of anaphylaxis during cold challenge. *N Engl J Med* 1976;294:687-90.
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