ERJ Express. Published on September 16, 2010 as doi: 10.1183/09031936.00049810

Genetic susceptibility to asbestos related fibrotic pleuropulmonary changes

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Keywords: asbestos, fibrosis, pleural plaques, polymorphism, xenobiotic metabolizing enzyme

ABSTRACT

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The objective of this study was to determine whether genetic polymorphisms in enzymes that metabolize oxidative agents modify the individual susceptibility to develop asbestos and smoking related pleuropulmonary changes. Nine polymorphisms of six genes (*EPHX1, GSTM1*, *GSTM3*, *GSTP1, GSTT1,* and *NAT2)* were genotyped from 1008 Finnish asbestos-exposed workers. The genotype data was compared to signs of lung fibrosis and pleural thickenings, as well as with total lung capacity (TLC), single breath diffusing capacity for carbon monoxide (DL_{CO}), and specific diffusing capacity (DL_{CO}/VA).

The *GSTT1* deletion polymorphism was associated with fibrotic changes (*p*=0.003), DL_{CO} (*p*=0.02), and DL_{CO}/VA (*p*=0.002), and the *GSTM1* deletion polymorphism with the greatest thickness of pleural plaques (*p*=0.009). In further analysis, the *GSTT1* null genotype was found to pose over three-fold risk for severe fibrotic changes (OR 3.12, 95% CI 1.51-6.43) and around 2-fold risks for decreased DL_{CO} (OR 1.77, 95% CI 1.06-2.95) and DL_{CO}/VA (OR 2.37, 95% CI 1.33-4.23). In addition, the *GSTM1* null genotype showed an elevated risk (OR 1.36 95% CI 1.03-1.80) for thicker pleural plaques.

Our data suggests that inherited detoxification capacity may affect the development and severity of asbestos and smoking related non-malignant pulmonary changes.

INTRODUCTION

The adverse pulmonary effects of asbestos exposure are well characterized; it has been shown to cause lung cancer and mesothelioma, as well as non-malignant pulmonary and pleural disorders. Although the exact mechanism by which inhalation of asbestos fibres leads to lung tissue injury is still unclear, this may involve a persistent inflammatory response mediated by reactive oxygen species, cytokines, and pro-inflammatory factors [1, 2]. Currently, it is believed that individual susceptibility to asbestos and smoking related pulmonary diseases is modified by genetic polymorphism of enzymes that metabolize oxidative agents.

Interstitial lung fibrosis, also called asbestosis, is caused by the deposition of asbestos fibers in the lungs. Asbestosis is characterized by shortness of breath and cough and it is known to associate with restrictive lung function impairment as well as with the impairment of gas exchange properties of lung parenchyma measured as decreased pulmonary diffusing capacity (DL_{CO}) [3].

Other lesions induced by asbestos are pleural plaques, circumscribed thickenings of the pleura that may calcify over long periods of time. However, pleural thickenings unrelated to asbestos exposure are also very common [4].

Both environmental and genetic factors are believed to participate in the development of asbestosis. Yet, a very limited number of reports have discussed the potential genetic background of asbestos induced non-malignant pulmonary diseases, such as lung fibrosis and pleural plaques [5]. In addition, the findings of these studies have remained contradictory.

Glutathione *S*-transferases (GSTs) consist of a superfamily of xenobiotic metabolizing enzymes (XMEs) involved in conjugation of glutathione with various electrophilic compounds and products of oxidative stress. A widely studied member of the family, GSTM1, is involved in the metabolism of diol-epoxide derivatives of polycyclic aromatic hydrocarbons (PAHs) and reactive oxygen species. GSTT1, on the other hand, can detoxify methylating agents, pesticides and many chemicals present in cigarette smoke [6]. The GSTP1 enzyme, which is the most abundantly expressed GST in human lungs, shares some substrate specificity with GSTM1, and is active towards many epoxides of PAHs including benzo(a)pyrene. [7].

The glutathione *S*-transferase M1 (*GSTM1*) null genotype (gene deficiency) is one of the proposed risk factors for asbestos induced non-malignant pulmonary diseases. [8]. Conversely, the *GSTT1* null genotype has been suggested as a protective factor against the development of asbestosis [9]. In addition to *GSTM1* and *GSTT1*, the *GSTP1* genotype has been associated to asbestos-related pulmonary fibrosis [10]. However, subsequent studies have not confirmed these findings [11-13].

N-acetyltransferases (NATs) are involved in the metabolism of various xenobiotics including the aromatic and heterocyclic amines present in tobacco smoke and the diet [14, 15].We have previously found an association between the ~~(~~*NAT2*~~)~~ slow acetylator genotype and increased risk for both malignant (mesothelioma) and non-malignant (asbestosis and pleural plaques) pulmonary disorders among asbestos exposed workers [16, 17]. In a subsequent study, however, the *NAT2* slow acetylator genotypes were associated with decreased risk of mesothelioma in an Italian study population [18]. Moreover, the low-activity genotypes of microsomal epoxide hydrolase (EPHX1), a critical biotransformation enzyme that plays a dual role in the activation and detoxification of exogenous chemicals, such as epoxides and PAHs [19], were

positively associated with malignant mesothelioma in the Italian study population while they were negatively associated with this malignancy in the Finnish study population [18].

We aimed to further investigate the potential genetic risk factors for pulmonary fibrosis and other asbestos-induced non-malignant lesions among 1008 Finnish Caucasian asbestos exposed workers. Based on previous association studies, seven polymorphisms were chosen to be analyzed from the five abovementioned ~~(XME)~~ genes (*GSTM1*, *GSTT1*, *GSTP1*, *EPHX1* and *NAT2)*. In addition, two relevant functional polymorphisms were analyzed from *GSTM3* gene, another important member of the GST-family [20, 21].

METHODS

Study population

This study combines data from two previous screening studies aiming to detect early occupational chest diseases among asbestos exposed workers. The first study group (n=602) was recruited in 1996-1997 and consisted of asbestos exposed subjects who lived in Helsinki area, and had an asbestos related occupational disease and a smoking history [22, 23]. The second study group (n=633) was recruited in 2003-2004 and consisted of asbestos exposed persons from three geographic areas (Helsinki, Tampere, Turku), who were heavily exposed, had previously been diagnosed with asbestos related occupational disease, or had visited clinics of occupational medicine in Helsinki and Tampere for a clinical follow-up [24].

Altogether 178 of the subjects recruited in 2003-2004 had already participated in the first study conducted in 1996-1997. They were therefore excluded from the second

patient group in the present study before combining the data. In the combined study population, blood samples were available for 1021 subjects, 1013 of whom the genotyping data was successfully achieved. However, five more subjects were excluded because of missing background information. Thus, the final study group consisted of 1008 subjects (992 males, 16 females).

In order to see whether the genotype frequencies in the case group were similar to those in the general Finnish population, a demographic reference group consisting of 2155 Finnish Caucasians recruited from South-western Finland was also included in the study. The recruitment of the referents has been described in detail earlier [25]. For this study, 49 control subjects were excluded because they had been diagnosed with some form of malignant disease and one because of missing background information. Thus, the final demographic reference group consisted of 2105 subjects (1051 males, 1054 females). Unfortunately, since neither asbestos exposure history nor clinical data were available for them and detailed data on smoking was obtained only from the current smokers, this group could only be used as demographic referents and not as a reference group in the further statistical analyses.

An approval for the study was obtained from the local ethics committee according to the legislation at the time of the original study. All subjects gave an informed consent to participate in the study.

Radiological examinations

The lungs of the study subjects were imaged prone in full inspiration with four different scanners: in 1996-1997 the Picker PO 2000 (Picker International, Cleveland, USA) device was used with both HRCT and spiral CT scanning of the lungs. In 2003-2004 the

patients were HRCT scanned with Siemens Somatom Balance (Siemens Medical, Erlangen, Germany) in Helsinki, Siemens Somatom Plus 4 (Siemens Medical) in Tampere, and GE Light-speed 16 Advantage (GE Healthcare, Milwaukee, WI, USA) in Turku. In 2003-2004 the patients with high cancer risk were also imaged with spiral CT. The HRCT images were printed as hard copies and analyzed blindly by two (2003-2004) or three (1996-1997) radiologists.

The radiologists scored visually the signs of fibrosis using an arbitrary semiquantitative scale from 0 to V including one subclass between each class. Several signs of pleural changes were also recorded: the extent (cm^2) and greatest thickness of pleural thickenings (ILO "width": 0= no plaques, 1 $\leq$ 5mm, 2=5-10mm, 3 $\geq$ 10mm), and their degree of calcification (0=no, 1=sparse, 2=a considerable part of the pleural thickenings, 3=nearly all). The more detailed methods including intra- and inter-reader consistencies of readings have been reported previously [22].

Lung function examinations

The single breath diffusing capacity for carbon monoxide (DL_{CO}), specific diffusing capacity (diffusing capacity related to alveolar volume DL_{CO}/VA), and the total lung capacity (TLC) with the helium single-breath dilution method were measured by using a Masterlab Transfer or a Compact Lab Transfer device (Erich Jaeger, Würzburg, Germany) according to ERS recommendations [26]. Correction of DL_{CO} was done according to patient's actual hemoglobin levels. The lung function variables were handled as percent of Viljanen reference values [27] based on the distribution of values in the reference population. The pulmonary diffusing capacity and the total lung capacity were considered as decreased if DL_{CO} or DL_{CO}/VA was $< 74\%$ of predicted, and TLC was $< 80\%$ of predicted, respectively [27].

Genotyping analyses

DNA was extracted mechanically (King Fisher mL, Thermo) from whole blood using Biosprint 15 DNA Blood Kit (Qiagen, Germany) and stored at -20°C until use.

The presence or absence of *GSTM1* and *GSTT1* genes was detected by using a multiplex PCR method [28]. The 3-bp deletion of the *GSTM3* gene (rs1799735), the *GSTP1* exon 5 genotype (Ile105Val, rs1695), and the *EPHX1* exon 4 genotype (His139Arg, rs2234922) were determined by using PCR-based restriction fragment length polymorphism (PCR-RFLP) methods [20, 29, 30]. The *GSTM3* promoter area genotype (rs1332018), as well as the *EPHX1* exon 3 genotype (Tyr113His, rs1051740) and two *NAT2* genotypes (C282T, rs1041983; T341C, rs45532639) were determined using TaqMan allelic discrimination assays [21, 31, 32].

The two *NAT2* polymorphisms studied are considered sufficient for reliable prediction of the NAT2 phenotype in Caucasian populations [33]. Consequently, the subjects were categorized to fast and slow NAT2-acetylators according to the number of wild-type and variant alleles in these two polymorphic loci; subjects with two variant alleles were considered as slow acetylators, all others were included in the fast acetylator category [34]. Similarly, The *EPHX1* diplotypes were categorized in the putative phenotype groups (high, intermediate, low and very low activity) essentially according to Benhamou et al. [35].

For quality control, two independent readers interpreted the results and a random selection of 10 % of all samples was re-tested. No discrepancies were discovered in the replicate tests.

Statistical analysis

The associations between genotypes, fibrosis, pleural plaques, and lung function parameters (TLC, DL_{CO}, DL_{CO}/VA) were evaluated by using linear regression analysis.

Logistic regression analysis was used to further study the risk for lung function impairment, fibrotic changes, pleural plaques and their severity with certain genotype. Covariates used in the analysis were: sex, age, pack years of smoking (PYs), and years of asbestos exposure for fibrosis; sex, age, PYs, years of asbestos exposure, and FEV_1 for TLC, DL_{CO} and DL_{CO}/VA. Occasionally lacking covariate data was replaced with the group mean value (47 replacements for asbestos exposure years and 20 replacements for pack-years). All of the data analyses were performed by using the SPSS version 15.0 (SPSS Inc., Chicago, IL).

Our study (n=1008) has 80% power to detect odds ratios (ORs) from 1.54 to 1.80 depending on the minor allele frequency (13-46%). After stratifying the subjects according to radiologic signs, the OR detected with 80% power ranges from 1.55 to 1.81 (mild changes, n=917) and from 2.02 to 2.42 (severe changes, n=313). The calculations are based on a two-sided alpha of 0.05.

The χ^2 analysis was used to test for a deviation from the Hardy-Weinberg equilibrium. Since our study is a case only study, the Hardy-Weinberg equilibrium (HWE) was only calculated for the demographic references; it is expected that HWE is distorted in the case sample in the region of association [36].

RESULTS

The demographics and HRCT characteristics of the asbestos exposed workers are summarized in Table 1.

The genotype and phenotype frequencies of the studied polymorphisms in the asbestos exposed workers were similar to those in the demographic referents (Supplementary Table 1), among whom all the studied polymorphisms were in Hardy-Weinberg equilibrium (data not shown).

The fibrosis score was found to be associated with the *GSTT1* deletion polymorphism (p=0.003) and the greatest thickness of pleural plaques with the *GSTM1* deletion polymorphism (p=0.009) (Table 2). In addition, tendencies, although failing to reach statistical significance, were detected between the *GSTM1* deletion polymorphism and the extent of pleural plaques (p=0.071), and *GSTP1* Ile105Val polymorphism and the calcification of plaques (p=0.080). Moreover, the DL_{CO}- and DL_{CO}/VA -values were found to be associated with *GSTT1* deletion polymorphism (p=0.021 and p=0.002, respectively) and *NAT2* C282T polymorphism (rs1041983) (p=0.007 and p=0.006, respectively) (Table 3). Tendencies were also seen between the *GSTM3* promoter area polymorphism (rs1332018) (p=0.098), and *NAT2* T341C polymorphism (rs45532639) (p=0.076) and DL_{CO}/VA.

The observed significant associations were further analysed by stratifying the cases according to the existence and severity of radiologic signs and pulmonary diffusing capacity. The signs of fibrosis were considered mild if the radiologic score was < 2 and severe if the score was ≥ 2. The greatest thickness of pleural plaques was categorized to <5 mm and ≥5 mm and their extent to ≤100 cm^2 and >100 cm^2.

In the stratified analysis, the *GSTT1* null genotype was found to pose a three-fold risk for severe fibrotic changes (OR 3.12, 95% CI 1.51-6.43) (Table 4). Similarly, the

GSTM1 null genotype was found to slightly elevate the risk for ≥ 5 mm thick pleural plaques (OR 1.36, 95% CI 1.03-1.80) as well as the extent (≥100 cm^2) of plaques (OR 1.27, 95% CI 0.98-1.65), although the latter association was only of borderline statistical significance. The *GSTT1* deletion was also found to pose a 1.8-fold risk (OR 1.77, 95% CI 1.06-2.95) for decreased DL_{CO} and 2.4-fold risk (OR 2.37, 95% CI 1.33-4.23) for decreased DL_{CO}/VA (Table 4). When only subjects with fibrotic changes were considered, the risk for lower pulmonary diffusing capacity was somewhat higher; the ORs were 1.88 (95% CI 1.10-3.21) for DL_{CO} and 2.81 (95% CI 1.55-5.12) for DL_{CO}/VA (data not shown).

The associations between polymorphisms and pulmonary changes were studied also separately in the two original study populations (Supplementary Tables 2 and 3). The only association that showed statistical significance in both the sub-cohorts was between *GSTT1* deletion and fibrotic changes. Moreover, when the other significant findings were further analysed after stratifying the cases according to the existence and severity of radiologic signs and pulmonary diffusing capacity, the only statistically significant associations were between *GSTT1* deletion and both DL_{CO} and DL_{CO}/VA in the first (1996-1997) study population (data not shown).

When the potential gene-gene interactions were studied, no significant combined effects were seen for any of the studied genotype combinations.

DISCUSSION

Our study is the first one demonstrating a significant association between the *GSTT1* null genotype and severe fibrotic changes of the lungs confirmed with HRCT. This association was observed both in the whole study population and independently in both

the studied sub-cohorts. The previous studies have suggested a protective, if any, role for *GSTT1* deletion against the development of asbestosis [9, 12, 13].

We also found an association between the *GSTT1* null genotype and low diffusing capacity for carbon monoxide (DL_{CO} and DL_{CO}/VA). Since decreased diffusing capacity is characteristic to asbestosis, due to fibrotic process of lung parenchyma, this finding could simply reflect the correlation that was found between fibrosis and *GSTT1*. However, the association was seen in the whole study group, not just among the workers with fibrotic changes. Therefore, this observation supports our finding suggesting an association between the *GSTT1* genotype and development of lung fibrosis.

In addition, a novel association between the *GSTM1* deletion and pleural plaques was detected; previously the *GSTM1* null genotype has been proposed as a risk factor for asbestos related parenchymal rather than pleural disease [8].

The *GSTP1* genotype has also been associated with risk of developing asbestosis [10]. We did not, however, find any statistically significant associations between *GSTP1* polymorphism and non-malignant pulmonary changes.

Although our findings contrast with previous studies, they are consistent with the functional consequences of the associated polymorphisms; deletion of an XME gene is presumed to lead to decreased detoxification capacity and thereby to accumulation of oxidative agents. Oxidative stress, in turn, may lead to lung tissue injury and development of pulmonary disorders.

There are several potential explanations for the discordance between the outcomes of the present and previous studies. First, the sampling of the patients and controls varies

considerably. In addition, the inclusion criteria are very heterogeneous; some studies have utilized a strict diagnosis of asbestosis, while others have studied various indicators of parenchymal and pleural disease independently.

A big difference between the present and previous research frames is also the sample size; our study population comprised over a thousand asbestos exposed workers of whom almost 800 showed different degrees of fibrosis. In earlier studies the amount of fibrosis patients has been much smaller, varying from a few tens to a couple of hundreds. Consequently, the elevated risk that was detected only for severe changes in the present study could have been left unrecognized in the previous studies with much smaller sample sizes, where stratification was not appropriate.

We have previously found an association between the *NAT2* slow acetylator genotype and both malignant and non-malignant pulmonary disorders among asbestos exposed workers [16, 17]. The risk for non-malignant disorders was over four-fold for patients with a combination of *NAT2* slow acetylator genotype and *GSTM1* null genotype. Unfortunately, we could not confirm this finding in the present study. This could be due to the different inclusion criteria for cases; in the previous study all non-malignant disorders were grouped together, while in the current study fibrotic changes and pleural plaques were studied separately. In addition, the discrepancy could be partly explained by the exposure level of the subjects; in our earlier study , the workers were all heavily exposed to asbestos and the exposure was confirmed with a lung fiber burden measurement. In the current study, the workers were exposed to various levels of asbestos and no fiber measurements were performed. The sample size was also remarkably smaller in our earlier study.

Our study also has potential limitations: the patients were enrolled in three cities during two separate primary studies, four different CT scanners were used, and seven radiologists participated in the image reading. However, since the Finnish population is very homogenous and the three big cities, where the patients were enrolled, are all located in the southern Finland very near to each other, we do not believe that geographic origin at the time of the examination has caused any significant bias in the data analysis. Moreover, any inconsistency in image reading causes inaccuracy and thus random noise to the results leading in loss of power rather than in a systematic error. This increases the error variance in computations and the detected associations are therefore likely to be underestimated.

Due to the multiple comparisons performed, false-positive associations may have been observed. Most of the methods correcting for multiple testing are very conservative, and it is not clear, for example, what is the number of comparisons you should adjust for [37]. Based on previous findings, we had an a priori hypothesis for each polymorphism chosen, which reduces the need for correction. However, these results should be considered with caution until replicated in another study population.

In summary, our results suggest that the *GSTT1* deletion is associated with severe pulmonary fibrosis. Additionally, the *GSTM1* deletion may have a role in the development of pleural plaques. These observations suggest that polymorphisms in XME genes may indeed be involved in the development of asbestos induced non-malignant pulmonary diseases and their severity. This information may be of great value for the ongoing efforts against asbestos associated disorders; due to the long latency period between asbestos exposure and the disease, there are still a great number of people in working population who have been exposed previously and are currently at risk of getting lung changes originating from the asbestos exposure.

Acknowledgements

The authors thank Sirpa Hyttinen for the assistance with genotyping and sample management, Pauliina Toivio for the help with data handling, and Anu Voho for her valuable advices.

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Table 1 Characteristics of the asbestos exposed workers

	Mean (SD) or N (%)
Age, years	63.3 (7.3)
Male sex	992 (98.4%)
Smoking history	
Never smoker	144 (14.3%)
Ex-smoker	634 (62.9%)
Current smoker	230 (22.8%)
Pack years (n=988)	20.4 (16.9)
Years of asbestos exposure (n=943)	23.9 (10.8)
Fibrosis score (n=775)	0.93 (0.66)
Pleural plaques	
Greatest thickness (n=1006)	1.88 (0.67)
Extent, cm^2 (n=1004)	103.5 (72.5)
Calcification (n=977)	1.34 (0.94)
Lung function	
TLC (n=958)	87.29 (13.65)
DL_{CO} (n=963)	90.98 (20.23)
DL_{CO}/VA (n=965)	98.18 (18.49)

DL_{CO} = Single breath diffusing capacity for carbon monoxide, % predicted; DL_{CO} /VA = specific diffusing capacity, % predicted; TLC = total lung capacity, % predicted

n= 1008 except as noted

Table 2 Association between genetic polymorphisms and pleural plaques

Phenotype	**Gene**	**Polymorphism**	**β**[#]	***p*-value**
Fibrosis	*GSTT1*	deletion	-0.09	0.003*
	GSTM1	deletion	-0.024	0.431
	GSTM3	rs1799735	-0.30	0.329
		rs1332018	0.005	0.180
	GSTP1	rs1695	0.002	0.937
	EPHX1	rs1051740	-0.025	0.410
		rs2234922	-0.014	0.656
		putative phenotype	0.017	0.571
	NAT2	rs1041983	0.001	0.962
		rs45532639	-0.046	0.130
		acetylator status	0.043	0.153
Pleural plaques				
Greatest thickness	*GSTT1*	deletion	0.016	0.604
	GSTM1	deletion	-0.008	0.009*
	GSTM3	rs1799735	0.008	0.802
		rs1332018	0.004	0.890
	GSTP1	rs1695	-0.003	0.912
	EPHX1	rs1051740	-0.013	0.676
		rs2234922	-0.004	0.903
		putative phenotype	0.010	0.750
	NAT2	rs1041983	-0.22	0.454
		rs45532639	-0.006	0.850
		acetylator status	0.039	0.189
Extent	*GSTT1*	deletion	0.017	0.588
	GSTM1	deletion	-0.055	0.071
	GSTM3	rs1799735	0.021	0.481
		rs1332018	-0.001	0.962
	GSTP1	rs1695	0.020	0.519
	EPHX1	rs1051740	-0.018	0.547
		rs2234922	-0.034	0.268
		putative phenotype	-0.004	0.900
	NAT2	rs1041983	-0.43	0.157
		rs45532639	0.005	0.858
		acetylator status	0.041	0.178
Calcification	*GSTT1*	deletion	0.042	0.146
	GSTM1	deletion	-0.016	0.573
	GSTM3	rs1799735	-0.029	0.316
		rs1332018	0.019	0.516
	GSTP1	rs1695	0.051	0.080
	EPHX1	rs1051740	-0.016	0.591
		rs2234922	-0.002	0.943
		putative phenotype	0.011	0.706
	NAT2	rs1041983	-0.037	0.199
		rs45532639	0.043	0.136
		acetylator status	-0.009	0.769

Covariates used in the analysis: sex, age, pack-years, and years of asbestos exposure
#Standardized coefficient β
* p<0,05

Table 3 Association between genetic polymorphisms and pulmonary function

Phenotype	Gene	Polymorphism	β#	*p*-value
TLC	*GSTT1*	deletion	0.027	0.421
	GSTM1	deletion	0.036	0.851
	GSTM3	rs1799735	0.007	0.585
		rs1332018	0.024	0.126
	GSTP1	rs1695	-0.025	0.146
	EPHX1	rs1051740	0.032	0.947
		rs2234922	0.010	0.765
		putative phenotype	-0.025	0.826
	NAT2	rs1041983	0.028	0.256
		rs45532639	-0.015	0.111
		acetylator status	0.003	0.314
DL_{CO}	*GSTT1*	deletion	0.062	0.021*
	GSTM1	deletion	0.010	0.971
	GSTM3	rs1799735	0.052	0.221
		rs1332018	-0.039	0.383
	GSTP1	rs1695	0.012	0.948
	EPHX1	rs1051740	0.047	0.616
		rs2234922	0.032	0.525
		putative phenotype	-0.018	0.974
	NAT2	rs1041983	-0.075	0.007*
		rs45532639	0.062	0.132
		acetylator status	0.007	0.319
DL_{CO}/VA	*GSTT1*	deletion	0.095	0.002*
	GSTM1	deletion	-0.013	0.650
	GSTM3	rs1799735	0.017	0.585
		rs1332018	-0.053	0.098
	GSTP1	rs1695	0.035	0.243
	EPHX1	rs1051740	0.047	0.171
		rs2234922	0.022	0.529
		putative phenotype	-0.022	0.540
	NAT2	rs1041983	-0.086	0.006*
		rs45532639	-0.057	0.076
		acetylator status	0.013	0.647

DL_{CO} = Single breath diffusing capacity for carbon monoxide, % predicted; DL_{CO}/VA = specific diffusing capacity, % predicted; TLC = total lung capacity, % predicted
Covariates used in the analysis: sex, age, pack-years, years of asbestos exposure, and FEV_1
#standardized coefficient β
* p<0,05

Table 4 Distribution of *GSTT1* and *GSTM1* genotypes according to the existence and severity of radiologic changes and pulmonary diffusing capacity

Fibrosis

Genotype	No radiologic changes[#]	Radiologic changes	OR (95 % CI)*	Mild[¶] changes	OR (95 % CI)*	Severe[+] changes	OR (95 % CI)*
GSTT1							
Present	198 (89.2)	681 (86.6)	1.0	611 (87.9)	1.0	70 (76.9)	1.0
Null	24 (10.8)	105 (13.4)	1.35 (0.83-2.18)	84 (12.1)	1.20 (0.73-1.95)	21 (23.1)	3.12 (1.51-6.43)

Pleural plaques

Greatest thickness				Extent			
Genotype	<5mm[#]	≥5mm	OR (95 % CI)*	Genotype	<100cm^{2}[#]	≥100cm^{2}	OR (95 % CI)*
GSTM1							
Present	181 (58.2)	353 (50.8)	1.0	Present	323 (55.6)	210 (49.6)	1.0
Null	130 (41.8)	342 (49.2)	1.36 (1.03-1.80)	Null	258 (44.4)	213 (50.4)	1.27 (0.98-1.65)

Diffusing capacity

DL_{CO}				DL_{CO}/VA			
Genotype	<74% pred[#]	≥ 74% pred	OR (95 % CI)[§]	Genotype	<74% pred[#]	≥ 74% pred	OR (95 % CI)[§]
GSTT1							
Present	145 (81.9)	686 (87.8)	1.0	Present	72 (77.4)	761 (87.8)	1.0
Null	32 (18.1)	95 (12.2)	1.77 (1.06-2.95)	Null	21 (22.6)	106 (12.2)	2.37 (1.33-4.23)

Data are presented as n (%) except as noted. DL_{CO} = Single breath diffusing capacity for carbon monoxide, % predicted; DL_{CO}/VA specific diffusing capacity, % predicted

[#] Reference category

* Adjusted for age, sex, pack-years and years of asbestos exposure

[¶] The changes were considered as mild if the radiologic score was < 2

[+] The changes were considered as severe if the radiologic score was ≥ 2

[§] Adjusted for age, sex, pack-years, years of asbestos exposure and FEV_1