

research article

The influence of genetic variability on the risk of developing malignant mesothelioma

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Background. Malignant mesothelioma is a rare cancer with poor outcome, associated with asbestos exposure. Reactive oxygen species may play an important role in the mechanism of carcinogenesis; therefore, genetic variability in antioxidative defence may modify an individual's susceptibility to this cancer. This study investigated the influence of functional polymorphisms of *NQO1*, *CAT*, *SOD2* and *hOGG1* genes, gene-gene interactions and gene-environment interactions on malignant mesothelioma risk.

Patients and methods. In total, 150 cases with malignant mesothelioma and 122 controls with no asbestos-related disease were genotyped for *NQO1*, *CAT*, *SOD2* and *hOGG1* polymorphisms.

Results. The risk of malignant mesothelioma increased with smoking, odds ratio (OR) 9.30 [95% confidence interval (CI): 4.83–17.98] and slightly with age, OR 1.10 (95% CI: 1.08–1.14). Medium and high asbestos exposures represented 7-times higher risk of malignant mesothelioma compared to low exposure, OR 7.05 (95% CI 3.59–13.83). *NQO1* rs1800566 was significantly associated with increased malignant mesothelioma risk, OR 1.73 (95% CI 1.02–2.96). Although there was no independent association between either *CAT* rs1001179 or *hOGG1* rs1052133 polymorphism and malignant mesothelioma, interaction between both polymorphisms showed a protective effect, OR_{int} 0.27 (95% CI 0.10–0.77).

Conclusions. Our findings suggest a role of both genetic variability in antioxidative defence and repair as well as the impact of gene-gene interactions in the development of malignant mesothelioma. The results of this study could add to our understanding of pathogenesis of malignant mesothelioma and contribute to prevention and earlier diagnosis of this aggressive cancer.

Key words: antioxidative enzymes; genetic polymorphism; malignant mesothelioma

Introduction

Malignant mesothelioma (MM) is a rare and aggressive disease with poor survival. It has been associated with occupational and/or environmental exposure to asbestos in more than 86% of patients with this disease.^{1,2} Malignant mesothelioma most commonly arises from pleura (65%–70%), peritoneum (30%) and very rarely other serous surfaces (1%).^{3,4} Global incidence is expected to peak 30 to 40 years after the peak of asbestos usage that oc-

curred in the 1960s and 1970s.^{3,5} However, recent studies still show a rise in incidence.⁶

The implication of asbestos exposure in MM has been validated, but the mechanism of carcinogenesis is not yet completely understood. Asbestos fibre components, specifically iron, are hypothesized to contribute to reactive oxygen species (ROS) production. Iron catalyses both Fenton and Haber-Weiss reactions which produce hydroxyl radical (HO) from peroxide (H₂O₂).⁷ Furthermore, all types of asbestos may cause frustrated phagocytosis in

the macrophages, which produces ROS, reactive nitrogen species (RNS), cytokines, chemokines, proteases and growth factors.^{8,9} This may lead to DNA damage, genomic instability and a malignant transformation of mesothelial cells.⁹ A number of studies show that ROS and RNS and inflammation could have a central role in asbestos fibre toxicity.¹⁰⁻¹³

On the other hand, antioxidative enzymes such as catalase (CAT), superoxide dismutases (SOD-s), and NAD(P)H quinone dehydrogenase 1 (NQO1) participate in the enzymatic defence against ROS and RNS.¹⁰ When the activity of these enzymes is decreased or changed, ROS concentrations increase and DNA damage may occur. One of the most important repair enzymes for oxidative DNA damage repair is human 8-oxoguanine glycosylase 1 (hOGG1). Functional polymorphisms that influence the expression level or activity have been reported in the genes coding for all these enzymes. CAT helps to maintain the oxidative balance by catalysing H₂O₂ to H₂O and O₂.^{14,15} Numerous polymorphisms of CAT gene (CAT) have been described, rs1001179 being the most commonly studied one. It causes cytosine (C) to thymine (T) change at position -262 in the promoter region (c.262C>T).¹⁴ Our previous study investigating the association of this polymorphism with asbestosis found a slight increase in the risk of the TT genotype.¹⁶ Superoxide dismutases (SOD) convert superoxide into H₂O₂ and O₂. SOD2 is found in mitochondria, where the amount of ROS is very high. The most common polymorphism is rs4880, resulting in C to T substitution at position 201 (c.201C>T), which causes the change of alanine to valine at position 16 (p.Ala16Val). Several studies associate the 6q25 chromosome deletion with certain forms of cancer, which is why some authors consider SOD2 to be a tumour suppressor gene.^{17,18} HOGG1 catalyses the repair of 8-oxoguanine that may result from ROS damage to the DNA. Functional polymorphisms of the hOGG gene may impact DNA repair. In rs1052133 polymorphism, C replaces G in exon 7, causing the substitution of serine with cysteine in codon 326 (p.Ser326Cys). Although a changed structure of the polymorphic enzyme has not been proved, several studies have shown the association between hOGG1 Ser326Cys polymorphism and lung cancer risk.^{19,20} NQO1 catalyses the reduction of quinones to hydroquinones, preventing the formation of free radicals. The most frequently studied single nucleotide polymorphism (SNP), rs1800566, results in C to T change (c.609C>T), which causes proline to serine substitution (p.Pro187Ser).²¹ Some studies found this polymorphism to be associated

with an increased risk of several malignant diseases: lung cancer, colorectal cancer, breast cancer and bladder cancer.^{22,23}

Only few studies have investigated the interplay between asbestos exposure and genetic variability in antioxidant defence system in MM so far.²⁴⁻²⁶ Nevertheless, the interaction between asbestos exposure and genetic susceptibility due to genetic polymorphism of antioxidant enzymes has been shown for asbestosis.²⁷ We have previously described the association between SOD2 Ala/Ala genotype and asbestosis²⁸ as well as association between CAT -262 TT genotype and asbestosis.¹⁶ Landi *et al.* reported on the association between SOD2 and the risk of MM²⁵, but according to our knowledge and available literature, the impact of NQO1, CAT and hOGG1 polymorphisms on the risk of developing MM has not been studied so far.

This study aimed to investigate whether functional polymorphisms in NQO1, CAT, SOD2 and hOGG1 genes influence the risk of MM, to investigate the interactions between genetic variability in antioxidative and DNA repair mechanisms and to investigate the interactions between asbestos exposure and the investigated polymorphisms in MM patients.

Patients and methods

Patients

The study included 159 MM patients (cases), treated at the Institute of Oncology Ljubljana between March 2007 and January 2013, along with 122 controls, who were occupationally exposed to asbestos in the asbestos cement manufacturing plant of Salonit Anhovo, Slovenia, but did not develop any disease associated with asbestos exposure. All patients and controls were from Central European Caucasian (Slovenian) population. The study was approved by the Slovenian Ethics Committee for Research in Medicine and was carried out according to the Helsinki Declaration. The subjects were included in the study after providing a written informed consent.

Methods

The diagnosis of MM was made by means of thoracoscopy or video-assisted thoracoscopic surgery (VATS) in patients with pleural MM and by means of laparoscopy or laparotomy in peritoneal MM. The diagnosis was confirmed histopathologically by an experienced pathologist.²

TABLE 1. Clinical characteristics of MM patients and controls

	Controls (n = 122)	MM patients (n = 159)	Test	p
Gender N (%)				
Male	88 (72.1)	123 (77.4)		
Female	34 (27.9)	36 (22.6)	$\chi^2 = 1.008$	0.315
Age (years), median (range)	54 (48–60.3)	65 (57–72)	U = 4392.000	< 0.001
No. of smokers ¹ (%)	13 (10.7)	80 (52.6)	$\chi^2 = 53.185$	< 0.001
Asbestos exposure ² N (%)				
No	0 (0.0)	25 (16.6)		
Yes	122 (100.0)	126 (83.4)		
Asbestos exposure ³ N (%)				
Low	96 (78.7)	22 (34.4)	$\chi^2 = 35.941$	< 0.001
Medium	11 (9.0)	21 (32.8)		
High	15 (12.3)	21 (32.8)		
Asbestos exposure ³ N (%)				
Low	96 (78.7)	22 (34.4)	$\chi^2 = 35.541$	< 0.001
Medium and high	26 (21.3)	42 (65.6)		

MM = Malignant mesothelioma

¹ data missing for 7 MM patients; ² data missing for 8 MM patients; ³ data available for all controls and 64 MM patients

The diagnosis of “no asbestos related disease” in the control group was confirmed by the experts of the Board for Recognition of Occupational Asbestos Diseases at the Clinical Institute of Occupational Medicine, which consisted of an occupational physician, pulmonologist and radiologist, as previously described.¹⁶

A personal interview with each of the subjects was conducted to get the data about smoking using a standardized questionnaire.²⁹ To determine asbestos exposure, a semiquantitative method was used. For all the controls, data on cumulative asbestos exposure in fibres/cm³-years were available from the previous study.²⁹ Data on cumulative asbestos exposure were also available for 27 MM patients. Based on these data, we divided the subjects into three groups: low (< 11 fibres/cm³-years), medium (11–20 fibres/cm³-years) and high (> 20 fibres/cm³-years) asbestos exposure. For the rest of the patients with MM, a thorough work history was obtained and where enough information was available, their exposures were compared with those from the group of patients with known cumulative asbestos exposure and were correspondingly divided into three groups with presumed low, medium and high asbestos exposures.² Thus, 37 MM patients were assigned to one of these three groups, but for 95 MM patients epidemiological data were not sufficient to allow the assignment of patients to one of the groups; consequently, they were only categorized as exposed or non-exposed. The influence of asbestos exposure on MM risk was determined in the subgroup of patients where the asbestos exposure was known or could be assessed.

DNA of the MM patients and some controls without asbestos related diseases was available from our previous studies^{2,30}, DNA of the rest of the controls was isolated from peripheral venous blood samples using FlexiGene DNA kit (Qiagen, Hilden, Germany).

Real-time polymerase chain reaction (PCR) based TaqMan assays were used for the analysis of *NQO1* rs1800566, *CAT* rs1001179, *SOD2* rs4880 and *hOGG1* rs1052133 polymorphisms as recommended by the manufacturer (Thermo Fisher Scientific, SNP genotyping assay C_2091255_30, C_11468118_10, C_8709053_10 and C_3095552_1_, respectively). Genotyping was performed blinded regarding the study endpoints and repeated in 20% of samples to check for genotyping accuracy and all the genotypes were concordant. Amplification was not successful in 11 subjects for *NQO1*, in 2 for *CAT*, in 6 for *SOD2* and in 7 subjects for *hOGG1* polymorphism.

Statistical methods

Standard descriptive statistics were first performed. Next, *t*-tests for differences of means of variables between the cases and controls were calculated, and Mann-Whitney (U) test was performed. The dominant genetic model was used for all the comparisons. To analyse the association between genotypes, cumulative asbestos exposure, and standard confounders (age, sex) and MM, univariate logistic regression was first used, followed by multivariate logistic regression modelling. A possible synergistic effect between genotypes and

TABLE 2. The distribution of antioxidative and repair gene polymorphisms in MM patients and controls and risk of MM

Polymorphism	Genotype	MM patients	Controls	Unadjusted risk		Adjusted by age		Adjusted by smoking		Adjusted by asbestos exposure	
		N (%)	N (%)	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p	OR (95% CI)	p
NQO1 rs1800566 ¹	CC	98 (62.0)	82 (73.9)	1.73 (1.02–2.96)	0.043	1.63 (0.91–2.95)	0.103	1.88 (1.04–3.41)	0.037	1.72 (0.83–3.57)	0.124
	CT	57 (36.1)	27 (24.3)								
	TT	3 (1.9)	2 (1.8)								
CAT rs1001179	CC	79 (50.0)	70 (57.4)	1.35 (0.84–2.17)	0.220	1.21 (0.71–2.05)	0.484	1.47 (0.86–2.51)	0.159	1.45 (0.73–2.88)	0.288
	CT	64 (40.5)	47 (38.5)								
	TT	15 (9.5)	5 (4.1)								
SOD2 rs4880 ²	CC	44 (27.7)	31 (25.8)	0.89 (0.52–1.52)	0.661	0.76 (0.41–1.39)	0.371	0.95 (0.52–1.73)	0.857	0.81 (0.38–1.73)	0.578
	CT	81 (50.9)	52 (43.3)								
	TT	31 (19.5)	37 (30.8)								
hOGG1 rs1052133 ³	CC	99 (62.3)	82 (70.1)	1.37 (0.82–2.29)	0.225	1.42 (0.80–0.51)	0.232	1.36 (0.77–2.41)	0.286	1.77 (0.85–3.66)	0.125
	CG	52 (32.7)	32 (27.4)								
	GG	6 (3.8)	3 (2.6)								

CAT = catalase; hOGG1 = human 8-oxoguanine glycosylase 1; MM = Malignant mesothelioma; NQO1 = NAD(P)H quinone dehydrogenase 1; OR = odds ratio; SOD2 = superoxide dismutase

For determining MM risk, carriers of at least one polymorphic allele were compared to non-carriers.
¹missing data for 10 patients; ²missing data for 2 patients; ³missing data for 4 patients

cumulative asbestos exposure was investigated by using dummy variables. *P*-values less than 0.05 were considered as statistically significant.

Results

The clinical characteristics of MM patients and controls are presented in Table 1. There was no statistical difference in gender between the cases and controls (*p* = 0.315), but MM patients were notably older (*p* < 0.001) and a much higher number of the patients were smokers (*p* < 0.001). All the controls (122) and 126 (83.4%) MM patients had been exposed to asbestos. For all the controls and 64 (50.8%) MM patients, asbestos exposure could be categorized into the groups. Among the subjects with known asbestos exposure, the MM patients had a significantly higher asbestos exposure compared to asbestos exposed subjects without any asbestos related disease (*p* < 0.001, Table 1).

Univariate regression logistic analysis has shown that the risk of MM was influenced by smoking, age and asbestos exposure, but not by gender. The risk of MM was increased in smokers (OR = 9.30; 95% CI = 4.83–17.98; *p* < 0.001) and older patients (OR = 1.10; 95% CI = 1.08–1.14; *p* < 0.001). Compared to a low exposure to asbestos, medium and high asbestos exposures increased the risk of MM 7-fold (OR = 7.05; 95% CI = 3.59–13.83; *p* < 0.001). Gender did not influence MM risk (OR = 0.76; 95% CI = 0.44–1.30; *p* = 0.316).

Genotype frequencies for controls and MM patients are presented in Table 2. Minor allele frequencies were 13.9% for NQO1 rs1800566, 22.4% for CAT rs1001179, 52.5% for SOD2 rs4880 and 18.8% for hOGG1 rs1052133. In controls, all SNPs were in Hardy-Weinberg equilibrium (all *p* > 0.05). The association between MM and the investigated polymorphisms was tested with univariate logistic regression using a dominant genetic model. The carriers of at least one polymorphic NQO1 allele

TABLE 3. Gene-gene interactions between rs1800566 NAD(P)H quinone dehydrogenase 1 (NQO1), rs1001179 catalase (CAT), rs4880 superoxide dismutase 2 (SOD2), and rs1052133 human 8-oxoguanine glycosylase 1 (hOGG1)

Gene 1			Gene 2			Interaction	
Genotypes	OR (95% CI)	p	Genotypes	OR (95% CI)	p	OR (95% CI)	p
NQO1 rs1800566 CT+TT vs.CC	1.73 (1.02–2.96)	0.043	hOGG1 rs1052133 CG+GG vs.CC	1.37 (0.82–2.29)	0.225	1.22 (0.36–4.13)	0.75
CAT rs1001179 CT+TT vs.CC	1.35 (0.84–2.17)	0.220	hOGG1 rs1052133 CG+GG vs.CC	1.37 (0.82–2.29)	0.225	0.27 (0.10–0.77)	0.014
SOD2 rs4880 CT+TT vs.CC	0.89 (0.52–1.52)	0.661	hOGG1 rs1052133 CG+GG vs.CC	1.37 (0.82–2.29)	0.225	0.78 (0.25–2.43)	0.669

OR = odds ratio

(CT and TT genotypes) had an increased risk of MM compared to those with CC genotype (OR = 1.73; 95% CI = 1.02–2.96; $p = 0.043$). No association was observed between MM and other genetic polymorphisms (Table 2).

Multivariate analysis was used to determine the combined effect of genetic determinants and clinical variables such as smoking, age and asbestos exposure. The association between *NQO1* and MM risk remained significant after adjustment for smoking, but the risk was slightly lower when adjusted by age or asbestos exposure. The association between other investigated polymorphisms and MM risk remained nonsignificant also after taking into account the effect of age, smoking and asbestos exposure (Table 2).

Next, gene-gene interactions between the investigated *NQO*, *CAT*, *SOD2* and *hOGG1* genotypes and the interactions between genotypes and asbestos exposure were calculated. The interaction between *CAT* rs1001179 and *hOGG1* rs1052133 had a protective effect on the risk of MM (OR_{int} = 0.27; 95% CI = 0.10–0.77; $p = 0.014$). On the other hand, no gene-gene interactions were observed between other investigated polymorphisms (Table 3).

Finally, we investigated the influence of interactions between polymorphisms and asbestos exposure on the risk of MM, but no interaction was found (Table 4).

Discussion

The association between asbestos exposure and MM has been clearly proved, but not much has been known about the influence of genetic polymorphisms that may modify the risk of developing this aggressive cancer. Our present study investigated the effect of genetic polymorphisms of some of the most important enzymes involved in removal of ROS and RNS (*NQO1*, *CAT*, *SOD2*) and DNA damage repair (*hOGG1*) on the risk of MM, as well as the impact of interactions between the observed genetic polymorphisms and between genetic polymorphisms and asbestos exposure on the risk of developing this cancer.

In the study, we have found that smoking increased the risk of MM. It has been well proved that exposure to asbestos fibres results in an increased generation of ROS.^{8,9} Many studies have also investigated the association between ROS and carcinogenesis, caused by tobacco smoke.³¹ According to the free radical hypothesis of aging, ROS and RNS can drive the accumulation of cell and DNA dam-

TABLE 4. The influence of interactions between the investigated polymorphisms and asbestos exposure on risk of malignant mesothelioma

Polymorphism	OR	CI (95%)	p
<i>NQO1</i> rs1800566	1.56	0.35–6.86	0.560
<i>CAT</i> rs1001179	1.57	0.39–6.29	0.522
<i>SOD2</i> rs4880	1.13	0.24–5.18	0.880
<i>hOGG1</i> rs1052133	0.50	0.12–2.14	0.352

CAT = catalase; *hOGG1* = human 8-oxoguanine glycosylase 1; *NQO1* = NAD(P)H quinone dehydrogenase 1; OR = odds ratio; *SOD2* = superoxide dismutase 2

age³² leading to carcinogenesis and cancer.^{33–36} The combined effect of both asbestos and smoking may thus greatly increase the amount of ROS in the cells and may cause more DNA damage than smoking or asbestos exposure alone. That could explain the observed higher risk of MM among smokers exposed to asbestos compared to non-smokers.

Our study also showed a slight increase in MM susceptibility in older patients, which is in line with other studies in which MM is found predominantly as disease of the elderly.³⁷ Mortality due to pleural MM increased between 75 and 89 years of age and in peritoneal MM between 65 and 84 years of age.³⁷ This may be due to the long latency time, which is the period from the first exposure to the diagnosis of MM, and can range from 20 to over 50 years.³⁸ There are many factors affecting the latency period, including dose response, age, gender and location of MM.³⁹

An important finding of our study is that medium and high asbestos exposures increase the risk of MM by 7-fold compared to low asbestos exposure. This is in line with the results of some studies that have also reported that the MM risk is related to the amount of exposure.^{40–43} An Australian study reported an increased risk of MM with higher and longer occupational or environmental exposure to asbestos.^{40,41} A Norwegian study also observed a correlation between the duration of occupational exposure and risk of MM^{42,43} However, in our previous study, low levels of asbestos exposure were reported in almost 36% of patients with MM.²

Another important finding of the current study indicates a higher risk of MM among subjects with the *NQO1* rs1800566 T allele. According to the available literature, the association between *NQO1* polymorphisms and MM has not been investigated yet. However, some studies have found an increased risk of lung cancer²¹, colorectal can-

cer⁴⁴ and bladder cancer²² among the carriers of the polymorphic allele. On the other hand, a risk of MM was not statistically significantly increased for other investigated polymorphisms. Contrary to the findings of this study, the only other study investigating the *SOD2* polymorphism in relation to MM risk²⁵ showed an increased risk of pleural MM in *SOD2* Ala/Ala genotype. Regarding other asbestos related diseases, we have previously reported an association between *SOD2* Ala/Ala genotype and higher risk of asbestosis.²⁸ Furthermore, a significantly higher risk of lung cancer was reported in carriers of both Ala/Val and Val/Val genotypes.^{45,46}

Even though there was no association between *CAT* rs1001179 or *hOGG1* rs1052133 alone and MM, one of the key findings of this study was that the interactions between *CAT* rs1001179 and *hOGG1* rs1052133 have a protective effect on the risk of MM. This can be explained by the fact that *CAT* as an antioxidative enzyme constitutes a part of the primary defence system against ROS, while *hOGG1* as a repair enzyme removes oxidized bases such as 8-oxoguanine. According to the above-mentioned mechanisms of defence against ROS, we can consider our observations as biologically plausible. We have also previously reported slightly increased risk of asbestosis among the carriers of the *CAT* rs1001179 TT genotype.¹⁶ Furthermore, Erculj *et al.* have observed an association between *hOGG1* Ser326Cys polymorphism and higher DNA damage levels in healthy young population.⁴⁷

A limitation of this study was that MM patients were significantly older than controls, however we accounted for that with adjustment for age in the statistical analysis. Furthermore, cumulative asbestos exposure could not be determined for all MM patients, as proper assessment is very difficult, especially for environmental or occasional exposure. Therefore, some of the analyses were only performed on the subgroup of MM patients. On the other hand, our study is one of the few that investigated gene-gene as well as gene-environment interactions in MM patients.⁴⁸ Neri *et al.* analysed a different set of genes, including several glutathione S-transferases that also contribute to antioxidative defence mechanisms and also showed the presence of gene-gene interactions as well as gene-environment interactions in the development of MM.⁴⁸ Therefore, further studies including a larger number of subjects with well-defined asbestos exposure are needed to elucidate the role of gene-environment interactions in the development of MM. Considering that pathogenesis of MM is still not completely understood, polymorphisms of other

enzymes that could affect the removal of ROS and RNS and other DNA repair mechanisms, also need to be investigated.

In conclusion, our study showed for the first time that *NQO1* polymorphism influences the risk of MM both independently and after adjustment by smoking. Another key observation is the protective effect of the interaction between *CAT* rs1001179 and *hOGG1* rs1052133 polymorphisms, indicating the importance of interaction between antioxidative and DNA repair mechanisms. The results of this and future studies will improve our understanding of MM pathogenesis and may consequently enable better preventive measures for the exposed populations, earlier diagnosis and new approaches to treatment of this aggressive malignant disease.

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