research article

The influence of genetic variability in *IL1B* and *MIR146A* on the risk of pleural plaques and malignant mesothelioma

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Background. Asbestos exposure is associated with the development of pleural plaques as well as malignant mesothelioma (MM). Asbestos fibres activate macrophages, leading to the release of inflammatory mediators including interleukin 1 beta (IL-1β). The expression of IL-1β may be influenced by genetic variability of *IL1B* gene or regulatory microRNAs (miRNAs). This study investigated the effect of polymorphisms in *IL1B* and *MIR146A* genes on the risk of developing pleural plaques and MM.

Subjects and methods. In total, 394 patients with pleural plaques, 277 patients with MM, and 175 healthy control subjects were genotyped for *IL1B* and *MIR146A* polymorphisms. Logistic regression was used in statistical analysis.

Results. We found no association between *MIR146A* and *IL1B* genotypes, and the risk of pleural plaques. *MIR146A* rs2910164 was significantly associated with a decreased risk of MM (OR = 0.31, 95% CI = 0.13–0.73, p = 0.008). Carriers of two polymorphic alleles had a lower risk of developing MM, even after adjustment for gender and age (OR = 0.34, 95% CI = 0.14–0.85, p = 0.020). Among patients with known asbestos exposure, carriers of at least one polymorphic *IL1B* rs1143623 allele also had a lower risk of MM in multivariable analysis (OR = 0.50, 95% CI = 0.28–0.92, p = 0.025). The interaction between *IL1B* rs1143623 and *IL1B* rs1071676 was significantly associated with an increased risk of MM (p = 0.050).

Conclusions. Our findings suggest that genetic variability of inflammatory mediator IL-1β could contribute to the risk of developing MM, but not pleural plaques.

Key words: asbestos; genetic variation; malignant mesothelioma; miRNA; pleural plaques

Introduction

Asbestos exposure is related to several pleural diseases, such as pleural plaques, diffuse pleural thickenings, pleural effusions and malignant mesothelioma (MM). MM is an aggressive form of cancer found on the mesothelium, generally on the pleura (65%), peritoneum (30%) or other serosal membranes (1%).[1,2]

MM is often diagnosed in its later stages, is rarely operable and can respond poorly to conventional chemotherapy.[3] Clinical signs and symptoms are uncharacteristic and reminiscent of many other pulmonary diseases. Patients often experience dyspnea, chest pain, weight loss and fatigue. Only a small proportion of MM patients are asymptomatic at the time of diagnosis.[2,4] Average life expectancy is around 7 months with support

therapy and 12 months with chemotherapy.[5] MM most often occurs in patients older than 65 years.[2,6] Epidemiological studies have shown that the main cause of MM is asbestos exposure, with the incidence of this cancer still increasing due to the long latent period.[7] Genetic factors have also been suggested to influence the development of MM; patients often have mutations in tumour suppressor genes, such as *BAP1*, *CDKN2A* and *NF2*.[8,9]

Along with MM, asbestos exposure is also related to the development of pleural plaques. Pleural plaques are white and yellow thickenings of pleura, often asymmetrical and bilateral. Histologically, they are acellular, composed of hyalinised collagen, which is covered by one layer of mesothelial cells. Half of the patients with a history of asbestos exposure develop pleural plaques, typically 20 to 30 years after exposure. The risk of pleural plaques rises with the length of asbestos exposure.[10] It has been proposed that inflammation caused by asbestos is involved in the pathogenesis of both pleural plaques and MM.[3,11] Asbestos fibres are known to trigger the release of inflammatory mediators, which leads to the downregulation of apoptosis.[3]

After inhalation, asbestos fibres reach pleural space and are deposited in mesothelial cells.[12] This leads to local inflammatory response and proliferation of mesothelial cells. *In vitro* studies have shown that the fibres induce inflammation and apoptosis and most of the tissue damage is related to elevated interleukin 1 beta (IL-1β).[3] Macrophages accumulate near asbestos deposits and release cytokines, such as IL-1β and tumour necrosis factor alpha (TNF-α).[8]

In response to asbestos, an intrinsic inflammatory mechanism triggers inflammation via inflammasome NLRP3, which is NLR family pyrin domain containing 3, activated by danger-associated molecular patterns (DAMP) or pathogen-associated molecular patterns (PAMP).[13,14] NLRP3 inflammasome is a protein complex of NLRP3, apoptosis-associated speck-like protein (ASC) and caspase-1, found in macrophages, which triggers a type of apoptosis, known as piroptosis.[11,13,15] The activation of NLRP3 inflammasome increases the production of IL-1β from its precursor, mediated by caspase-1 and pro-inflammatory mediators from macrophages.[8,15,16] IL-1β, coded by *IL1B* gene, is an inflammatory mediator, found during chronic inflammation and a key player in carcinogenesis.[17,18] It promotes neutrophil recruitment and transcription of NF-κB (nuclear factor kappa B), the latter being known to influence tumour growth and response to chemotherapy.[18] *In vitro* studies showed that IL-1β plays an important part in increasing proliferation, leading to a malignant transformation.[8]

IL-1β release can also be regulated by miRNAs, 21-23 nucleotides long non-coding RNAs, which inhibit translation by binding to the 3′-untranslated region (3′-UTR) of mRNA.[19] miRNAs are involved in networks of gene regulation and their expression often changes in cancerous tissue, including MM.[20-25] A key miRNA, influencing the expression of *IL1B*, is miRNA-146. Two human variants are found; miRNA-146a and miRNA-146b, both assumed to play a role in toll-like receptor (TLR) based signalling and cytokine response.[21,22,26] Previous studies found that miRNA-146a has an anti-inflammatory function, with its silencing leading to an increase in IL-1β and its induction having the opposite effect.[22,26,27]

Genetic factors, such as single nucleotide polymorphisms (SNPs), may influence protein expression.[17,28,29] *IL1B* rs16944 (c.-511C>T), located in 5′ untranslated region (UTR), influences the binding of transcription factors.[30] Higher levels of IL-1β were found in homozygotes with polymorphic allele, leading to a higher risk of developing chronic inflammation-related diseases, such as diabetes mellitus type 2 and breast cancer.[31,32] *IL1B* rs1143623 (-1464G>C) is also located in 5′ UTR and affects the binding of transcription factors.[30] Its polymorphic C allele was associated with a lower risk of developing lung and colorectal carcinoma, due to a lower production and release of IL-1β.[28,29] The relationship between *IL1B* rs1071676, located in 3′UTR, and carcinogenesis has not yet been established, but as it affects miRNA binding site, it could also influence *IL1B* expression. SNPs have also been found in genes coding for miRNAs, such as *MIR146A* rs2910164, which has been related to both higher[33] and lower risks[34] of malignant transformations, according to previous research.[35]

To the best of our knowledge, the role of *IL1B* and *MIR146A* genetic variability in the development of asbestos-related diseases has not been evaluated so far. The aim of the present study was therefore to evaluate the influence of *IL1B* and *MIR146A* polymorphisms on the risk of developing pleural plaques and MM.

Subjects and methods

Subjects

The retrospective case-control study included 277 patients with histologically confirmed pleu-

ral or peritoneal MM, treated at the Institute of Oncology Ljubljana between 1 January 2001 and 30 September 2018, 394 patients with pleural plaques and 175 healthy control subjects, all of whom were previously exposed to asbestos. The control group and those with pleural plaques were occupationally exposed to asbestos by working in the factory Salonit Anhovo, Slovenia, and were presented at the State Board for the Recognition of Occupational Asbestos Diseases between January 1999 and December 2003. In 2018, the subjects from the control group were found not to have any asbestos-related disease.

The study was approved by the Slovenian Ethics Committee for Research in Medicine and was carried out according to the Declaration of Helsinki.

Clinical diagnosis

Patients with pleural plaques have been diagnosed based on X-ray and high-resolution computed tomography (HRCT), while MM diagnosis was confirmed by a pathologist based on the histopathology of samples gathered thoracoscopically in the case of the pleural and laparoscopically in the case of the peritoneal type of MM.[2,36,37]

Asbestos exposure and smoking

A semiquantative method was used to assess the asbestos exposure. The data on cumulative asbestos exposure expressed in fibres/cm^3-years were available for all control subjects, all subjects with pleural plaques except for 6, and for 40 subjects with MM. Based on these data, the asbestos exposure in these subjects was categorised into three groups: low (< 11 fibres/cm^3-years), medium (11–20 fibres/cm^3-years) and high (> 20 fibres/cm^3-years) asbestos exposure. For additional 49 subjects with MM who lacked the data on cumulative asbestos exposure a thorough work history was obtained by an interview performed by a single expert experienced in asbestos exposure assessment. Their exposures were compared with the exposures from the group of patients with known cumulative asbestos exposure and were categorized accordingly into three groups with presumed low, medium and high asbestos exposure.[2] For the remaining 188 MM patients, exact data on asbestos exposure were not available.

An interview based on a standardized questionnaire was conducted with the control group and patients with pleural plaques to collect data on smoking, while the medical documentation of the Institute of Oncology of Ljubljana was used to obtain this piece of data for patients with MM.[2,38]

Single nucleotide polymorphism (SNP) selection

Using LD Tag SNP Selection,[30] dbSNP,[39] Ensembl[40] and LDlink[41] we identified *IL1B* SNPs, which had the minor allele frequency (MAF) greater than 0.05 in the European population, could influence the expression of *IL1B* and were located less than 5000 base pairs up- or downstream from the gene. Polymorphism rs1071676, located in 3'UTR as well as rs16944 and rs1146323, located in 5'UTR matched our criteria. Based on miRDB,[42] miRTarBase[43] and Variation Viewer we identified miRNAs, that could influence *IL1B* expression and SNPs in the genes coding for these miRNAs. Based on the inclusion criteria, we selected rs2910164, a SNP in miRNA-146a.

Molecular genetic analysis

We isolated DNA from venous blood of 44 patients with MM using E.Z.N.A.® SQ II Blood DNA Kit (Omega Bio-tek, Inc., Norcross, Georgia, USA) following the manufacturer's instructions. DNA samples of all other subjects had been isolated during previous studies.[44] Genotyping was performed using competitive allele-specific PCR (KASP), the KASP Master mix (LGC, Middlesex, UK) and custom KASP Genotyping Assay (LGC, Middlesex, UK) according to the manufacturer's instructions.

Statistics

Median and interquartile range were used to describe continuous variables, while frequencies were used for categorical variables. To compare the distribution of categorical variables, Fisher's exact test was performed, while non-parametric Kruskal-Wallis test was used for continuous variables. Deviation from the Hardy-Weinberg equilibrium (HWE) was evaluated using chi-square test. Both additive and dominant genetic models were used in statistical analyses. Univariable and multivariable logistic regression was used to analyse the association between genotypes and asbestos-related diseases (pleural plaques and MM). For the analysis of multiplicative interactions between genotypes, logistic regression models using dummy variables were used. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) for Windows, version 21.0 (IBM Corporation, Armonk, NY, USA).

Results

Characteristics of patients with MM and pleural plaques as well as the control group are shown in Table 1. There was a statistically significant difference between the groups in respect to age ($p < 0.001$) and asbestos exposure ($p < 0.001$). MM patients were significantly older than the control group or patients with pleural plaques. Among the subjects with known asbestos exposure, 51.7% of patients with MM had medium or high exposure compared to 23.4% of the control group and 28.4 % of patients with pleural plaques. There was no statistically significant difference between groups regarding gender ($p = 0.410$) and smoking status ($p = 0.267$) (Table 1).

A further analysis of asbestos exposure showed that medium and high levels of asbestos exposure were associated with an increased risk of MM compared both to the control group (odds ratio [OR] = 3.50; 95% confidence interval [CI] = 2.03–6.02; $p < 0.001$) and patients with pleural plaques (OR = 2.70; 95% CI = 1.69–4.33; $p < 0.001$).

Among the patients with MM, 19 (6.9 %) had stage I MM, 61 (22.1 %) were in stage II of the disease, 83 (30.1 %) had stage III and 81 (29.3 %) stage IV MM. Thirty-two patients (11.6 %) had the peritoneal subtype of MM, where stage was not determined and in one patient, the MM stage could not be determined. In our cohort, the most prevalent histological subtype of MM was the epithelioid subtype (206; 74.4 %); however some of the patients had either the biphasic (26; 9.4 %) or sarcomatoid subtype (26; 9.4 %) and in the case of a few patients (19; 6.6 %), the histological subtype was not determined.

A comparison of patients with pleural plaques and healthy controls revealed no statistically significant influence on the risk of pleural plaques for any of the selected polymorphisms, neither in univariable analysis nor after adjustments for age, gender and asbestos exposure (Supplementary Table 1).

The analysis of the association between genetic polymorphisms and MM has shown statistically significant influence of polymorphism *MIR146A* rs2910164 on the risk of developing MM. Carriers of two polymorphic alleles (genotype CC) had a lower risk of developing MM (OR = 0.31; 95% CI = 0.13–0.73; $p = 0.008$). There was no influence of other genetic polymorphisms on the development of MM (Table 2).

In multivariable analysis, polymorphism *MIR146A* rs2910164 remained associated with a decreased risk of developing MM after adjustment for age and gender (OR = 0.34; 95% CI = 0.14–0.85; $p = 0.020$). However, in the subgroup with asbestos exposure data, *MIR146A* rs2910164 polymorphism no longer showed statistically significant influence on the risk of MM after adjustment for age, gender and asbestos exposure (OR = 0.39; 95% CI = 0.11–1.38; $p = 0.144$) (Table 2). Carriers of at least one polymorphic *IL1B* rs1143623 (genotype GC or CC) showed a significantly decreased risk of MM after adjustment for age, gender and asbestos exposure (OR = 0.50; 95% CI = 0.28–0.92; $p = 0.025$) (Table 2).

A comparison of patients with MM and pleural plaques showed that polymorphism *MIR146A* rs2910164 was statistically significantly associated with the risk of the development of MM compared to pleural plaques (Table 3). Patients that had two polymorphic *MIR146A* rs2910164 alleles (geno-

TABLE 1. Characteristics of subjects included in the study

Characteristics		Control group N = 175	Pleural plaques N = 394	Malignant mesothelioma N = 277	Test	p
Gender	Male, N (%)	119 (68.0)	271 (68.8)	202 (72.9)	1.757[a]	0.410
	Female, N (%)	56 (32.0)	123 (31.3)	75 (27.1)		
Age	Median (25%–75%)	55.3 (48.6–63.7)	54.9 (48.8–62.7)	66.0 (59.0–73.0)	151.666[b]	**< 0.001**
Asbestos exposure	Low, N (%)	134 (76.6)	278 (71.6) [6]	43 (48.3) [188]	26.891[a]	**< 0.001**
	Medium, N (%)	13 (7.4)	41 (10.6)	24 (27.0)		
	High, N (%)	28 (16.0)	69 (17.8)	22 (24.7)		
Smoking	No, N (%)	94 (53.7)	194 (49.4) [1]	150 (55.6) [7]	2.640[a]	0.267
	Yes, N (%)	81 (46.3)	199 (50.6)	120 (44.4)		

[a] calculated using Fisher exact test; [b] calculated using Kruskal-Wallis test; number of missing data is presented in [] brackets

TABLE 2. Association between selected polymorphisms and the risk of developing malignant mesothelioma

SNP	Genotype	Controls N (%)	MM N (%)	OR (95% CI)	p	OR (95% CI)$_{adj1}$	p_{adj1}	OR (95% CI)$_{adj2}$	p_{adj2}
IL1B rs1143623	GG	88 (50.3)	152 (54.9)	reference		reference			
	GC	67 (38.3)	97 (35.0)	0.84 (0.56–1.26)	0.396	0.82 (0.52–1.29)	0.388	0.56 (0.29–1.05)	0.072
	CC	20 (11.4)	28 (10.1)	0.81 (0.43–1.52)	0.514	0.69 (0.35–1.38)	0.294	0.34 (0.11–1.04)	0.060
	GC+CC	87 (49.7)	125 (45.1)	0.83 (0.57–1.22)	0.341	0.79 (0.52–1.20)	0.266	**0.50 (0.28–0.92)**	**0.025**
IL1B rs16944	TT	21 (12.0)	36 (13.0)	1.10 (0.60–2.02)	0.756	0.94 (0.48–1.82)	0.849	0.53 (0.20–1.43)	0.210
	TC	75 (42.9)	118 (42.6)	1.01 (0.67–1.51)	0.960	0.98 (0.63–1.52)	0.911	0.67 (0.36–1.24)	0.198
	CC	79 (45.1)	123 (44.4)	reference		reference			
	TC+TT	96 (54.9)	154 (55.6)	1.03 (0.70–1.51)	0.878	0.97 (0.64–1.47)	0.873	0.63 (0.35–1.13)	0.122
IL1B rs1071676	GG	105 (60.0)	165 (59.6)	reference		reference			
	GC	60 (34.3)	98 (35.4)	1.04 (0.69–1.56)	0.851	0.97 (0.62–1.51)	0.895	1.00 (0.53–1.89)	0.993
	CC	10 (5.7)	14 (5.1)	0.89 (0.38–2.08)	0.789	0.96 (0.38–2.41)	0.931	2.03 (0.70–5.90)	0.194
	GC+CC	70 (40.0)	112 (40.4)	1.02 (0.69–1.50)	0.927	0.97 (0.64–1.48)	0.885	1.15 (0.64–2.08)	0.632
MIR146A rs2910164	GG	94 (53.7)	158 (57.0)	reference		reference			
	GC	64 (36.6)	110 (39.7)	1.02 (0.69–1.53)	0.913	0.91 (0.59–1.41)	0.672	0.67 (0.36–1.25)	0.209
	CC	17 (9.7)	9 (3.2)	**0.31 (0.13–0.73)**	**0.008**	**0.34 (0.14–0.85)**	**0.020**	0.39 (0.11–1.38)	0.144
	GC+CC	81 (46.3)	119 (43.0)	0.87 (0.60–1.28)	0.488	0.79 (0.52–1.21)	0.278	0.62 (0.34–1.11)	0.109

adj1 = adjustment for age and gender; adj2 = adjustment for age, gender and asbestos exposure; CI = confidence interval; MM = malignant mesothelioma; OR = odds ratio; SNP = single nucleotide polymorphism

TABLE 3. Association between selected polymorphisms and the risk of developing malignant mesothelioma compared to pleural plaques

SNP	Genotype	Pleural plaques N (%)	MM N (%)	OR (95 % CI)	p	OR (95 % CI)$_{adj1}$	p_{adj1}	OR (95 % CI)$_{adj2}$	p_{adj2}
IL1B rs1143623	GG	205 (52.0)	152 (54.9)	reference		reference			
	GC	157 (39.8)	97 (35.0)	0.83 (0.60–1.16)	0.277	0.88 (0.61–1.27)	0.499	0.67 (0.39–1.15)	0.151
	CC	32 (8.1)	28 (10.1)	1.18 (0.68–2.04)	0.554	1.07 (0.58–1.99)	0.827	0.65 (0.22–1.86)	0.418
	GC+CC	189 (48.0)	125 (45.1)	0.89 (0.66–1.21)	0.467	0.92 (0.65–1.29)	0.617	0.67 (0.40–1.11)	0.123
IL1B rs16944	TT	50 (12.7)	36 (13.0)	1.02 (0.63–1.67)	0.923	0.96 (0.56–1.67)	0.897	0.54 (0.22–1.34)	0.184
	TC	169 (42.9)	118 (42.6)	0.99 (0.71–1.38)	0.969	0.98 (0.68–1.42)	0.929	0.70 (0.41–1.19)	0.186
	CC	175 (44.4)	123 (44.4)	reference		reference			
	TC+TT	219 (55.6)	154 (55.6)	1.00 (0.73–1.36)	0.998	0.98 (0.69–1.38)	0.905	0.66 (0.40–1.10)	0.110
IL1B rs1071676	GG	233 (59.1)	165 (59.6)	reference		reference			
	GC	145 (36.8)	98 (35.4)	0.95 (0.69–1.32)	0.778	0.93 (0.65–1.34)	0.708	0.92 (0.53–1.60)	0.774
	CC	16 (4.1)	14 (5.1)	1.24 (0.59–2.60)	0.578	1.29 (0.56–2.97)	0.553	**2.83 (1.04–7.71)**	**0.042**
	GC+CC	161 (40.9)	112 (40.4)	0.98 (0.72–1.34)	0.911	0.97 (0.68–1.37)	0.849	1.09 (0.66–1.82)	0.731
MIR146A rs2910164	GG	196 (49.7)	158 (57.0)	reference		reference			
	GC	163 (41.4)	110 (39.7)	0.84 (0.61–1.15)	0.276	0.84 (0.58–1.20)	0.326	0.65 (0.38–1.11)	0.118
	CC	35 (8.9)	9 (3.2)	**0.32 (0.15–0.68)**	**0.003**	**0.33 (0.15–0.75)**	**0.008**	0.34 (0.11–1.09)	0.069
	GC+CC	198 (50.3)	119 (43.0)	0.75 (0.55–1.02)	0.063	0.74 (0.53–1.05)	0.092	**0.59 (0.36–0.99)**	**0.046**

adj1 = adjustment for age and gender; adj2 = adjustment for age, gender and asbestos exposure; CI = confidence interval; MM = malignant mesothelioma; OR = odds ratio; SNP = single nucleotide polymorphism

type CC) had a significantly decreased risk of MM compared to pleural plaques (OR = 0.32; 95% CI = 0.15–0.68; p = 0.003). Similarly, after adjustment for gender and age, patients who were homozygotes for polymorphic *MIR146A* rs2910164 still showed a lower risk of developing MM (OR = 0.33; 95% CI = 0.15–0.75; p = 0.008). In the subgroup with available asbestos exposure data, *MIR146A* rs2910164 was significantly associated with a decreased MM risk only in the dominant model (OR = 0.59; 95% CI = 0.36–0.99; p = 0.046) (Table 3). Additionally, patients that were homozygotes for the *IL1B* rs1071676 polymorphism (CC genotype) had an increased risk of developing MM when patients with pleural plaques were used as a control group and the analysis was adjusted for age, gender and asbestos exposure (OR = 2.83; 95% CI = 1.04–7.71; p = 0.042) (Table 3).

In further logistic regression modelling, the interactions between polymorphisms showed no significant influence on the risk of pleural plaques (data not shown). The analysis of the influence of the interaction between *IL1B* rs1143623 and *IL1B* rs1071676 polymorphisms showed significant influence on the increased MM risk (OR = 2.24, 95% CI = 1.00–5.00, p = 0.050). No other interactions between polymorphisms had a statistically significant influence on the risk of MM (Supplementary Table 2).

Discussion

The association between MM and asbestos exposure has first been described in 1960 and, although very few genetic factors have been studied, multiple factors have since then been considered to influence the pathogenesis of MM.[45] In the present study, we evaluated the effect of polymorphisms of IL-1β and miRNA-146a genes on the risk of developing MM and pleural plaques. The key finding of the present study was the association between *MIR146A* rs2910164 and lower risk of the development of MM.

Consistent with the previous studies, the average age of MM patients was found to be higher than that of the patients with pleural plaques or the control group, probably due to the long latency period between the first asbestos exposure and MM.[2,6,44] Our study showed no significant association between smoking and MM, which is in agreement with previous findings.[2,44,46] Subjects with high or medium exposure to asbestos had a higher risk of developing MM, compared to the group with pleural plaques or the control group. Regardless of that, almost half (48.3%) of MM patients were exposed to low levels of asbestos, which is consistent with previous studies claiming there is no threshold level for the development of MM.[47,48]

It is not yet clear to what an extent the pleural plaques present a risk factor for MM. The studies performed so far suggested that pleural plaques are more a sign of asbestos exposure, than a carcinogenic factor.[49,50] This hypothesis is in agreement with the findings of this study as the genotype frequency distribution of patients with pleural plaques was found to be more similar to that of the control group, rather than the genotype frequency distribution of patients with MM.

Compared to both the control group and the patients with pleural plaques, homozygotes with polymorphic *MIR146A* rs2910164 C allele were at a lower risk of developing MM, even after adjustment for age and gender. In the subgroup of patients with known asbestos exposure, carriers of at least one polymorphic *MIR146A* rs2910164 allele had a lower risk of MM in comparison to patients with pleural plaques.

According to our knowledge, the relation between *MIR146A* rs2910164 and MM has not yet been studied, but the polymorphism itself has already been associated with several other malignant diseases. Previous studies suggested that the polymorphic allele C had a protective function in the oncogenesis of melanoma[51] and non-small cell lung carcinoma[33], while the same was found for the G allele in case of papillary thyroid tumour.[52] The association of rs2910164 with the pathogenesis of MM could be explained with its influence in miRNA expression: CC genotype was previously associated with a greater production of miRNA-146a in cancerous tissue.[53-55] Increased expression of miRNA-146a in turn leads to the suppression of inflammatory pathways, reducing the expression of proinflammatory cytokines IL-1β, IL-6 and TNFα,[56] while miRNA-146a inhibition has been shown to increase production of those cytokines, resulting in greater inflammatory response to asbestos, promoting carcinogenesis and increasing the risk of MM.[17,18] The role and expression of miRNA-146a in carcinogenesis is still unclear, as some studies found the levels of miRNA-146a to be decreased in cancerous tissue of the lung[57] and stomach carcinoma,[58] while other studies found increased levels in the cases of melanoma,[51] cervical cancer[59] and papillary thyroid cancer.[52] It is possible that miRNA-146a has a tissue-specific function, so further studies are required.

Another important finding of this study has been the association between the polymorphic *IL1B* rs1143623 allele and a lower risk of developing MM. In the subgroup of subjects with known asbestos exposure, subjects with at least one polymorphic C allele had a lower risk of developing MM compared to the control group. *IL1B* rs1143623 is located at the biding site of the transcription factors and can lower the expression of *IL1B*.[30,39] Lower levels of IL-1β result in a less intensive inflammatory reaction caused by the asbestos fibres, which could have a protective effect. The former is in agreement with the studies that showed subjects with one polymorphic C allele having a lower risk of developing lung cancer[28] and homozygotes for the polymorphic C allele having a lower risk of developing colorectal cancer.[29]

Finally, this study has shown that the interaction between *IL1B* rs1143623 and *IL1B* rs1071676 is associated with a higher risk of developing MM, even though *IL1B* rs1071676 independently had no effect on the risk of MM, while *IL1B* rs1143623 was associated with a lower risk of MM within the subgroup of subjects with known asbestos exposure. *IL1B* rs1143623 was associated with a lower risk of MM only among carriers of two wild type *IL1B* rs1071676 alleles. As *IL1B* rs1143623 can influence the binding of transcription factors and rs1071676 can influence the binding of miRNA, the interaction of both polymorphisms could result in a greater expression of IL-1β, however this has not been studied yet.[30] Further studies are needed to explain the role of *IL1B* rs1143623 and its interactions with other polymorphisms and environmental factors in MM.

Lack of asbestos exposure information for all the subjects has been identified as the limitation of our study. Therefore, the subgroup for which asbestos exposure has been taken into consideration, was smaller than the overall sample. This could account for the discrepancy between the results of the analysis adjusted for asbestos exposure and the results of the analysis that did not take asbestos exposure into account. The strength of this study is its large sample size. To the best of our knowledge, this is also the first study researching the effect of *IL1B* and *MIR146A* polymorphisms on the risk of developing MM.

In conclusion, our results suggest that *IL1B* and *MIR146A* polymorphisms may contribute to the risk of MM development. Further studies, possibly evaluating serum or tissue protein expression, are needed to confirm these associations in independent patient cohorts and elucidate the role of IL-1β and miRNA-146a in the development of asbestos-related diseases.

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