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Gene–asbestos interaction in malignant pleural mesothelioma susceptibility

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Abstract

Asbestos exposure is the main risk factor for malignant pleural mesothelioma (MPM), a rare aggressive tumor. Nevertheless, on average less than 10% of subjects highly exposed to asbestos develop MPM, suggesting the possible involvement of other risk factors. To identify the genetic factors that may modulate the risk of MPM, we conducted a gene–environment interaction analysis including asbestos exposure and 15 single nucleotide polymorphisms (SNPs) previously identified through a genome-wide association study on Italian subjects. In the present study, we assessed gene–asbestos interaction on MPM risk using relative excess risk due to interaction and synergy index for additive interaction and V index for multiplicative interaction. Generalized multifactor dimensionality reduction (GMDR) analyses were also performed. Positive deviation from additivity was found for six SNPs (rs1508805, rs2501618, rs4701085, rs4290865, rs10519201, rs763271), and four of them (rs1508805, rs2501618, rs4701085, rs10519201) deviated also from multiplicative models. However, after Bonferroni correction, deviation from multiplicative model was still significant for rs1508805 and rs4701085 only. GMDR analysis showed a strong MPM risk due to asbestos exposure and suggested a possible synergistic effect between asbestos exposure and rs1508805, rs2501618 and rs5756444. Our results suggested that gene–asbestos interaction may play an additional role on MPM susceptibility, given that asbestos exposure appears as the main risk factor.

Introduction

Malignant pleural mesothelioma (MPM) is a rare, aggressive tumor, characterized by treatment resistance and poor prognosis (1). Although rare in the past, MPM frequency increased, in relation to asbestos use (1). The only clearly established risk factors for MPM are exposure to asbestos and other asbestiform minerals such as erionite and, to a lesser extent, ionizing radiation for medical purposes (1,2).

A genetic component in the etiology of the disease (3) might in part explain the relative rarity of MPM also in heavily exposed

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Abbreviations

CI	confidence interval
CVC	cross-validation consistency
GMDR	generalized multifactor dimensionality reduction
GWAS	genome-wide association study
HWE	Hardy–Weinberg equilibrium
MPM	malignant pleural mesothelioma
OR	odds ratio
PCA	Principal component analysis
RERI	relative excess risk due to interaction
SI	synergy index
SNP	single nucleotide polymorphism
TBA	testing balanced accuracy

cohorts (2), the reports of familial clustering (4–6) and the results of candidate–gene association studies (3,7).

Matullo *et al.* (8) identified 15 single nucleotide polymorphisms (SNPs) (5 of them imputed and confirmed after genotyping) associated to MPM in a genome-wide association study (GWAS) on an Italian study sample of 407 MPM cases and 389 healthy controls, and concluded that genetic risk factors may play an additional role in the development of MPM (8). Cadby *et al.* (9) in a companion GWAS study on MPM observed other associated SNPs but failed to replicate results from Matullo *et al.* (8). They hypothesized the lack of replication might be explained by differences in population genetic structure, type of asbestos exposure or different asbestos exposure assessment.

The present study further investigates the interactions between candidate SNPs (8) and asbestos exposure, and their effects in modulating MPM risk in Italian population.

Methods

Ethics statement

All MPM cases and controls included in the present paper gave written informed consent. This study was performed according to the principles of the Declaration of Helsinki and in agreement with ethical requirements. Approval was obtained from the Istituto Nazionale per la Ricerca sul Cancro Ethics Committee for the studies in Genoa and La Spezia, and from the Human Genetics Foundation (HuGeF) Ethics Committee for the studies in Casale Monferrato and Turin.

Study sample

Interaction analysis was conducted using genotypic frequencies and sample information from the GWAS by Matullo *et al.* (8). The study sample was composed of MPM cases and controls from cities located in Northern Italy: Casale Monferrato and Turin in the Piedmont Region, and Genoa and La Spezia in the Liguria Region. Casale Monferrato sample was a population-based MPM case–control study (10), and included 241 MPM patients and 252 population controls. Turin sample was a hospital-based MPM case–control study (7), and consisted of 91 MPM patients and 56 controls (non-neoplastic and non-respiratory conditions). The hospital-based study in Genoa and La Spezia included 75 incident MPM cases (11), and 81 controls (healthy subjects or patients hospitalized for non-neoplastic and non-respiratory conditions). All study subjects were of Italian origin and Caucasian ethnicity. Criteria of eligibility also included: residing in the study area at the time of diagnosis and pathological confirmation of the diagnosis (based on histology or cytology with immunohistochemical staining). For practical reasons, the study in Turin was limited to cases and controls admitted to the main metropolitan hospitals. Cases and controls were individually matched by age and gender.

After reviewing the individual occupational histories, collected during questionnaire-based personal interviews, asbestos exposure was classified by an expert (D.M.) as 'absent/unlikely' (no acknowledged occupational or environmental exposure), 'low' (low exposure probability, or definite exposure at low level) and 'high' (definite and high exposure, corresponding in principle to asbestos–cement and asbestos-textile workers, insulators, shipyard workers and dockers and similar activities). Further details on the exposure assessment process were given in Magnani *et al.* (12).

SNP genotyping and genotyping quality controls

Whole-genome genotyping was performed using a HumanCNV370-Quad BeadChip (Illumina, San Diego, CA) for 716 samples. The remaining 80 samples were tested on a Human610-Quad BeadChip (which includes 100% of the HumanCNV370 BeadChip SNPs) as the HumanCNV370-Quad had been discontinued. Genotypes assignment was done by GenomeStudio v. 2011.1 (Illumina, San Diego, CA). Five SNPs initially identified through imputation analysis (rs2236304, rs7632718, rs9833191, rs10815216, rs73034881) were subsequently fully genotyped by 5′ nuclease assay (TaqMan® Assay, Life Technologies). For replication purposes, 62 MPM cases from Piedmont, 225 healthy Italian subjects belonging to another ongoing study and 127 MPM cases and controls from a Finnish occupational-health study were genotyped with a 5′ nuclease assay for 4 SNPs (Table 2, SNPs #2,5,9,10) out of the 10 genotyped by CNV370 BeadChip. In our hands, genotypic and allele frequencies of these four SNPs were consistent between the TaqMan® genotyped subgroups and the CNV370 group. Quality controls were conducted in the main GWAS (8) and are only summarized here. A cut-off genotyping call rate of 0.98 was set, leading to the exclusion of 18 study subjects. Identity by descent estimation using the identity by state distance was used to check genotypic identity or relatedness among subjects (13). Subjects with identity by descent ≥ 0.05 ($n = 16$) were considered consanguineous and excluded from further analyses. We additionally excluded three samples with an X chromosome inbreeding homozygosity estimate of about 0.5. In total, 37 subjects (4.64%) were removed from the analysis, leaving 759 subjects (392 cases and 367 controls). SNPs with minor allele frequency, <1% ($n = 15\,252$), those having > 0.05 missing genotypes ($n = 11\,535$) and those deviating from Hardy–Weinberg equilibrium (HWE) in the control population ($P \leq 0.001$, $n = 1157$) were excluded from the analysis, for a final study data set of 330 879 genotyped SNPs.

Population structure and SNPs selection

Analyses in the present study were focused on the 15 SNPs identified by Matullo *et al.* (8) and their interaction mode with asbestos. Fifteen SNPs (rs2236304, rs742109, rs1508805, rs2501618, rs4701085, rs4290865, rs9536579, rs7632718, rs9833191, rs3801094, rs7841347, rs10519201, rs5756444, rs10815216, rs73034881) resulted associated to MPM risk in the GWAS analyses (8). These SNPs were selected by logistic regression analysis on per allele additive model adjusting by age, gender, principal component analysis (PCA) cluster, center of recruitment and asbestos exposure level (8).

Statistical analysis

The relationship between SNPs and asbestos exposure in MPM risk was analyzed by logistic regression method adjusting by age, gender, PCA cluster and center. A binary classification was used both for asbestos exposure (exposed versus unexposed) and for genotypes (homozygous for major allele versus one or two copies of the minor allele). Subjects with exposure coded as 'absent/unlikely exposure' were considered as unexposed, while subjects coded as 'low' or 'high' were considered as exposed. MPM risk for a given SNP and asbestos exposure was expressed by $OR_{i,j}$ where the first index (*i*) indicated the asbestos exposure coded as 0 for unexposed and 1 for exposed subjects; the second index (*j*) indicated the SNP genotype coded as 0 for subjects homozygous for the major allele and 1 for subjects bearing one or two copies of the minor allele. Subjects unexposed and homozygous for the major allele were considered as reference group, thus coding their MPM risk as $OR_{00} = 1$.

Interaction was analyzed in respect to both additive and multiplicative models based on the ORs obtained by logistic regression. Deviation from an additive model was explored as the relative excess risk due to interaction (RERI) and the synergy index (SI) (14). Confidence intervals (CIs) were calculated by the delta method (15). RERI was defined as $[OR_{11} - OR_{01} - OR_{10} + 1]$ and SI as $[OR_{11} - 1]/[(OR_{01} - 1) + (OR_{10} - 1)]$ where the $OR_{i,j}$ represents the odds ratio estimated using logistic regression adjusted by age, gender, PCA cluster and center of recruitment.

Under the null hypothesis of no interaction under the additive model, RERI is not significantly different from 0, whereas SI is not significantly

different from 1. RERI > 0 indicates positive interaction and RERI < 0 negative interaction. SI > 1 means positive interaction and SI < 1 negative interaction. Synergistic interaction (positive interaction) implies that the combined action between two factors in an additive model is greater than the sum of individual effects. On the contrary, antagonistic interaction means that in the presence of two factors in an additive model, the action of one exposure variable reduces the effects of the other (14).

Deviations from a multiplicative model were explored by multivariable logistic regression models including: asbestos exposure, one SNP at time and the corresponding interaction term (SNP × exposure); logistic regression models were adjusted for age, gender, PCA cluster and center of recruitment. *P* values for multiplicative interaction were calculated by comparing the full model including a multiplicative interaction term to a reduced model without it, using the likelihood ratio test (16).

The Multiplicativity index $V = OR_{11}/(OR_{01} \times OR_{10})$ (17) was also calculated. A V = 1 suggested a multiplicative joint effect, whereas V values greater or lower than 1 indicated an interaction that is greater than or lesser than multiplicative, respectively.

The generalized multifactor dimensionally reduction (GMDR) (18) method was applied to analyze high order SNP–exposure interactions (GMDR v7, software obtained from http://www.ssg.uab.edu/gmdr/). GMDR is a model-free method. Potential confounders (age, gender, PCA cluster and center of recruitment) were included as covariates. The data were divided in 10 sets: 9 for training and 1 for testing. *N* (from 1 to 5) factors were selected from the training set and their combinations were represented in *n*-dimensional space. The GMDR classified each combination (multifactorial class) as 'high risk' or 'low risk', thus reduced the *n*-dimensional space to one dimensional with two levels. For each possible model size (one-factor, two-factors, etc.), the model with the lowest misclassification error was selected. 'Leave-one-out cross-validation' was used to calculate the prediction error and to evaluate the predictive ability of the model to fit the test set. The result was a set of models, and the Testing Balanced Accuracy (TBA) and cross-validation consistency (CVC) indexes were used to determinate the overall best model. TBA was calculated as (sensitivity + specificity)/2. ORs were computed by 'low risk' versus 'high risk' combinations. *P* values were determined by sign test, a robust non-parametric test implemented in GMDR software (19). The model with higher TBA and CVC and *P* value less of equal to 0.05 derived from sign test was considered as the best one.

In controls, HWE was reassessed for the 15 SNPs comparing the observed genotype frequencies with the expected frequencies using Chi-squared test with significance level at $P < 0.05$.

Statistical significance was set at $P < 0.05$, CIs were estimated at 95%.

Results

After standard GWAS quality control procedure, as reported in (8), we considered eligible for the statistical analyses 759 subjects, 367 MPM cases and 392 controls. The general characteristics of the sample are reported in Table 1. For each SNP we performed the gene–environment interaction analysis considering only subjects with known asbestos exposure and genotype.

All the polymorphisms were in HWE at $P < 0.05$, except rs2236304 ($P = 0.022$), rs9536579 ($P = 0.034$) and rs10815216 ($P = 0.004$). This may be due to the small size of the sample or to chance.

The baseline association of covariates with MPM onset (Supplementary Table 1, available at *Carcinogenesis* Online) showed statistically significant differences for age ($P < 0.001$), PCA cluster ($P = 0.040$) and centre Turin versus Casale Monferrato $P = 0.001$ and multivariable analyses were adjusted for accordingly.

Stratified analysis

The joint effect of exposure and each of the candidate SNPs, assessed by multivariable logistic regressions, controlling by possible confounders (age, PCA cluster, centre and sex), is shown in Table 2.

For example, considering the SNP rs2236304 the risk of mesothelioma was increased (OR = 3.34 95%CI 1.00–11.09) in unexposed subjects carrying the minor allele compared to unexposed and homozygous for the major allele (reference category), and it was also increased in exposed individuals homozygous for the major allele (OR = 16.00 95%CI 5.45–46.98). The effect further increased (OR = 28.87 95%CI 9.95–83.78) when both factors (exposure and minor allele) were present. Evidence of synergistic interaction between the minor allele of this polymorphism and asbestos exposure is further evaluated by indicators showed in Table 3 (see later).

Considering 'unexposed and non-carrying at risk alleles' as the reference group, ORs among 'exposed and non-carrying' subjects ranged from 4.56 (rs7632718) to 35.83 (rs5756444); among 'exposed-carrying' subjects they ranged from 3.92 (rs10815216) to 28.87 (rs223604) (Table 2). Rs1508805, rs2501618, rs4701085, rs4290865, rs10519201 (*SHC4*) and rs7632718 (*SLC74A14*) showed a similar pattern: for the carrying subjects we observed null effect or protection among unexposed subjects and risk increase among those exposed.

For rs5756444, in the absence of exposure, the minor allele triplicated the risk (OR 3.37, borderline statistical significance) while it showed no effect among the exposed (OR from 35.83 to 21.34 with overlapping CIs).

Table 1. Summary statistics of subjects included in the interaction analysis

	Centre						Overall sample	
	Casale Monferrato		Genoa		Turin			
	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Gender								
Females	75 (31.65)	75 (32.61)	19 (25.33)	6 (8.22)	17 (30.91)	27 (30.34)	111 (30.25)	108 (27.55)
Males	162 (68.35)	155 (67.39)	56 (74.67)	67 (91.78)	38 (69.09)	62 (69.66)	256 (69.75)	284 (72.45)
Total	237 (50.75)	230 (49.25)	75 (50.68)	73 (49.32)	55 (38.19)	89 (61.81)	367 (48.35)	392 (51.65)
Asbestos exposure								
No	54 (22.78)	4 (1.74)	41 (54.67)	10 (13.70)	18 (32.73)	3 (3.37)	113 (30.79)	17 (4.34)
Yes	183 (77.22)	190 (82.61)	34 (45.33)	63 (86.30)	37 (67.27)	86 (96.67)	254 (69.21)	339 (86.48)
Non-available		36 (15.65)						36 (9.18)
Age								
(Mean ± SD)	63.36 ± 11.06	67.61 ± 11.14	58.59 ± 15.03	69.64 ± 9.64	68.31 ± 8.80	68.74 ± 8.84	63.11 ± 12.01	68.25 ± 10.39

Percentages are reported for cases and controls groups.

Table 2. OR, SNP genotypes and asbestos-exposure frequencies in MPM cases and controls

	SNP	EXP	Genotype (code)	Cases (*N*)	Controls (N)	OR[a]	(95%CI)
1	rs2236304 (*MMP14*)	0	CC (0)	4	57	1	
		0	CG/GG (1)	13	56	3.34	(1.00–11.09)
		1	CC (0)	108	116	16.00	(5.45–46.98)
		1	CG/GG (1)	231	138	28.87	(9.95–83.78)
2	rs742109	0	GG (0)	9	43	1	
		0	AG/AA (1)	8	70	0.52	(0.18–1.48)
		1	GG (0)	120	67	10.25	(4.50–23.35)
		1	AG/AA (1)	219	187	6.62	(3.03–14.46)
3	rs1508805	0	GG (0)	15	63	1	
		0	AG/AA (1)	2	50	0.19	(0.04–0.87)
		1	GG (0)	156	158	5.22	(2.74–9.94)
		1	AG/AA (1)	183	96	10.33	(5.35–19.93)
4	rs2501618 (*CEP350*)	0	GG (0)	14	76	1	
		0	AG/AA (1)	3	37	0.53	(0.14–1.99)
		1	GG (0)	212	202	7.20	(3.78–13.73)
		1	AG/AA (1)	126	52	17.41	(8.61–35.21)
5	rs4701085	0	AA (0)	13	56	1	
		0	AG/GG (1)	4	57	0.31	(0.09–1.03)
		1	AA (0)	132	158	4.64	(2.31–9.32)
		1	AG/GG (1)	207	106	10.47	(5.17–21.20)
6	rs4290865	0	CC (0)	9	64	1	
		0	AC/CC (1)	7	49	0.86	(0.29–2.52)
		1	CC (0)	186	182	7.97	(3.73–17.03)
		1	AC/CC (1)	148	71	17.21	(7.81–37.91)
7	rs9536579	0	GG (0)	11	60	1	
		0	AG/AA (1)	6	53	0.55	(0.19–1.64)
		1	GG (0)	221	130	10.93	(5.30–22.54)
		1	AG/AA (1)	118	124	5.80	(2.79–12.08)
8	rs3801094 (*ETV1*)	0	GG (0)	5	59	1	
		0	AG/AA (1)	12	54	2.94	(0.95–9.14)
		1	GG (0)	130	128	15.58	(5.83–41.68)
		1	AG/AA (1)	197	122	25.13	(9.42–67.07)
9	rs7841347 (*PVT1*)	0	AA (0)	7	37	1	
		0	AG/GG (1)	10	76	0.66	(0.23–1.91)
		1	AA (0)	97	54	11.56	(4.60–29.06)
		1	AG/GG (1)	242	200	7.42	(3.10–17.75)
10	rs10519201 (*SHC4*)	0	CC (0)	14	86	1	
		0	AC/AA (1)	3	27	0.62	(0.16–2.39)
		1	CC (0)	240	215	8.03	(4.23–15.23)
		1	AC/AA (1)	99	39	20.21	(9.76–41.87)
11	rs5756444	0	AA (0)	3	47	1	
		0	AG/GG (1)	14	66	3.37	(0.90–12.64)
		1	AA (0)	146	81	35.83	(10.44–112.96)
		1	AG/GG (1)	193	173	21.34	(6.66–71.95)
12	rs7632718 (*SLC74A14*)	0	GG (0)	5	28	1	
		0	GA/AA (1)	12	85	0.68	(0.21–2.16)
		1	GG (0)	72	88	4.56	(1.62–12.92)
		1	GA/AA (1)	267	166	10.04	(3.67–27.64)
13	rs9833191 (*THRB*)	0	TT (0)	7	47	1	
		0	TC/CC (1)	10	66	1.22	(0.42–3.55)
		1	TT (0)	146	64	19.79	(8.10–48.33)
		1	TC/CC (1)	193	190	9.29	(3.91–22.06)
14	rs10815216	0	AA (0)	13	38	1	
		0	AC/CC (1)	4	75	0.17	(0.05–0.59)
		1	AA (0)	151	80	6.95	(3.33–14.51)
		1	AC/CC (1)	188	174	3.92	(1.19–8.05)
15	rs73034881 (*SDK1/FOXK1*)	0	GG (0)	14	72	1	
		0	AG/AA (1)	3	41	0.34	(0.09–1.29)
		1	GG (0)	253	154	9.73	(5.11–18.51)
		1	AG/AA (1)	86	100	5.16	(2.61–10.20)

The risks were estimated for each of the 15 SNPs and asbestos exposure. For asbestos exposure (EXP) '1' indicates exposure, whereas '0' indicates non-exposure. Subject who were unexposed (0) and homozygous for the major allele were considered as reference group.

[a]OR (odds ratio) adjusted by age, gender, PCA cluster, center.

For rs1508805 (Table 2), in the absence of exposure, the minor allele conferred protection (OR = 0.19), whereas exposure doubled the risk (from 5.22 to 10.33).

Analysis without adjusting for confounding (age, sex, PCA cluster and center) give similar results (Supplementary Table 2, available at *Carcinogenesis* Online).

Additive and multiplicative interaction

RERI, SI, V and the statistical significance of the interaction term in the multiplicative logistic regression model are reported in Table 3, for each SNP.

In respect to deviation from additivity, significant positive interaction between SNP and exposure was found for rs1508805, rs2501618 (*CEP350*), rs4701085, rs4290865, rs10519201 (*SHC4*), rs7632718 (*SLC74A14*) according to RERI and SI indexes. Rs73034881 (*SDK1/FOXK1*) showed borderline RERI and statistically significant SI, with negative interaction. Significant negative interaction between SNP and exposure was also found according to SI index for rs9536579, rs5756444, rs10815216, rs9833191 (*THRB*) and rs7303881 (*SDK1/FOXK1*). After accounting for multiple comparisons using Bonferroni correction, SI index was still statistically significant for eight SNPs (rs1508805, rs2501618 (*CEP350*), rs4701085, rs4290865, rs9536579, rs10519201, rs5756444, rs9833191 (*THRB*)) but RERI was no longer statically significant for any SNPs.

Statistically significant deviation from the multiplicative model was observed for: rs1508805, rs2501618 (*CEP350*), rs4701085, rs10519201 (*SHC4*), rs575644, and rs10815216. Except for rs575644 (V = 0.18), all these deviations from multiplicative model indicated a more than multiplicative interaction (V > 1) between SNP and exposure. After accounting for multiple comparisons using Bonferroni correction of $P = 0.003$ (0.05/15) the interaction remained statistically significant on the multiplicative scale, for rs150885 and rs471085.

Similar trends were observed in unadjusted association analyses (Supplementary Table S3, available at *Carcinogenesis* Online).

GMDR

Table 4 shows the results obtained from the GMDR analysis for one-to five-factors models adjusted by covariates; for each 1 to 5 factors combination the best model is reported. According to GMDR selection model (19), the best model is the one with maximum TBA, maximum CVC and *P* value derived from sign test less or equal to 0.05. There was no single model with all of these characteristics. The model with maximum CVC (10/10), included exposure only, had the second highest TBA (63.39%), OR = 9.71

Table 3. Results for gene–environment interaction analysis for each candidate SNP and asbestos exposure, adjusted for age, gender, PCA cluster and centre

		Deviation from additive model		Deviation from multiplicative model	
	Gene	RERI 95%CI	SI 95%CI	V	P[a]
1	rs2236304 (*MMP14*)	10.53 (–2.30 to 23.36)	1.61 (1.11 to 2.33)	0.54	0.309
2	rs742109	–3.15 (–7.87 to 1.57)	0.64 (0.41 to 0.99)	1.24	0.780
3	rs1508805[b,c,d]	5.92 (1.72 to 10.12)	2.74 (1.60 to 4.69)	10.60	<0.001
4	rs2501618 (*CEP350*)[b,d]	10.69 (2.11 to 19.26)	2.87 (1.77 to 4.63)	4.56	0.016
5	rs4701085[b,c,d]	6.52 (1.87 to 11.18)	3.21 (1.76 to 5.88)	7.28	<0.001
6	rs4290865[d,e]	9.38 (1.15 to 17.62)	2.37 (1.52 to 3.70)	2.51	0.106
7	rs9536579[d]	–4.68 (–9.57 to 0.21)	0.51 (0.33 to 0.77)	0.96	0.951
8	rs3801094 (*ETV1*)	7.61 (–2.17 to 17.39)	1.46 (1.01 to 2.11)	0.55	0.333
9	rs7841347 (*PVT1*)	–3.80 (–9.73 to 2.13)	0.63 (0.40 to 0.99)	0.97	0.964
10	rs10519201 (*SHC4*)[b,d]	12.56 (1.78 to 23.46)	2.89 (1.75 to 4.77)	4.06	0.043
11	rs5756444[d,f]	–16.85 (–41.05 to 7.34)	0.54 (0.38 to 0.78)	0.18	0.005
12	rs7632718 (*SLC74A14*)[e]	5.87 (0.38 to 11.17)	2.78 (1.35 to 5.71)	3.24	0.070
13	rs9833191 (*THRB*)[d]	–10.73 (–22.41 to 0.96)	0.45 (0.29 to 0.65)	0.42	0.097
14	rs10815216[f]	–2.20 (–5.23 to 0.83)	0.57 (0.35 to 0.92)	3.32	0.048
15	rs73034881 (*SDK1/FOXK1*)	–3.91 (–7.84 to 0.02)	0.51 (0.33 to 0.81)	1.56	0.537

[a]Likelihood ratio test for multiplicative interaction term.
[b]RERI > 0, SI > 1 and V > 1 statistically significant.
[c]V index statistically significant after Bonferroni correction.
[d]After Bonferroni correction SI index statistically significant: rs1508805 95%CI 1.21–6.13; rs2501618 95%CI 1.39–5.89; rs4701085 95%CI 1.30–7.95; rs4290865 95%CI 1.22–4.62; rs9536579 95%CI 0.27–0.94; rs10519201 95%CI 1.36–6.13; rs5756444 95%CI 0.33–0.93; rs9833191 95%CI 0.24–0.79.
[e]RERI >0, SI >1 is statistically significant but V index is not statistically significant.
[f]V index statistically significant.

Table 4. Interaction test of multiple SNPs and asbestos exposure in MPM risk: best models assessed by GMDR for one- to five-factors combinations

	TBA	Sign test (*P*)	CV	OR (95%CI)	χ^2 (p)
EXP	**0.6339**	**10 (0.001)**	**10/10**	**9.71 (4.20–22.44)**	**37.32 (<0.001)**
EXP rs7632718	0.6166	10 (0.001)	6/10	3.88 (2.38–6.30)	31.22 (<0.001)
EXP rs4701085 rs1508805	0.6269	10 (0.001)	3/10	5.29 (3.18–8.80)	43.85 (<0.001)
EXP rs1508805 rs2501618 rs5756444	**0.6445**	**10 (0.001)**	**5/10**	**6.64 (3.60–11.30)**	**53.17 (<0.001)**
EXP rs1508805 rs2501618 rs5756444 rs7632718	0.6264	10 (0.001)	3/10	7.13 (4.25–11.97)	60.21 (<0.001)

The two best models (see text for details) are in bold.

(95%CI 4.02–22.44) and sign test $P < 0.001$. The model including SNP rs2501618, rs1508805, rs5756444 and exposure had the third CVC (5/10) and the first maximum TBA (64.45%), sign test $P < 0.001$ and OR = 6.64 (95%CI 3.60–11.30) (Table 4).

ORs estimated by GMDR using a classification ('high risk' versus 'low risk') corresponded, as expected, to the ORs estimated by logistic regression using the same classification (results not shown).

Discussion

This is the first study systematically examining interactions between asbestos exposure and a set of candidate SNPs emerging from a GWAS on MPM. We considered for each SNP both additive and multiplicative interactions with asbestos exposure. The age differences between cases and controls observed in Casale Monferrato and Genoa studies were due to different participation of cases and controls invited to the study. Cases and controls were age and sex matched but we observed a lower participation of controls in older ages, in particular among women (7). The main analyses were therefore always adjusted for age, gender, PCA cluster and center.

Interaction analysis is dependent on the selection of the joint effect of interest. In the absence of '*a priori*' knowledge and of theoretical reason for choosing either, both additive and multiplicative models were tested. Interaction on the additive scale is present when the joint effect of the two risk factors is different from the sum of the individual effects. Interaction on the multiplicative scale is characterized by joint effect of the two risk factors different from the product of the individual effects (14). In this study, deviation from additive model was assessed by RERI and SI indexes. All of the selected SNPs had SI indexes with significant values, suggesting deviation from additivity. The RERI index, on the contrary, was more restrictive. Indeed, as noted by Assmann *et al.* (20), SI is generally statistically more unstable than RERI, when estimated using ORs instead of relative risks, as in the present study. When multiple comparisons were considered using Bonferroni correction SI remained statistically significant for eight SNPs (rs1508805, rs2501618 (*CEP350*), rs4701085, rs4290865, rs9536579, rs10519201, rs5756444, rs9833191 (*THRB*)) and multiplicative interaction for two SNPs (rs1508805 and rs10519201 (*SHC4*)). We note that this correction is conservative and may lead to false negative results (21).

The GMDR analysis suggested that the major contribution to the development of MPM was due to asbestos exposure, even after taking into account the selected SNPs. In fact, the model with the highest TBA value (a four-factors model including: exposure, rs1508805, rs2501618, rs5756444) had a low value of CVC (5/10); the model including only asbestos exposure had an only slightly lover TBA value (64.45% versus 63.39%) but a much better CVC value (10/10), so it is the best one. Without adjusting for confounding variables the GMDR selected the same two models and included the same SNPs that were selected by the model adjusted by confounders (Supplementary Table 4, available at *Carcinogenesis* Online).

The majority of the selected SNPs from GMDR analysis were also selected by additive or multiplicative interaction analysis; however the results we obtained from the GMDR analysis indicated the preeminent role of asbestos exposure and offer limited support for an interaction between asbestos exposure and some variant alleles of some SNPs.

In the present study, we selected the significant SNPs from our published GWAS (8), as no other evidence was previously reported in the literature. In a recent publication, Cadby *et al.* (9) also investigated MPM risk with a GWAS, but their findings were not replicated in our Italian sample, apart some evidence of replication in the *SDK1* gene region (3q26.2).

As reported in Matullo *et al.* (8), SNPs included in the present study have only a limited '*a priori*' association with MPM risk or other asbestos health effects. The rs2501618, located in *CEP350* gene, and selected as deviating from both additive and multiplicative models, was found associated to atopy in a previous paper (22) studying potential candidate genes for asthma or atopy. In our work, rs2501618 reduced the MPM risk in unexposed subjects but increased the risk in exposed subjects with a synergistic interaction between asbestos exposure and the minor allele.

Deviation from additive interaction was found for SNP rs10519201, located in *SHC4* gene. SI additive interaction index remained significant after Bonferroni correction. This SNP showed association with psychiatric illness (eating-disorder) in Boraska *et al.* (23).

Rs7632718 is located in *SLC7A14* (solute carrier family 7 member 14), which lies in 3q26.2 region, that was one of the replicating regions in Cadby *et al.* (9). Although no link with MPM risk had been previously reported for *SLC7A14*, a chromosomal gain of this region has been described in MPM (24), suggesting a possible involvement of other neighboring genes.

Cadby *et al.* (9) indicated *SDK1* and the region around this gene as most consistently associated with MPM risk in both Australian and Italian studies. In the present analysis, although not statistically significant for all the interaction indexes, the rs73034881, located in *SDK1/FOXK1* region, is suggestive of negative (protective) additive interaction between the variant allele and asbestos exposure. It is interesting to note that FOXK1 is an interactor of BAP1, whose deleterious mutations are responsible for a cancer prone syndrome that includes mesothelioma in its phenotype.

In these analyses we found a possible interaction, both additive and multiplicative, between asbestos exposure and both rs2501618 (*CEP350*) and rs10519201 (*SHC4*). Interaction is also suggested by four-factors GMDR interaction analysis that included exposure, rs2501618 (*CEP350*), rs1508805 and rs5756444. According to the Variant Effect Predictor software (http://www.ensembl.org/info/docs/tools/vep/index.html) used to determine the effect of the SNPs, rs10519201 and rs5756444 are localized in regulatory regions suggesting putative functional consequences. CEP350 interacts directly with FGFR1 oncogene partner (FOP), a critical protein in the myeloproliferative disorders (25) and SHC4 has been reported to activate both Ras-dependent and Ras-independent migratory pathways in melanomas (26). Their involvement in cancer suggests a possible role in MPM pathogenesis, interacting with asbestos exposure.

THRB and *MMP14* are reported as deregulated in MPM (27,28). *THRB* encodes for thyroid hormone receptor beta (TRb), which could function as a tumor suppressor, and *MMP14* (matrix metallopeptides 14) has been reported to influence overall survival in MPM cases (28) but we did not find any significant interaction with asbestos exposure in relation to MPM risk. *PVT1* (Pvt1 oncogenic-non-protein coding) gene is involved in several types of cancer (29,30) but no significant interaction between asbestos and *PVT1*-rs7841347 was found.

None of the SNPs deviating from HWE showed a clear interaction with asbestos exposure in MPM development, although rs9536579 and rs2236304 showed deviation from the additive model for SI index, and rs10815216 showed borderline deviation from both the multiplicative model and the additive model for SI index. Moreover, after Bonferroni correction neither SI index

nor V index were statistically significant for rs1081521, and no significance was found for rs2236304 SI index.

Several limitation of the current study should be acknowledged. The statistical power is limited: the sample size is critical in general for all gene–environment interaction studies, and in particular for MPM, a rare disease where only few cases are not exposed to asbestos. This may influence the estimated ORs and their CIs and increase the variability of interaction indexes. Moreover, we cannot exclude either other SNPs with weaker effect in MPM risk might interact with asbestos-exposure, or the involvement of rare variants that could not be assessed with the platform used in our original GWAS. To address these issues a larger study would be required. The availability of methods for complete genome sequencing will allow to circumvent the problem of rare variants.

In conclusion, our results provide some suggestions that the genetic background of an individual may modulate asbestos-related carcinogenesis of the pleura, but the interpretation of a specific interaction model is made difficult by the limited 'a priori' evidence of a functional role of the investigated genetic variants. Asbestos however remains the major risk factor for MPM.

Supplementary material

Supplementary Table 1–4 can be found at http://carcin.oxfordjournals.org/

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