Original Article

Germline mutations in DNA repair genes predispose asbestos-exposed patients to malignant pleural mesothelioma

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ABSTRACT

Malignant pleural mesothelioma (MPM) is a rare, aggressive cancer caused by asbestos exposure. An inherited predisposition has been suggested to explain multiple cases in the same family and the observation that not all individuals highly exposed to asbestos develop the tumor. Germline mutations in *BAP1* are responsible for a rare cancer predisposition syndrome that includes predisposition to mesothelioma. We hypothesized that other genes involved in hereditary cancer syndromes could be responsible for the inherited mesothelioma predisposition. We investigated the prevalence of germline variants in 94 cancer-predisposing genes in 93 MPM patients with a quantified asbestos exposure. Ten pathogenic truncating variants (PTVs) were identified in *PALB2, BRCA1, FANCI, ATM, SLX4, BRCA2, FANCC, FANCF, PMS1* and *XPC*. All these genes are involved in DNA repair pathways, mostly in homologous recombination repair. Patients carrying PTVs represented 9.7% of the panel and showed lower asbestos exposure than did all the other patients (p = 0.0015). This suggests that they did not efficiently repair the DNA damage induced by asbestos and leading to carcinogenesis.

This study shows that germline variants in several genes may increase MPM susceptibility in the presence of asbestos exposure and may be important for specific treatment.

Introduction

Malignant pleural mesothelioma (MPM) is a rare and aggressive cancer caused by exposure to a single carcinogen, asbestos [1]. The frequency of MPM is dramatically higher in asbestos-polluted areas, as exemplified by the MPM epidemic in the northern Italy town of Casale Monferrato caused by the presence of an asbestos

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cement factory (1907–1986). In this area, the average annual incidence in 2009–2013 was 51.2 among men and 20.2 among women (per 100,000, per year), approximately 10 times higher than the corresponding Italian incidence rates (http://cpo.it/workspace/files/pleural-mesothelioma-incidence-574400b9b1625.pdf).

Asbestos induces carcinogenesis by directly interfering with mitotic spindle formation and by inducing chronic inflammation [2–4] with the production of cytokines and reactive oxygen species (ROS) by activated macrophages. ROS are also generated by the iron contained in asbestos fibers [5].

As in cases of exposure to other carcinogens, not all individuals exposed to high level of asbestos develop cancer [6]. This observation and the reports of families with multiple cases suggest that an inherited predisposition may play a role, even though common asbestos exposure must be considered [7–10]. As for other tumors [11,12], low-risk susceptibility factors have been identified by Genome Wide Association studies (GWAs) on the germline genome of MPM patients [13,14].

The occurrence of a dominant inherited predisposition, termed a 'high-risk predisposition', is a well known concept in cancer and has been clearly demonstrated for several cancer types [15–18]. The most studied high-risk factor for MPM is inherited mutations in *BAP1*, a tumor suppressor gene that encodes a deubiquitinase involved in the modulation of transcription and DNA repair [19]. To date, 79 families in which individuals carry one of 65 germline loss-of-function (LOF) mutations in *BAP1* have been identified worldwide [20–24]. The carriers are at high risk of a number of tumors, including mesothelioma, cutaneous and uveal melanoma, clear cell renal carcinoma, and basal cell carcinoma. Patients are also prone to develop peculiar cutaneous tumors, called melanocytic BAP1-mutated atypical intradermal tumors (MBAITs), which are considered to be a marker of BAP1 syndrome [25].

Investigations on mesothelioma cases with germline *BAP1* mutations suggest that these patients require asbestos exposure to develop mesothelioma and that these tumors most often have an epithelioid histotype and may be associated with a long survival [21,26].

The identification of the BAP1 syndrome prompted us to analyze *BAP1* in 18 families with familial MPM or mesothelioma and melanoma. We found germline *BAP1* mutations in only two families [21,22], suggesting that other genes may play a role in the mesothelioma predisposition.

We decided to investigate the overall genetic predisposition conferred by 94 genes associated with cancer in 93 patients with MPM who lived in areas subjected in the past to high asbestos exposure. Asbestos exposure was quantitatively evaluated in all study participants.

Materials and methods

Patients and controls

The study included 93 Italian patients with MPM. Diagnosis was made as described in Betti et al. [22]. Seventy-seven patients were randomly selected from the previously reported case-control studies [27,28] and were classified as sporadic, whereas sixteen patients had a family history of mesothelioma. Six familial and five sporadic patients were studied for mutations in *BAP1* and other genes involved in familial melanoma, and they were found to be mutation-negative [22].

All patients lived in Piedmont (northern Italy) and signed an informed consent form. The study was approved by the local ethics committee.

Clinical details on gender, age at diagnosis, survival, histotype, asbestos exposure and family history for mesothelioma for all patients were collected from their oncologist and/or from the Malignant Mesothelioma Registry of the Piedmont Region (RMM) (Table 1). Information on family history was limited to first- and second-degree relatives. Information on asbestos exposure at work, at home and in the general environment was collected by the RMM using a standardized questionnaire [29], which was administered by trained interviewers. Asbestos exposure was classified in the following categories: occupational, para-occupational, environmental and household, as previously described [21]. Moreover, exposure was assessed quantitatively by considering the entire exposure history of every study subject [30]. In brief, an exposure index was computed for each exposure circumstance by multiplying frequency, intensity and duration of exposure. The sum of the indices provided an estimate of life-long cumulative asbestos exposure.

Table 1
Clinical features of 93 MPM patients.

Clinical features	MPM Patients (N = 93) N (%)	Patients with PTVs (N = 9) N (%)	Patients without PTVs (N = 84) N (%)	OR* (95% CI)
Gender				
Male	65 (69.9%)	6 (66.7%)	59 (70.2%)	0.8 (0.2–3.6)
Female	28 (30.1%)	3 (33.3%)	25 (29.8%)	1 (reference)
Histotype				
Epithelioid	62 (66.7%)	8 (88.9%)	54 (64.3%)	4 (0.5–33.6)
Biphasic	16 (17.2%)	1 (11.1%)	15 (17.9%)	1 (ref: biphasic and sarcomatoid)
Sarcomatoid	12 (12.9%)	–	12 (14.3%)	
Unknown	2 (2.1%)	–	2 (2.4%)	
Not available	1 (1.1%)	–	1 (1.1%)	
Asbestos exposure				
Occupational	53 (57%)	5 (44.4%)	48 (42.9%)	0.8 (0.2–3.4)
Para-occupational	17 (18.3%)	3 (33.3%)	14 (16.7%)	1 (ref: para-occupational,
Environmental	19 (20.4%)	1 (11.1%)	18 (21.4%)	environmental and household)
Household	1 (1.1%)	–	1 (1.2%)	
Not available	3 (3.2%)	–	3 (3.6%)	
History of cancer				
At least one first-/second-degree relative with mesothelioma	16 (17.2%)	2 (22.2%)	14 (16.7%)	
Not reported	75 (80.6%)	7 (77.8%)	68 (80.9%)	
Not available	2 (2.2%)	–	2 (2.4%)	
Age at diagnosis, years				**p (Mann-Whitney test)**
Mean ± SD	68 ± 12.3^	73.9 ± 7.2	67.4 ± 12.6^	0.13
Survival				**p (Log rank test)**
1-year (95% CI)	57% (46–67)	25% (4–56)	59% (47–70)	0.25
2-year (95% CI)	30% (20–40)	12% (1–42)	30% (20–41)	
Quantitative asbestos exposure				**p (Student's t-test)**
Mean ± SD	22.8 ± 137.9^^	3.8 ± 9.5	24.9±145.3^^	
Mean ± SD (after logarithmic transformation)	0.9 ± 1.8	−0.8 ± 2.3	1.1 ± 1.6	**0.0015**

Abbreviations: PTVs, pathogenic truncating variants; OR, odds ratio; CI, confidence interval; SD, standard deviation.
*Patients with PTVs *versus* Patients without PTVs; ^Not available for 3 patients; ^^Not available for 3 patients.

For familial cases, information on cancer in relatives was gathered from their attending clinician's reports, clinical records and a section of the RMM questionnaire.

The study also included an appropriate control group of 96 Italian subjects. Seventy-one controls were healthy subjects, whereas twenty-five controls were hospitalized for non-neoplastic/non-respiratory conditions. One control per patient, matched for date of birth (±5 years), gender and Piedmont town (Casale Monferrato or Turin), was randomly selected from case-control studies previously reported by our group [27,28]. The sample size was chosen to estimate a possible pathogenic truncating variants (PTVs) prevalence of 1% with a confidence interval width of 100.0% of the point estimate (i.e. from 0 to 8%).

Next-generation sequencing (NGS) and variant validation

Genomic DNA was extracted from peripheral blood using the QIAamp® DNA Blood Maxi Kit (QIAGEN Inc., Valencia, CA, USA) according to the manufacturer's protocol.

Targeted next-generation sequencing (NGS) was performed on patient and control genomic DNAs (gDNA) using the TruSight® Cancer sequencing panel (Illumina Inc., San Diego, CA, USA) which targets 94 genes involved in common and rare cancer syndromes. In addition, the set includes 284 SNPs found to be associated with cancer through GWAs. Detailed protocols for the library preparation and data analyses are described in the Supplementary Methods.

Loss of heterozygosity (LOH) analysis in tumor samples was performed using Sanger sequencing and microsatellite analyses (Table S1). Protein expression was evaluated by immunohistochemistry (IHC) (Supplementary Methods).

Statistical analyses

To evaluate the association between patients mutational status and clinical characteristics, i.e., histological type (epithelioid *versus* others) and asbestos exposure (occupational *versus* environmental/para-occupational), a binary logistic regression was performed. The odds ratio (ORs) and the 95% confidence intervals (95% CIs) were calculated.

A Student's 2-tailed *t*-test after normalization using logarithmic transformation was used to compare the mean cumulative asbestos exposure among patients. The transformation was used in order to meet the normal distribution assumption of the variable required to perform the Student's *t*-test.

The follow-up time was analyzed from the date of diagnosis to the date of death or the latest follow-up date. Overall survival (OS) was calculated using a Kaplan-Meier analysis stratified by group, and the OS difference was determined using the log-rank test.

The 95% confidence interval of the prevalence of PTVs was computed using the Poisson distribution [31].

A *p* value ≤ 0.05 was considered statistically significant. Statistical analysis was performed using STATA v12 (Stata Corporation, College Station, TX, USA).

Results

Patient clinical features

The clinical features of the 93 MPM patients are reported in Table 1. Asbestos exposure was evaluated as occupational, para-occupational, environmental and household in approximately 57%, 18%, 20% and 1% of patients, respectively. No information about asbestos exposure was available for three patients (3.2%). The mean follow-up time of the 93 MPM patients was 1.8 years (SD 2.2). Seventy-two patients died, 12 were alive and 9 patients were lost at follow-up. At one year from diagnosis, 57% of the patients were alive, and at two years only 30% of the patients were still alive.

Variant detection and characterization

On average, 70% of the mapped NGS reads were on target regions. Most samples had over 93% of reads with coverage above 20x, while the mean per-target depth of coverage across all samples was 215x.

We identified 772 variants, including 12 indels, five nonsense, 402 missense and 353 synonymous variants. Only ten variants were considered PTVs and were subjected to further study (Table 2). Two in-frame deletions and eleven missense variants fulfilled the filter criteria described in Supplementary Methods, and they are reported in Table 3.

All the PTVs, the in-frame deletions and the missense variants were successfully validated with Sanger sequencing on DNA obtained from peripheral blood.

Pathogenic truncating variants

Nine MPM patients (9.7%) harbored heterozygous PTVs in the following ten genes: *PALB2, BRCA1, FANCI, ATM, SLX4, BRCA2, FANCC, FANCF, PMS1* and *XPC* (Table 4).

One patient was a double heterozygote for two truncating mutations in different genes: a nonsense mutation in *BRCA1* (c.3001G > T p.Glu1001*) and a frameshift mutation in *FANCI* (c.3846_3850delCACCT p.Ser1282fs*18).

A nonsense mutation in *PALB2* (c.691A > T p.Lys231*) was carried by another patient, whereas frameshift deletions were carried by seven patients, affecting *BRCA2* (c.6591_6592delTG p.Glu2198Asnfs*4), *ATM* (c.8436delT p.Ser2812fs), *SLX4* (c.2819delG p.Gly940fs), *PMS1* (c.1380delT p.Ser460fs), *FANCC* (c.1344delC p.Ala448fs), *FANCF* (c.604delC p.Leu202fs) and *XPC* (c.524_528delCAAGA p.Thr175fs).

We assessed whether these PTVs were reported in gene- or disease-databases. Only two PTVs were reported and classified as pathogenic, i.e. the nonsense mutation in *BRCA1* (c.3001G > T p.Glu1001*) and the frameshift mutation in *BRCA2* (c.6591_6592delTG p.Glu2198Asnfs*4) (Table S2).

Table 2
PTVs identified in this study.

Gene	Transcript	Mutation	Mutation type	Mutation effect	FFPE tumor sample	DNA repair pathway
ATM	NM_000051.3	c.8436delT p.Ser2812fs	Deletion	Frameshift	LOH-nuclear protein expression (IHC)	HRR
BRCA1	NM_007294.3	c.3001G > T p.Glu1001*	Substitution	Nonsense	Cytoplasmatic protein expression (IHC)	HRR
BRCA2	NM_000059.3	c.6591_6592delTG p.Glu2198Asnfs*4	Deletion	Frameshift	LOH	HRR
FANCC	NM_000136.2	c.1344delC p.Ala448fs	Deletion	Frameshift	na	HRR
FANCF	NM_022725.3	c.604delC p.Leu202fs	Deletion	Frameshift	na	HRR
FANCI	NM_001113378.1	c.3846_3850delCACCT p.Ser1282fs*18	Deletion	Frameshift	na	HRR
PALB2	NM_024675.3	c.691A > T p.Lys231*	Substitution	Nonsense	na	HRR
PMS1	NM_000534.4	c.1380delT p.Ser460fs	Deletion	Frameshift	na	MMR
SLX4	NM_032444.2	c.2819delG p.Gly940fs	Deletion	Frameshift	Cytoplasmatic protein expression (IHC)	HRR
XPC	NM_004628	c.524_528delCAAGA p.Thr175fs	Deletion	Frameshift	na	NER

Abbreviations: PTVs, pathogenic truncating variants; FFPE, Formalin-Fixed Paraffin-Embedded; IHC, immunohistochemistry; LOH, loss of heterozygosity; HRR, homologous recombination repair; MMR, mismatch repair; NER, nucleotide excision repair; na, not available; *ATM*, ataxia telangiectasia mutated; *BRCA1*, early-onset breast cancer gene 1; *BRCA2*, early-onset breast cancer gene 2; *FANCC*, Fanconi Anemia Complementation Group C; *FANCF*, Fanconi Anemia Complementation Group F; *FANCI*, Fanconi Anemia Complementation Group I; *PALB2*, partner and localizer of BRCA2; *PMS1*, postmeiotic segregation increased 1; *SLX4*, Structure-Specific Endonuclease Subunit; *XPC*, Xeroderma pigmentosum, complementation group C.
All changes are heterozygous.

Table 3
Variants of unknown significance (VUS) identified in this study that fulfilled the filter criteria.

Gene	Transcript	Variant	Variant type	Variant effect
FANCA	NM_000135.2	c.1874G > C p.Cys625Ser	Substitution	Missense
FANCA	NM_000135.2	c.3665C > T p.Pro1222Leu	Substitution	Missense
FANCA	NM_000135.2	c.1255T > G p.Phe419Val	Substitution	Missense
HNF1A	NM_000545.5	c.1226C > A p.Pro409His	Substitution	Missense
MLH1	NM_000249.3	c.1852_1853delAAinsGC p.Lys618Ala	Substitution	Missense
MLH1	NM_000249.3	c.1136A > G p.Tyr379Cys	Substitution	Missense
MSH6	NM_000179.2	c.3727A > T p.Thr1243Ser	Substitution	Missense
MUTYH	NM_001048171.1	c.1145G > A p.Gly382Asp	Substitution	Missense
TSC2	NM_000548.3	c.1915C > T p.Arg639Trp	Substitution	Missense
TSC2	NM_000548.3	c.2278A > C p.Thr760Pro	Substitution	Missense
FANCM	NM_020937.2	c.1313_1318delATAAAA p.Asp438_Asn440del	Deletion	In-frame
ERCC3	NM_000122.1	c.786_791delAGAAGA p.Glu262_Glu264del	Deletion	In-frame

Abbreviations: *FANCA*, Fanconi Anemia Complementation Group A; *HNF1A*, Hepatocyte Nuclear Factor 1-Alpha; *MLH1*, MutL Homolog 1; *MSH6*, mutS homolog 6; *MUTYH*, MutY DNA Glycosylase; *TSC2*, Tuberous Sclerosis Complex 2; *FANCM*, Fanconi Anemia Complementation Group M; *ERCC3*, ERCC excision repair 3, TFIIH core complex helicase subunit.
All changes are heterozygous.

Table 4
Clinical features of patients carrying PTVs.

Patient ID	PTV	Gender	Histotype	Age at onset, years	Age at death, years	Asbestos exposure	Cancers in relatives
MPM125AL	*PALB2* c.691A > T p.Lys231*	M	Biphasic	82	83	Para-occupational	Intestinal cancer (brother)
MPM156AL	*BRCA1* c.3001G > T p.Glu1001* *FANCI* c.3846_3850delCACCT p.Ser1282fs*18	F	Epithelioid	70	na	Occupational	Breast cancer (sister)
MPM1115	*ATM* c.8436delT p.Ser2812fs	M	Epithelioid	72	75	Occupational	nr
MPM1135	*SLX4* c.2819delG p.Gly940fs	F	Epithelioid	64	65	Para-occupational	MPM (sister), lung cancer (mother, father)
MPM88TO	*BRCA2* c.6591_6592delTG p.Glu2198Asnfs*4	M	Epithelioid	65	68	Occupational	nr
MPM85AL	*FANCC* c.1344delC p.Ala448fs	F	Epithelioid	79	79	Para-occupational	nr
MPM122AL	*FANCF* c.604delC p.Leu202fs	M	Epithelioid	81	81	Occupational	nr
MPM87TO	*PMS1* c.1380delT p.Ser460fs	M	Epithelioid	67	68	Occupational	nr
MPM155AL	*XPC* c.524_528delCAAGA p.Thr175fs	M	Epithelioid	80	82	Environmental	MM (mother)

Abbreviations: PTVs, pathogenic truncating variants; M, male; F, female; MM, malignant mesothelioma; MPM, malignant pleural mesothelioma; na, not available; nr, not reported.

We also checked the PTV frequencies in public population databases, on the hypothesis that pathogenic mutations are very rare. Only the deleterious mutation in *FANCI* (c.3846_3850delCACCT p.Ser1282fs*18) is reported in ExAC with an MAF (minor allele frequency) lower than 0.001% (Table S2). The MAF of each PTV in our dataset was higher than the MAF of all LOF variants in the corresponding gene reported by ExAC and EVS databases ($p < 0.05$). These investigations, performed on databases including thousands of subjects, confirmed that these PTVs are very rare in these genes.

When we looked at all the LOF variants in the ten genes in controls, only one control was found to carry a frameshift mutation in *BRCA2* (different from that carried by our MPM patient), but no further information (e.g. vital status, cause of death) is available.

Since clinical phenotype is not shared in the population databases, we also evaluated an appropriate control panel of 96 Piedmont subjects. No PTVs were found in our control panel, supporting a PTV role in mesothelioma risk. The 95% confidence interval of the prevalence of PTVs in controls was from 0 to 3.8%.

The clinical data for the patients are reported in Table 4. Patients with *SLX4* and *XPC* PTVs had familiarity for mesothelioma in first- or second-degree relatives, whereas the patient with *BRCA1* and *FANCI* PTVs had familiarity for breast cancer, and the patient with *PALB2* PTV for intestinal cancer.

Functional studies on tumor samples

Cancer-predisposing genes are most often tumor suppressor genes whose bi-allelic loss is due to an additional somatic variant in the tumor that complements the inherited LOF variant.

To evaluate the expression of the protein in an FFPE (Formalin-Fixed Paraffin-Embedded) tumor sample, IHC was performed using specific antibodies.

IHC using the anti-BRCA1 antibody on an FFPE tumor sample of MPM156AL showed a diffuse cytoplasmatic staining instead of the expected nuclear staining (data not shown). Since the inherited variant was a PTV, the results suggest that a second mutation leads to a variant non-functional protein. This patient also carried a PTV in *FANCI* that was found in tumor DNA, but the FFPE tumor specimen amount was not enough to perform IHC.

The patient who carried a PTV in *BRCA2* showed LOH for the D13S1701 microsatellite marker and a decreased amount of the D13S171 allele in the FFPE tumor sample (data not shown). This suggests that a somatic event causes loss of the gene and abolishes the protein.

The patient who carried a PTV in *SLX4* validated in tumor DNA showed cytoplasmatic positivity in the FFPE tumor sample instead of the expected nuclear staining, suggesting the presence of a second somatic variant leading to the production of a non-functional protein.

The patient with a PTV in *ATM* showed a decreased amount of an allele for the sole informative microsatellite marker (D11S1778) in the tumor DNA (data not shown), suggesting LOH. However, IHC with the anti-ATM antibody showed normal nuclear staining (data not shown), suggesting the presence of a wild type allele. Since we did not identify the PTV in the tumor tissue, overall, this result suggests that the mutated allele was lost in the tumor. This behavior has also been reported for breast cancers harboring *ATM* PTVs [32], possibly because during cancer

progression, allele deletion was more advantageous than the original PTV.

No tumor specimen was available from the patients with PTVs in *PALB2*, *XPC*, *FANCC*, *FANCF* and *PMS1*.

Variants of unknown significance (VUS)

Two in-frame deletions and eleven missense variants that fulfilled the filter criteria were found in eight different genes: *ERCC3*, *FANCM*, *FANCA*, *MLH1*, *MSH6*, *MUTYH*, *TSC2* and *HNF1A* (Table 3).

The two in-frame deletions (*FANCM* c.1313_1318delATAAAA p.Asp438_Asn440del; *ERCC3* c.786_791delAGAAGA p.Glu262_Glu264del) were carried by a single patient.

Two variants were identified in *MLH1*. One patient carried the c.1136A > G p.Tyr379Cys variant, whereas two other patients carried a variant that affects two consecutive base pairs in *cis* leading to a Lys618Ala missense variant (c.1852_1853delAAinsGC) already reported as a VUS in genomic databases (Table S3).

The variants *HNF1A* c.1226C > A p.Pro409His and *MSH6* c.3727A > T p.Thr1243Ser were found in two and one patients, respectively.

Three variants were found in *FANCA* (c.1874G > C p.Cys625Ser, c.3665C > T p.Pro1222Leu, c.1255T > G p.Phe419Val) in four patients. One of them also carried a *TSC2* variant (c.1915C > T p.Arg639Trp). Another missense variant in *TSC2* (c.2278A > C p.Thr760Pro) was carried by a different patient.

LOF mutations in *TSC1* and *TSC2* are the cause of tuberous sclerosis syndrome (TS), a generally severe genodermatosis inherited as an autosomal dominant trait and characterized by mental retardation, seizures and angiofibromas of the skin. The c.1915C > T p.Arg639Trp variant is reported as pathogenic in the TS database-Leiden Open Variation Database. We cannot rule out that these patients were affected by TS since information about concurrent inherited disease was not reported in the Registry and during the interview. It is intriguing that one patient with TS has been reported to develop a primary pericardial mesothelioma [33]. Moreover, *TSC1* has been found to be somatically mutated in mesothelioma, and mice with *TSC1* and *TP53* mutations develop mesothelioma [34].

We assessed whether these VUS were reported in gene- or disease-databases. Only one of the missense variants (*MUTYH* c.1145G > A p.Gly382Asp), carried by three patients, is considered pathogenic in the ClinVar database (Table S3). One of these patients also carried a PTV in *SLX4*.

In the population databases, the VUS showed an MAF lower than 0.001 (Table S3). In our control panel, only the two missense variants, *HNF1A* c.1226C > A p.Pro409His and *MLH1* c.1852_1853delAAinsGC p.Lys618Ala were found, each mutation in one control (prevalence 1.04%, 95% CI from 0.03 to 5.80%).

The clinical features of the patients are reported in Table 5. Patients with two missense variants (*MUTYH* c.1145G > A p.Gly382Asp; *MLH1* p.Lys618Ala) respectively had familiarity for

Table 5
Clinical features of patients carrying VUS.

Patient ID	Variant	Gender	Histotype	Age at onset, years	Age at death, years	Asbestos exposure	Cancers in relatives
MPM1114	*FANCA* c.1874G > C p.Cys625Ser	M	Biphasic	67	68	Environmental	Rectal cancer (father)
MPM124AL	*FANCA* c.1874G > C p.Cys625Ser	M	Epithelioid	84	84	Para-occupational	nr
MPM1110	*FANCA* c.1255T > G p.Phe419Val	M	Epithelioid	70	71	Occupational	nr
MPM99AL	*FANCA* c.3665C > T p.Pro1222Leu *TSC2* c.1915C > T p.Arg639Trp	M	Biphasic	80	81	Environmental	Breast cancer (mother)
MPM1119	*HNF1A* c.1226C > A p.Pro409His	M	Epithelioid	50	51	Environmental	Colon cancer (mother)
MPM82TO	*HNF1A* c.1226C > A p.Pro409His	M	Sarcomatoid	69	70	Occupational	nr
MPM173AL	*MLH1* c.1852_1853delAAinsGC p.Lys618Ala	M	Sarcomatoid	na	na	Occupational	MPM (father), ovarian cancer (mother), lung cancer (paternal uncle)
MPM1120	*MLH1* c.1852_1853delAAinsGC p.Lys618Ala	M	Biphasic	69	70	Occupational	nr
MPM1129	*MLH1* c.1136A > G p.Tyr379Cys	M	Epithelioid	59	63	Occupational	nr
MPM94TO	*MSH6* c.3727A > T p.Thr1243Ser	M	Biphasic	69	70	Occupational	nr
MPM97TO	*MUTYH* c.1145G > A p.Gly382Asp	M	Epithelioid	66	68	Occupational	MPM (paternal cousin), bone sarcome (sibling)
MPM126AL	*MUTYH* c.1145G > A p.Gly382Asp	M	Biphasic	74	75	Occupational	nr
MPM1135	*MUTYH* c.1145G > A p.Gly382Asp	F	Epithelioid	64	65	Para-occupational	MPM (sister), lung cancer (mother, father)
MPM1132	*TSC2* c.2278A > C p.Thr760Pro	M	Epithelioid	58	—	Occupational	Lung cancer (father), cancer of unknown site (son)
MPM82AL	*FANCM* c.1313_1318delATAAAA p.Asp438_Asn440del *ERCC3* c.786_791delAGAAGA p.Glu262_Glu264del	M	Biphasic	71	71	Para-occupational	nr

Abbreviations: VUS, variants of unknown significance; M, male; F, female; MPM, malignant pleural mesothelioma; nr, not reported; na, not available.

MPM in first- or second-degree relatives. Familiarity for other cancers was found in patients with *FANCA*, *HNF1A* and *TSC2* variants.

Because the data are not unequivocal, all these variants should be considered VUS until their effect is evaluated by segregation and functional studies.

Other findings

A group of variants that did not fulfill the selected filter criteria are nevertheless mentioned here because they have been reported as low-risk factors in the literature.

MITF c.952G > A p.Glu318Lys (rs149617956), which is considered a low-risk factor for familial melanoma [35], was found in a single MPM patient who also carried *MUTYH* c.1145G > A p.Gly382Asp.

We also found a *BAP1* missense variant (c.944A > C p.Glu315Ala) (rs149974450) in one patient. This variant is reported in ExAC and EVS with an MAF lower than 0.02%, but four out of six *in silico* prediction tools considered it as benign. A functional assay is needed to evaluate the effect of this VUS.

Statistical analyses

Patients were divided into three groups, i.e. patients carrying PTVs, patients carrying VUS and patients who were non-carriers (i.e. patients who did not carry either PTV or VUS). Statistical analyses were performed to evaluate the differences among these groups (Table 1, Tables S4 and S5).

The mean of the quantitative asbestos exposure was 3.8 (SD 9.5) for the group with PTVs and 24.9 (SD 145.3) for the group without PTVs. A statistically significant difference of the mean values of quantitative exposure between these two groups was detected (p = 0.0015) (Table 1). A statistically significant difference was also present when the quantitative exposure of the PTV group was compared with either the 70 non-carrier patients (p = 0.0039) (Table S4) or the VUS group (p = 0.008) (Table S5a). No statistically significant difference of the mean value of quantitative exposure was found when the 14 patients with VUS were compared with the 70 non-carrier patients (p = 0.38, Table S5b).

No statistically significant association was found for histotype or the categories of asbestos exposure in the groups with and without PTVs. Considering the group without PTVs as a reference, the OR for patients with sarcomatoid and biphasic histologies compared with those who had epithelioid histology was 4 (95% CI 0.5—33.6), and the OR among those with occupational exposure compared with those with environmental, para-occupational and household asbestos exposure was 0.8 (95% CI 0.2—3.4) (Table 1). The mean age at diagnosis was 73.9 (SD 7.2) for the group with PTVs and 67.4 (SD 12.6) for the other group. No significant differences in age at diagnosis or survival were found between the two groups (p = 0.13 and p = 0.25, respectively) (Table 1).

Discussion

Our study shows, for the first time, that a substantial proportion (9.7%) of MPM patients carry PTVs in DNA repair genes, and that these patients show a statistically significant lower intensity of asbestos exposure. This suggests that although asbestos exposure is necessary to develop MPM, in these patients even a low exposure to asbestos is sufficient to induce tumorigenesis.

Interestingly, eight of these mutated genes are involved in homologous recombination repair (HRR) (*BRCA1*, *BRCA2*, *PALB2*, *ATM*, *FANCI*, *FANCC*, *FANCF*, *SLX4*), like *BAP1*, whereas two (*XPC* and *PMS1*) are involved in nucleotide excision repair (NER) and mismatch repair (MMR), respectively. HRR is the mechanism used to repair double-strand breaks (DSBs) induced by asbestos fibers. On the other hand, asbestos may also cause DNA damage repaired by base excision repair (BER), NER or MMR.

It is thus possible to hypothesize that these patients, because of their defect in DNA repair, were less able to repair DNA damage induced by asbestos.

Moreover, 14 patients (15%) carried rare missense and in-frame variants considered damaging by *in silico* prediction tools and located in *FANCA*, *TSC2*, *HNF1A*, *MLH1*, *MSH6*, *MUTYH*, *ERCC3* and *FANCM*. Although a stringent approach cannot consider these mutations as pathogenic without functional analyses, it is intriguing to note that they are located in genes involved in the same DNA repair pathways reported for the PTVs, i.e. HRR (*FANCA*, *FANCM*), NER (*ERCC3*) and MMR (*MLH1*, *MSH6*, *MUTYH*).

Our approach of variant classification was very conservative, since we did not consider the possible pathogenic missense variants in the analysis. Consequently, we may have underestimated the prevalence of mutation carriers. Moreover, the NGS approach that we used does not reveal large rearrangements, for which a whole genome NGS or CGH-based approach would be more appropriate [36,37]. Last and not least, mutations in genes that are not included in the TruSight Cancer panel were not screened. Thus, it is possible that patients who did not carry variants in the 94 genes that were the object of our study harbor variants in genes that were not screened. In order to completely characterize all genes conferring increased risk of MPM, a whole exome approach is needed.

Our data show, for the first time, that predisposition to MPM is very heterogeneous, similar to that reported for pancreatic cancer and other cancer types [17,18,38]. Also the proportion of patients carrying PTV is similar.

Our study suggests that MPM could be added to the constellation of tumors resulting from mutations in *BRCA1*, *BRCA2*, *ATM*, *SLX4*, *FANCC*, *FANCI*, *PALB2*, *FANCF*, *PMS1* and *XPC*. Most probably the type of carcinogen exposure is relevant for the cancer type that will be developed by variant carriers, as suggested for *BAP1* [21].

The characterization of mutation carriers within these families is of particular clinical importance because all carriers are at risk for a certain cancer spectrum and should be included in specific high-risk surveillance and secondary prevention programs [39]. Finally, identification of inherited predisposition for mesothelioma may prove to be important for treatment in the future. This was the case of ovarian cancers due to predisposing germline *BRCA1* and *BRCA2* variants, where a different response to PARP inhibitors was identified [40,41]. Similarly, the identification of subsets of patients who carry predisposing mutations in the homologous recombination pathway may distinguish patients who can benefit from drugs that induce synthetic lethality [42,43].

Conflict of interest statement

Marta Betti, Elisabetta Casalone, Daniela Ferrante, Anna Aspesi, Giulia Morleo, Alessandra Biasi, Marika Sculco, Giuseppe Mancuso, Luisella Righi, Federica Grosso, Roberta Libener, Mansueto Pavesi, Narciso Mariani, Caterina Casadio, Renzo Boldorini, Dario Mirabelli, Barbara Pasini, Corrado Magnani, Giuseppe Matullo, Irma Dianzani.
The authors have no conflict of interest.

CM and DM were expert witness for the public prosecutor in criminal trials regarding the occurrence of asbestos-related diseases following occupational and environmental exposures to asbestos.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.canlet.2017.06.028.

References

[1] S. Novello, C. Pinto, V. Torri, L. Porcu, M. Di Maio, M. Tiseo, et al., The third Italian consensus conference for malignant pleural mesothelioma: state of the art and recommendations, Crit. Rev. Oncol. Hematol. 104 (2016) 9–20, http://dx.doi.org/10.1016/j.critrevonc.2016.05.004.

[2] D.W. Kamp, S.A. Weitzman, The molecular basis of asbestos induced lung injury, Thorax 54 (1999) 638–652 (Accessed 28 March 2017), http://www.ncbi.nlm.nih.gov/pubmed/10377212.

[3] C.G. Jensen, L.C. Jensen, C.L. Rieder, R.W. Cole, J.G. Ault, Long crocidolite asbestos fibers cause polyploidy by sterically blocking cytokinesis, Carcinogenesis 17 (1996) 2013–2021 (Accessed 28 March 2017), http://www.ncbi.nlm.nih.gov/pubmed/8824529.

[4] H. Fung, Y.W. Kow, B. Van Houten, B.T. Mossman, Patterns of 8-hydroxydeoxyguanosine formation in DNA and indications of oxidative stress in rat and human pleural mesothelial cells after exposure to crocidolite asbestos, Carcinogenesis 18 (1997) 825–832 (Accessed 28 March 2017), http://www.ncbi.nlm.nih.gov/pubmed/9111221.

[5] M. Carbone, H. Yang, Molecular pathways: targeting mechanisms of asbestos and erionite carcinogenesis in mesothelioma, Clin. Cancer Res. 18 (2012) 598–604, http://dx.doi.org/10.1158/1078-0432.CCR-11-2259.

[6] M. Neri, D. Ugolini, I. Dianzani, F. Gemignani, S. Landi, A. Cesario, et al., Genetic susceptibility to malignant pleural mesothelioma and other asbestos-associated diseases, Mutat. Res. 659 (2008) 126–136, http://dx.doi.org/10.1016/j.mrrev.2008.02.002.

[7] V. Ascoli, C. Carnovale-Scalzo, F. Nardi, C. Efrati, M. Menegozzo, A one-generation cluster of malignant mesothelioma within a family reveals exposure to asbestos-contaminated jute bags in Naples, Italy, Eur. J. Epidemiol. 18 (2003) 171–174 (Accessed 28 March 2017), http://www.ncbi.nlm.nih.gov/pubmed/12733840.

[8] V. Ascoli, D. Cavone, E. Merler, P.G. Barbieri, L. Romeo, F. Nardi, et al., Mesothelioma in blood related subjects: report of 11 clusters among 1954 Italy cases and review of the literature, Am. J. Ind. Med. 50 (2007) 357–369, http://dx.doi.org/10.1002/ajim.20451.

[9] V. Ascoli, E. Romeo, C. Carnovale Scalzo, I. Cozzi, L. Ancona, F. Cavariani, et al., Familial malignant mesothelioma: a population-based study in central Italy (1980-2012), Cancer Epidemiol. 38 (2014) 273–278, http://dx.doi.org/10.1016/j.canep.2014.02.014.

[10] A.U. Dogan, Y.I. Baris, M. Dogan, S. Emri, I. Steele, A.G. Elmishad, et al., Genetic predisposition to fiber carcinogenesis causes a mesothelioma epidemic in Turkey, Cancer Res. 66 (2006) 5063–5068, http://dx.doi.org/10.1158/0008-5472.CAN-05-4642.

[11] M.R. Chandler, E.P. Bilgili, N.D. Merner, A review of whole-exome sequencing efforts toward hereditary breast cancer susceptibility gene discovery, Hum. Mutat. 37 (2016) 835–846, http://dx.doi.org/10.1002/humu.23097.

[12] V.N. Giri, J.L. Beebe-Dimmer, Familial prostate cancer, Semin. Oncol. 43 (2016) 560–565, http://dx.doi.org/10.1053/j.seminoncol.2016.08.001.

[13] G. Matullo, S. Guarrera, M. Betti, G. Fiorito, D. Ferrante, F. Voglino, et al., Genetic variants associated with increased risk of malignant pleural mesothelioma: a genome-wide association study, PLoS One 8 (2013) e61253, http://dx.doi.org/10.1371/journal.pone.0061253.

[14] G. Cadby, S. Mukherjee, A.W.B. Musk, A. Reid, M. Garlepp, I. Dick, et al., A genome-wide association study for malignant mesothelioma risk, Lung Cancer 82 (2013) 1–8, http://dx.doi.org/10.1016/j.lungcan.2013.04.018.

[15] S. Zhang, R. Royer, S. Li, J.R. McLaughlin, B. Rosen, H.A. Risch, et al., Frequencies of BRCA1 and BRCA2 mutations among 1,342 unselected patients with invasive ovarian cancer, Gynecol. Oncol. 121 (2011) 353–357, http://dx.doi.org/10.1016/j.ygyno.2011.01.020.

[16] N. Tung, N.U. Lin, J. Kidd, B.A. Allen, N. Singh, R.J. Wenstrup, et al., Frequency of germline mutations in 25 cancer susceptibility genes in a sequential series of patients with breast cancer, J. Clin. Oncol. 34 (2016) 1460–1468, http://dx.doi.org/10.1200/JCO.2015.65.0747.

[17] R.C. Grant, I. Selander, A.A. Connor, S. Selvarajah, A. Borgida, L. Briollais, et al., Prevalence of germline mutations in cancer predisposition genes in patients with pancreatic cancer, Gastroenterology 148 (2015) 556–564, http://dx.doi.org/10.1053/j.gastro.2014.11.042.

[18] N.J. Roberts, A.L. Norris, G.M. Petersen, M.L. Bondy, R. Brand, S. Gallinger, et al., Whole genome sequencing defines the genetic heterogeneity of familial pancreatic cancer, Cancer Discov. 6 (2016) 166–175, http://dx.doi.org/10.1158/2159-8290.CD-15-0402.

[19] J.R. Testa, M. Cheung, J. Pei, J.E. Below, Y. Tan, E. Sementino, et al., Germline BAP1 mutations predispose to malignant mesothelioma, Nat. Genet. 43 (2011) 1022–1025, http://dx.doi.org/10.1038/ng.912.

[20] K. Rai, R. Pilarski, C.M. Cebulla, M.H. Abdel-Rahman, Comprehensive review of BAP1 tumor predisposition syndrome with report of two new cases, Clin. Genet. 89 (2016) 285–294, http://dx.doi.org/10.1111/cge.12630.

[21] M. Betti, E. Casalone, D. Ferrante, A. Romanelli, F. Grosso, S. Guarrera, et al., Inference on germline BAP1 mutations and asbestos exposure from the analysis of familial and sporadic mesothelioma in a high-risk area, Genes. Chromosom. Cancer 54 (2015) 51–62, http://dx.doi.org/10.1002/gcc.22218.

[22] M. Betti, A. Aspesi, A. Biasi, E. Casalone, D. Ferrante, P. Ogliara, et al., CDKN2A and BAP1 germline mutations predispose to melanoma and mesothelioma, Cancer Lett. 378 (2016) 120–130, http://dx.doi.org/10.1016/j.canlet.2016.05.011.

[23] J.A. Ohar, M. Cheung, J. Talarchek, S.E. Howard, T.D. Howard, M. Hesdorffer, et al., Germline BAP1 mutational landscape of asbestos-exposed malignant mesothelioma patients with family history of cancer, Cancer Res. 76 (2016) 206–215, http://dx.doi.org/10.1158/0008-5472.CAN-15-0295.

[24] K. Rai, R. Pilarski, G. Boru, M. Rehman, A.H. Saqr, J.B. Massengill, et al., Germline BAP1 alterations in familial uveal melanoma, Genes. Chromosom. Cancer 56 (2017) 168–174, http://dx.doi.org/10.1002/gcc.22424.

[25] M. Carbone, L.K. Ferris, F. Baumann, A. Napolitano, C.A. Lum, E.G. Flores, et al., BAP1 cancer syndrome: malignant mesothelioma, uveal and cutaneous melanoma, and MBAITs, J. Transl. Med. 10 (2012) 179, http://dx.doi.org/10.1186/1479-5876-10-179.

[26] F. Baumann, E. Flores, A. Napolitano, S. Kanodia, E. Taioli, H. Pass, et al., Mesothelioma patients with germline BAP1 mutations have 7-fold improved long-term survival, Carcinogenesis 36 (2015) 76–81, http://dx.doi.org/10.1093/carcin/bgu227.

[27] M. Betti, D. Ferrante, M. Padoan, S. Guarrera, M. Giordano, A. Aspesi, et al., XRCC1 and ERCC1 variants modify malignant mesothelioma risk: a case-control study, Mutat. Res. 708 (2011) 11–20, http://dx.doi.org/10.1016/j.mrfmmm.2011.01.001.

[28] I. Dianzani, L. Gibello, A. Biava, M. Giordano, M. Bertolotti, M. Betti, et al., Polymorphisms in DNA repair genes as risk factors for asbestos-related malignant mesothelioma in a general population study, Mutat. Res. 599 (2006) 124–134, http://dx.doi.org/10.1016/j.mrfmmm.2006.02.005.

[29] C. Magnani, A. Agudo, C.A. González, A. Andrion, A. Calleja, E. Chellini, P. Dalmasso, A. Escolar, S. Hernandez, C. Ivaldi, D. Mirabelli, J. Ramirez, D. Turuguet, M. Usel, B. Terracini, Multicentric study on malignant pleural mesothelioma and non-occupational exposure to asbestos, Br. J. Cancer. 83 (2000) 104–111, http://dx.doi.org/10.1054/bjoc.2000.1161.

[30] D. Ferrante, D. Mirabelli, S. Tunesi, B. Terracini, C. Magnani, Pleural mesothelioma and asbestos exposure: a case-control study with quantitative risk assessment-response to Marsh and Benson's letter, Occup. Environ. Med. 74 (2017) 157–158, http://dx.doi.org/10.1136/oemed-2016-104091.

[31] N.E. Breslow, N.E. Day, Statistical methods in cancer research. Volume I - the analysis of case-control studies, IARC Sci. Publ. (1980) 5–338 (Accessed 31 May 2017), http://www.ncbi.nlm.nih.gov/pubmed/7216345.

[32] D.E. Goldgar, S. Healey, J.G. Dowty, L. Da Silva, X. Chen, A.B. Spurdle, et al., Rare variants in the ATM gene and risk of breast cancer, Breast Cancer Res. 13 (2011) R73, http://dx.doi.org/10.1186/bcr2919.

[33] A. Naramoto, N. Itoh, M. Nakano, H. Shigematsu, An autopsy case of tuberous sclerosis associated with primary pericardial mesothelioma, Acta Pathol. Jpn. 39 (1989) 400–406 (Accessed 28 March 2017), http://www.ncbi.nlm.nih.gov/pubmed/2481938.

[34] Y. Guo, L.R. Chirieac, R. Bueno, H. Pass, W. Wu, I.A. Malinowska, et al., Tsc1-Tp53 loss induces mesothelioma in mice, and evidence for this mechanism in human mesothelioma, Oncogene 33 (2014) 3151–3160, http://dx.doi.org/10.1038/onc.2013.280.

[35] V.K. Hill, J.J. Gartner, Y. Samuels, A.M. Goldstein, The genetics of melanoma: recent advances, Annu. Rev. Genomics Hum. Genet. 14 (2013) 257–279, http://dx.doi.org/10.1146/annurev-genom-091212-153429.

[36] T. Judkins, B. Leclair, K. Bowles, N. Gutin, J. Trost, J. McCulloch, et al., Development and analytical validation of a 25-gene next generation sequencing panel that includes the BRCA1 and BRCA2 genes to assess hereditary cancer risk, BMC Cancer 15 (2015) 215, http://dx.doi.org/10.1186/s12885-015-1224-y.

[37] J. Staaf, T. Törngren, E. Rambech, U. Johansson, C. Persson, G. Sellberg, et al., Detection and precise mapping of germline rearrangements in BRCA1, BRCA2, MSH2, and MLH1 using zoom-in array comparative genomic hybridization (aCGH), Hum. Mutat. 29 (2008) 555–564, http://dx.doi.org/10.1002/humu.20678.

[38] C. Hu, S.N. Hart, W.R. Bamlet, R.M. Moore, K. Nandakumar, B.W. Eckloff, et al., Prevalence of pathogenic mutations in cancer predisposition genes among

pancreatic cancer patients, Cancer Epidemiol. Biomarkers Prev. 25 (2016) 207—211, http://dx.doi.org/10.1158/1055-9965.EPI-15-0455.

[39] H. Vasen, I. Ibrahim, C.G. Ponce, E.P. Slater, E. Matthäi, A. Carrato, et al., Benefit of surveillance for pancreatic cancer in high-risk individuals: outcome of long-term prospective follow-up studies from three european expert centers, J. Clin. Oncol. 34 (2016) 2010—2019, http://dx.doi.org/10.1200/JCO.2015.64.0730.

[40] A. George, S. Kaye, S. Banerjee, Delivering widespread BRCA testing and PARP inhibition to patients with ovarian cancer, Nat. Rev. Clin. Oncol. (2016), http://dx.doi.org/10.1038/nrclinonc.2016.191.

[41] E.M. Swisher, K.K. Lin, A.M. Oza, C.L. Scott, H. Giordano, J. Sun, et al., Rucaparib in relapsed, platinum-sensitive high-grade ovarian carcinoma (ARIEL2 Part 1): an international, multicentre, open-label, phase 2 trial, Lancet. Oncol. 18 (2017) 75—87, http://dx.doi.org/10.1016/S1470-2045(16)30559-9.

[42] P.C. Fong, D.S. Boss, T.A. Yap, A. Tutt, P. Wu, M. Mergui-Roelvink, et al., Inhibition of poly(ADP-ribose) polymerase in tumors from BRCA mutation carriers, N. Engl. J. Med. 361 (2009) 123—134, http://dx.doi.org/10.1056/NEJMoa0900212.

[43] C.J. Lord, A. Ashworth, BRCAness revisited, Nat. Publ. Gr. 16 (2016), http://dx.doi.org/10.1038/nrc.2015.21.