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

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To the Editor of the *Cancer Letters*

Dear Sir,

We are submitting our manuscript entitled "Germline mutations in DNA repair genes predispose asbestos-exposed patients to malignant pleural mesothelioma."

We studied the prevalence of germline mutations in 94 genes involved in cancer syndromes in 93 patients with malignant pleural mesothelioma. To our knowledge this is the first study of this kind.
A main asset of our study was the thorough quantification of asbestos exposure in the studied patients.
We observed that 9.7% of the patients carried pathogenic truncating variants in DNA repair genes. Moreover, these patients were exposed to a smaller amount of asbestos as compared with all the other patients. This difference is statistically significant.
Our study suggests that patients with a DNA repair defect are more susceptible to asbestos carcinogenesis because they cannot efficiently repair the DNA damage induced by asbestos.
Our study has clinical relevance because these patients might respond to drugs that induce synthetic lethality. Moreover, patients' relatives who carry the same variants should undergo surveillance for the constellation of cancers specific for the mutated genes.
We wish to underline that mesothelioma is a rare cancer; thus, our 93-patient study panel is noteworthy.

We were wondering whether we could exclude the following scientists as reviewers, because they are direct competitors:

- Luciano Mutti, RCPI Medical Oncology, Chair In Cancer Research, University of Salford

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-Harvey Pass, Department of Cardiothoracic Surgery, New York University Langone Medical Center, New York, NY, 10065, USA.

We hope that our paper may find a place in the *Cancer Letters.*

Yours sincerely,
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Highlights

1. We show that germline mutations in DNA repair genes may predispose to MPM.
2. Mutation carriers showed lower asbestos exposure as compared with the other patients.
3. Abnormal DNA repair may favour MPM carcinogenesis due to asbestos exposure.
4. Carriers may benefit from drugs that induce synthetic lethality.
5. MPM should be included in the spectrum of several cancer predisposition syndromes.

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 reasoned that other genes 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 all the other patients (p=0.0017). This suggests that they do not efficiently repair the DNA damage induced by asbestos, leading to carcinogenesis.

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

Keywords
Mesothelioma
Asbestos exposure
Germline mutation
DNA repair genes
Homologous recombination repair

1. 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 cement factory (1907-1986). In this area, the average annual incidence in 2009–2013 was 51.2 among men and 20.2 (per 100,000, per year) among women, 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].

Similar to 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 a possible role for an inherited predisposition, 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, so called 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]. So far, 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 for 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), that are considered to be a marker of BAP1 syndrome [25].

Cancer is induced because of the loss of the tumor suppressor activity by a second somatic mutation [26].

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 [21,27].

We recently identified mesothelioma as being in the cancer spectrum of the *CDKN2A* syndrome, together with familial melanoma and pancreatic cancer [22]. Our study also showed that twelve families with familial mesothelioma did not carry germline mutations either in *BAP1* or melanoma predisposition genes, suggesting that other genes could play a role [22].

Here, 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.

2. Materials and Methods

2.1 Patients

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 [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 were found to be mutation-negative [22].

All patients lived in Piedmont (northern Italy) and signed an informed consent. 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 I). 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 whole 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.

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.

2.2 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 genomic DNA (gDNA) using the TruSight® Cancer sequencing panel (Illumina Inc., San Diego, CA, USA) that 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. Protein expression was evaluated by immunohistochemistry (IHC) (Supplementary Methods).

2.3 Statistical Analyses

Patients were divided in two groups, those carrying pathogenic truncating variants (PTVs) and all other patients.

To evaluate the association between the two groups of patients 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 between groups.

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.

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

3. Results

3.1 Patient clinical features

Clinical features of the 93 MPM patients are reported in Table I. 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.

3.2 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 17 PTVs (12 indels and five nonsense variants) and 402 missense and 353 synonymous variants. Seven of the PTVs should be considered variants of unknown significance (VUS) until a functional assay evaluates their effect (Table S2).

Therefore, only ten PTVs were considered pathogenic and were subjected to further study (Table II). Eleven missense variants fulfilled the filter criteria described in Supplementary Methods (Table III).

3.3 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 IV).

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).

The nonsense mutation in *BRCA1* is classified as pathogenic by ClinVar and ENIGMA. The frameshift mutation in *BRCA2* is classified as pathogenic by ClinVar, ENIGMA and ARUP. The deleterious mutation in *FANCI* is reported in ExAC with an MAF (minor allele frequency) lower than 0.001%.

The other eight PTVs were not reported in gene- or disease-specific databases (Table S3).

The MAF of each PTV in our dataset was higher than the MAF of all LOF variants in the corresponding gene in the ExAC and EVS databases ($p<0.0001$), except for *BRCA2* ($p=0.004$) in EVS and *PMS1* ($p=0.017$) in ExAC.

All the variants were successfully validated with Sanger sequencing on DNA obtained from peripheral blood.

The clinical data for the patients are reported in Table IV. 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.

No truncating mutations were found in *BAP1* and *CDKN2A* in our cohort.

3.4 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 that leads to the production of a non-functional protein.

The patient with a PTV in *ATM* showed a decreased amount of an allele for the only 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 have not identified the PTV in the tumor tissue, overall, this result suggests that the mutated allele was lost in the tumor. This behavior was also reported for breast cancers harboring ATM PTVs [31], 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*.

3.5 Missense variants

Eleven missense variants were found in six different genes: *FANCA, MLH1, MSH6, MUTYH, TSC2* and *HNF1A* (Table III, Table V).

Only one of these variants (*MUTYH* c.1145G>A p.Gly382Asp), carried by three patients, is considered pathogenic by ClinVar. One of these patients also carried a PTV in *SLX4*.

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 S4).

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 this concurrent inherited disease was not thoroughly reported in the Registry and during the interview.

It is intriguing that a patient with TS has been reported to develop a primary pericardial mesothelioma [32]. Moreover, *TSC1* has been found to be somatically mutated in mesothelioma, and mice with *TSC1* and *TP53* mutations develop mesothelioma [33].

A variant (c.1226C>A p.Pro409His) in *HNF1A* was found in two patients.

A variant (c.3727A>T p.Thr1243Ser) in *MSH6* was found in another patient, and two missense variants (*MUTYH* c.1145G>A p.Gly382Asp, *MLH1* p.Lys618Ala) were found in patients with familial MPM. Familiarity for other cancers was found in patients with *FANCA, HNF1A* and *TSC2* variants.

Because the data are not unequivocal, all these missense variants should be considered VUS until their effect is evaluated by a functional assay.

3.6 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 [34], was found in a single MPM patient who also carried also *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.

3.7 Statistical analyses

Statistical analyses were performed to evaluate the differences between patients carrying PTVs and the other patients.

The mean of the quantitative asbestos exposure was 3.8 (SD 9.5) for the group with PTVs and 25.7 (SD 148) for the other group. A statistically significant difference of the mean values of quantitative exposure between the two groups was detected (p=0.0017) (Table I). A statistically significant difference was also present when the quantitative exposure of the PTV group was compared to that of the 71 patients who did not carry either PTVs or missense variants (p=0.0037) (Table S5).

No statistically significant association was found for histotype or the categories of asbestos exposure in the two groups. 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 and para-occupational asbestos exposure was 1.2 (95% CI 0.3-4.7) (Table I).

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 I).

4. Discussion

Our study shows, for the first time, that a substantial proportion (9.7%) of MPM patients carry PTVs in DNA repair genes, and 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, 13 patients (14%) carry rare missense variants considered damaging by *in silico* prediction tools and located in *FANCA, TSC2*, *HNF1A*, *MLH1*, *MSH6*, and *MUTYH*.

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 systems reported for the PTVs, i.e., HRR (*FANCA*) 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. In this way, we may have underestimated the prevalence of mutation carriers. The NGS approach we used does not reveal large rearrangements, for which a whole genome NGS or CGH-based approach would be more appropriate [35,36]. Last and not least, mutations in genes that are not included in the TruSight Cancer panel have not been 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.

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 [17,18,37]. Also the proportion of patients carrying PTV is similar.

Our study suggests that MPM must 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 relevance because all carriers are at risk for a certain cancer spectrum and should be included in specific high-risk surveillance and secondary prevention programs [38]. However, in order to completely characterize all genes conferring increased risk of MPM, a whole exome approach is needed.

Finally, the identification of inherited predisposition for mesothelioma may turn out to be relevant for treatment in the future. This was the case for ovarian cancers due to predisposing germline *BRCA1* and *BRCA2* variants, where a different response to PARP inhibitors was identified [39,40]. 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 [41,42].

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Table(s)

Table I. 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%)	1 (reference)
Female	28 (30.1%)	3 (33.3%)	25 (29.8%)	1.2 (0.3-5.1)
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	12 (12.9%)	-	12 (14.3%)	sarcomatoid)
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%)	1.2 (0.3-4.7)
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	1 (1.1%)	-	1 (1.2%)	household)
Not available	3 (3.2%)	-	3 (3.6%)	
History of cancer				
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	23.4±140.2^^	3.8±9.5	25.7±148.0^^	
Mean± SD (after logarithmic transformation)	0.9±1.8	-0.8±2.3	1.1±1.6	0.0017

Abbreviations: PTVs, pathogenic truncating variants; OR, odd ratio; CI, confidence interval; SD, standard deviation

*****Patients with PTVs *versus* Patients without PTVs; ^Not available for 3 patients;^^ Not available for 6 patients

Table(s)

Table II. PTVs identified in this study

Gene	Transcript	Mutation	Mutation type	Mutation Effect	FFPE tumor sample	DNA repair pathway
PALB2	NM_024675.3	c.691A>T p.Lys231*	Substitution	Nonsense	na	HRR
BRCA1	NM_007294.3	c.3001G>T p.Glu1001*	Substitution	Nonsense	Cytoplasmatic protein expression (IHC)	HRR
FANCI	NM_001113378.1	c.3846_3850delCACCT p.Ser1282fs*18	Deletion	Frameshift	na	HRR
ATM	NM_000051.3	c.8436delT p.Ser2812fs	Deletion	Frameshift	LOH-nuclear protein expression (IHC)	HRR
SLX4	NM_032444.2	c.2819delG p.Gly940fs	Deletion	Frameshift	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
PMS1	NM_000534.4	c.1380delT p.Ser460fs	Deletion	Frameshift	na	MMR
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; *PALB2*, partner and localizer of BRCA2; *BRCA1*, early-onset breast cancer gene 1; *FANCI*, Fanconi Anemia Complementation Group I; *ATM*, ataxia telangiectasia mutated; *SLX4*, Structure-Specific Endonuclease Subunit; *BRCA2*, early-onset breast cancer gene 2; *FANCC*, Fanconi Anemia Complementation Group C; *FANCF*, Fanconi Anemia Complementation Group F; *PMS1*, postmeiotic segregation increased 1; *XPC*, Xeroderma pigmentosum, complementation group C.
All changes are heterozygous.

Table(s)

Table III. Missense variants identified in this study that fulfilled the filter criteria

Gene	Transcript	Variant
FANCA	NM_000135.2	c.1874G>C p.Cys625Ser
		c.3665C>T p.Pro1222Leu
TSC2	NM_000548.3	c.1255T>G p.Phe419Val
		c.1915C>T p.Arg639Trp
		c.2278A>C p.Thr760Pro
HNF1A	NM_000545.5	c.1226C>A p.Pro409His
MLH1	NM_000249.3	c.1852_1853delAAinsGC p.Lys618Ala
		c.1136A>G p.Tyr379Cys
MSH6	NM_000179.2	c.3104G>T p.Arg1035Leu
		c.3727A>T p.Thr1243Ser
MUTYH	NM_001048171.1	c.1145G>A p.Gly382Asp

Abbreviations: *FANCA*, Fanconi Anemia Complementation Group A; *TSC2*, Tuberous Sclerosis Complex 2; *HNF1A*, Hepatocyte Nuclear Factor 1-Alpha; *MLH1*, MutL Homolog 1; *MSH6*, mutS homolog 6; *MUTYH*, MutY DNA Glycosylase.
All changes are heterozygous.

Table(s)

Table IV. 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.

Table(s)

Table V. Clinical features of patients carrying missense variants

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)

Abbreviations: *FANCA*, Fanconi Anemia Complementation Group A; *TSC2*, Tuberous Sclerosis Complex 2; *HNF1A*, Hepatocyte Nuclear Factor 1-Alpha; *MLH1*, MutL Homolog 1; *MSH6*, mutS homolog 6; *MUTYH*, MutY DNA Glycosylase; M, male; F, female; MPM, malignant pleural mesothelioma; nr, not reported; na, not available.

Supplementary File

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Conflict of interest statement

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

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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.